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Letter to the Editor

Chloramphenicol activity against carbapenemase producing *Enterobacterales*

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The dearth of new drugs against carbapenemase-producing Enterobacterales (CPE), especially against metallo-β-lactamase-producers, has prompted reconsidering of old antimicrobials as potential options [1]. Among these, chloramphenicol has been less investigated, likely due to concerns with risk of toxicity. However, it has sometimes been considered as a potential anti-CPE option [1,2].

In this study, we evaluated chloramphenicol activity, with reference broth microdilution (BMD) and a commercial gradient diffusion (GD) system, against a collection of CPE of different clonal lineages and carbapenemase genotypes. The collection included 58 KPC-producing *Klebsiella pneumoniae* (KP-KPC) of 7 clonal lineages (ST11, ST15, ST101, ST258, ST307, ST512 and ST1685), 27 NDM-producing *K. pneumoniae* (KP-NDM) of 3 clonal lineages (ST147, ST39 and ST11), 5 VIM-producing *K. pneumoniae* (KP-VIM) of 4 clonal lineages (ST101, ST147, ST870 and ST2217), and 15 NDM-producing *Escherichia coli* (EC-NDM) of 6 clonal lineages (ST38, ST125-like, ST167, ST361 and ST405), isolated in Italy during nationwide surveys or outbreaks, and previously characterized by whole genome sequencing (Table S1).

Chloramphenicol susceptibility was assessed using reference BMD (ISO 20776-1:2019, https://www.iso.org/standard/70464.html) and MIC Test Strip GD system (Liofilchem, Roseto degli Abruzzi, Italy), following manufacturer's instructions, starting from the same 0.5 McFarland suspension in 0.9% normal saline. GD MIC values falling between doubling dilutions were rounded up to the nearest doubling dilution. Isolates were categorized as wild-type (WT) and non-WT, based on the ECOFF value of 16 mg/L (https://www.eucast.org/clinical_breakpoints).

GD results were compared to those of reference BMD according to ISO 20776-2:2021 (https://www.iso.org/standard/79377.html), measuring bias (B, acceptability $\leq\pm30\%$), essential agreement (EA, acceptability $\geq90\%$), and category agreement (CA, acceptability $\geq90\%$, referred to the ECOFF value). Categorical discrepancies were also evaluated with reference to the ECOFF value in terms of category overestimation (CO, when a WT strain was categorized as non-WT) or category underestimation (CU, when a non-WT strain was categorized as WT) rates.

Overall, reference BMD showed that 43/105 (41%) of the isolates were classified as WT, with 85.2% of KP-NDM isolates (23/27), followed by 66.7% of EC-NDM isolates (10/15), 20% of KP-VIM isolates (1/5) and 15% of KP-KPC isolates (9/58). The overall chloramphenicol $MIC_{50/90}$ were 32/>256 mg/L, while those for KP-KPC and KP-NDM isolates were >256/>256 mg/L and 8/32 mg/L, respectively (Table 1).

Results of comparison between BMD and GD showed that the latter system presented a bias of 85.3% (58/68), EA and CA rates of 89.5% (94/105) and 84.8% (89/105), respectively, and a CO of 15.2% (16/105) (with no CU) (Table S2), denoting a low accuracy of the GD system which exhibited a clear trend to overestimate MIC values.

Chloramphenicol susceptibility testing showed a variable behaviour among CPE, with a high rate of KP-NDM (85%) being WT. This result could be of interest given the lack of activity of new beta-lactamase inhibitors combinations (e.g., ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam) against MBL producers, and the increasing number of reports of cefiderocol resistance among NDM-KP [3,4]. On the other hand, non-WT strains were more prevalent among other types of CPE. Previous reports on chloramphenicol susceptibility patterns among CPE were consistent with our study [5,6].

Analysis of acquired chloramphenicol resistance genes in our CPE collection revealed a notable diversity. The most common gene was *catA1* ($n = 42$ in KP-KPC), followed by *catB3* ($n = 25$ in KP-NDM), *catB2* ($n = 5$ KP-VIM), *catB4* ($n = 4$, KP-KPC) and *floR* ($n = 3$, EC-NDM). Multiple resistance genes were detected in three strains (one KP-NDM presenting *catB4* and *catII*, one KP-KPC presenting *catA1, catB3* and *floR*, and one KP-KPC presenting *catA1, cmlA5* and *floR*), while 22 strains ($n = 12$ EC-NDM, $n = 10$ KP-KPC) presented no acquired resistance genes (Table S1). Overall, chloramphenicol geometric mean MIC value (GMM) of strains carrying one or more acquired chloramphenicol resistance genes ($N = 79$) was significantly higher than that of strains lacking those genes ($N = 22$) (GMM, 86.4 vs. 20.6 mg/L, $P < 0.01$). Other mechanisms of chloramphenicol resistance (e.g., porin alterations, overexpression of efflux pumps and alterations in ribosomal genes) were not investigated in this study.

In conclusion, this study suggests that chloramphenicol might still deserve some interest as a potential anti-CPE agent, possibly in combination with other anti-CPE molecules [7], even if its use might contribute to an increase of resistance selection. However, this conclusion should be interpreted cautiously, and further investigations including broader collections of CPE and other CPE species are needed to confirm and extend present findings.

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Table 1
Results of chloramphenicol susceptibility testing by reference broth microdilution.

	MIC (mg/L)								
	4	8	16	32	64	128	256	>256	Total
Number of isolates (%/cumulative%)									
All strains	2 (2/2)	23 (22/24)	18 (17/41)	11 (10/51)	6 (6/57)	3 (3/60)	5 (5/65)	37 (35/100)	105 (100)
K. pneumoniae KPC	–	2 (3/3)	7 (12/15)	9 (16/31.5)	3 (5/36.5)	2 (3/39.5)	1 (1/40.5)	34 (60/100)	58 (100)
K. pneumoniae NDM	2 (7/7)	19 (71/78)	2 (7/85)	2 (7/92)	–	–	1 (4/96)	1 (4/100)	27 (100)
K. pneumoniae VIM	–	1 (20/20)	0 (0/20)	0 (0/20)	1 (20/40)	1 (20/60)	2 (40/100)	–	5 (100)
E. coli NDM	–	1 (7/7)	9 (60/67)	–	2 (13/80)	–	1 (7/87)	2 (13/100)	15 (100)

The vertical line indicates WT cut-off values.
The grey zone indicates MIC values above ECOFF
No strains showed MIC values between ≤0.125 and 2 mg/L.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.02.003.

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