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Enterobacter hormaechei Carrying *bla*$_{NDM}$ Predominates Among Carbapenem-resistant *Enterobacter* spp. Collected During the SENTRY Antimicrobial Surveillance Program (2016–2021)

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Introduction

- *Enterobacter* spp. have emerged as one of the leading reservoirs of carbapenemase genes, but a lack of knowledge surrounds the distribution of these genes among specific *Enterobacter* lineages owing in part to
  - Imprecise physiological and biochemical identification in microbiology laboratories
  - Genomic typing methods that rely on small numbers of intrinsic markers
  - Survey methodology that focuses narrowly on carbapenemase type or geographic locale
- Proper classification may reveal important differences contributing to reduced antibiotic susceptibility via intrinsic or acquired resistance mechanisms among *Enterobacter* lineages.
- This study characterised a global collection of carbapenem-resistant *Enterobacter* (CREnt) from the SENTRY Antimicrobial Surveillance Program using next generation sequencing (NGS) and comparative genomics to survey temporal and geographic shifts in carbapenemase carriage.

Materials and Methods

Bacterial organisms, susceptibility testing, and NGS qualification

- 242 CREnt and 63 non-CREnt (1/patient infection episode) collected from 96 centres in 29 countries across 4 regions from 2016–2021 were included in this study.
- Isolates were screened for carbapenem non-susceptibility (MICs >1 mg/L for imipenem, IMI, and/or meropenem, MER) using standard broth microdilution methods and interpreted according to Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.
- NGS-based identification and resistance mechanism characterization was performed for all isolates.

Screening of carbapenemase genes and comparative genomics

- MLST and Kraken2 identification were performed using publicly available databases.
- A core genome MLST (cgMLST) for *Enterobacter* was created using chewBBACA v2.8.5.
  - 117 complete *Enterobacter* spp. genomes (20/species; *E. ludwigii* *n* = 17) were used for scheme creation (2,783 loci, 95% genome presence); an EHOR-specific cgMLST scheme was created using 89 complete genomes (2,906 loci, 95% genome presence).
  - A concatenated alignment of all loci for each study isolate was produced with MUSCLE v3.8.1551; maximum-likelihood phylogenetic tree of all isolates was created with FastTree 2.1.11. and edited using iTOL v6.7.
  - cgMLST schema comparisons were performed and unique gene sets for each scheme were assigned to clusters of orthologous genes (COGs) using DeepNOG.

Results

- CREnt accounted for 2.6% of *Enterobacter* spp. (245/9,354) and 8.3% (245/2,945) of all carbapenem-resistant *Enterobacteriaceae* (Table 1 and data not shown).
- *E. hormaechei* (EHOR) accounted for 74.0% of CREnt and were the most prevalent species isolated in all years (Fig. 1 and Table 1).
  - 58.9% of carbapenemase-producing (CPE) EHOR bore a metallo-β-lactamase (MBL) (Table 1); 80.6% of those strains carried NDM-type enzymes (47.5% overall).
  - KPC-type enzymes were detected in 34.1% of EHOR (61.7% KPC-3; 30.0% KPC-2).
  - MBL carriage in EHOR rose from 50.0% in 2016 to 72.0% in 2021 while KPC prevalence declined over that period (Table 1).
  - Among non-EHOR CREnt, *E. cloacae* (ECL) was the most common species (31.7%) followed by *E. roggenkampii* (17.4%); KPCs (84.4%), specifically KPC-2 (56.3%), predominated in this group (Table 1).
  - Non-CPE CREnt accounted for 14.9% of all CREnt (11.2% EHOR; 25.4% non-EHOR) and were more frequently isolated from Europe (Fig. 2).
  - IMI-intermediate/MER-susceptible MICs were more common in non-CREnt non-EHOR species (70.6%, 24/34); 100% of those strains were non-CPE (data not shown).
- cgMLST analysis delineated distinct divisions between EHOR and non-EHOR lineages (Fig. 3).
  - Kraken2 species designation generally agreed with cgMLST-based clustering for most groups, but failed to speciate 8 isolates within the *E. asburiae* cgMLST cluster.
  - Kraken2 assigned 16 isolates to ECL that clustered within EHOR by cgMLST.
    - 75.0% of ST93 or ST89 isolates were called ECL by Kraken2.
    - 100% of ST89 members were non-CPE isolates from Poland; no characteristic (geography, resistance mechanism) united ST93 isolates.
  - Compared to a cgMLST scheme comprised of multiple *Enterobacter* lineages, an EHOR-specific scheme contained a higher representation of genes potentially involved in nutrient transport and metabolism (24), secretion and trafficking (14), and numerous genes of unknown function (13) (Table 2).

Conclusions

- *E. hormaechei* (EHOR) is the dominant species among CREnt, and clones tended to cluster by geography and carbapenem-resistance mechanism.
  - From 2016-2021, MBL carriage in EHOR was consistent, while KPC carriage declined.
- Kraken2 speciation generally agreed with cgMLST; Kraken2 designated *E. cloacae* strains (ST93 and ST89) were identified within the EHOR lineage by cgMLST.
- In non-EHOR CREnt, KPCs were the most common resistance mechanism detected (42.9%).
  - KPC-2 was more common among non-EHOR CREnt compared to higher rates of KPC-3 carriage in EHOR.
  - Carbapenemase carriage displayed no pattern in these isolates; IMI-intermediate/MER-susceptible phenotypes were common in non-CREnt non-CPE non-EHOR isolates.
- The EHOR core genome contained more genes associated with Type-II and Type-VI secretion and nutrient transport/metabolism, pointing to potential survival strategies underlying EHOR success in clinical settings.

Table 1. Distribution of carbapenemases among CREnt collected worldwide from 2016–2021 through the SENTRY Antimicrobial Surveillance Program

Organism/Carbapenemase[a]	Year						Grand Total
	2016	2017	2018	2019	2020	2021	
Enterobacter spp.[b]	1,723	1,602	1,585	1,575	1,493	1,376	9,354
CREnt[c] (%)	35 (2.0)[d]	48 (3.0)	35 (2.2)	47 (3.0)	33 (2.2)	44 (3.2)	242 (2.6)
CR *E. hormaechei* (%)	21 (60.0)	35 (72.9)	28 (80.0)	39 (84.8)	27 (81.8)	29 (65.9)	179 (74.0)
MBL	8	17	16	17	17	18	93
NDM	7	13	14	13	13	15	75
KPC	8	13	9	17	6	7	60
KPC-2	1	3	1	8	4	1	18
KPC-3	6	10	8	7	1	5	37
CR *E. cloacae* (%)	5 (14.3)	3 (6.3)	2 (5.7)	1 (2.1)	3 (9.1)	6 (13.6)	20 (8.2)
MBL	3	1	1	1		2	8
NDM	2	1				2	5
KPC	2	2	1			3	8
KPC-2			1			3	4
KPC-3	2	2					4
Other CR *Enterobacter* spp.[e] (%)	9 (25.7)	10 (20.8)	5 (14.3)	7 (14.9)	3 (9.1)	9 (20.5)	43 (17.8)
MBL		1	1	1	1		4
NDM		1	1				2
KPC	3	2	2	4	1	7	19
KPC-2	1	2	1	2	1	7	14
KPC-3	1		1	2			4

[a] Carbapenemases identified: IMI-1 (1), IMI-12 (1), IMI-4 (1), IMP-8 (1), KPC-2 (36), KPC-3 (45), KPC-4 (3), KPC-6 (3), NDM-1 (60), NDM-4 (8), NDM-5 (2), NDM-7 (10), NMC-A (2), OXA-48 (9), VIM-1 (21), and VIM-4 (1). For isolates with NDM-1/OXA-48 (1) or NDM-5/OXA-48 (1) profile, only the NDM-allele is represented in Table 1.
[b] Isolates are generally referred to as "*Enterobacter* spp." unless otherwise identified via genome sequencing.
[c] Only the CREnt isolates subjected to next-generation sequencing are shown. Additional CREnt isolates collected in 2017 (1), 2019 (2), and 2021 (2) were excluded from this analysis.
[d] % CREnt of total *Enterobacter* spp. collected in any year or the % of each species or species group of all CREnt.
[e] Other CREnt isolates: *E. asburiae* (7), *E. bugandensis* (2), *E. chengduensis* (3), *E. kobei* (9), *E. ludwigii* (3), *E. roggenkampii* (11), and unspeciated *Enterobacter* (8).

Figure 1. Kraken2 speciation among CREnt in the SENTRY Antimicrobial Surveillance collection.

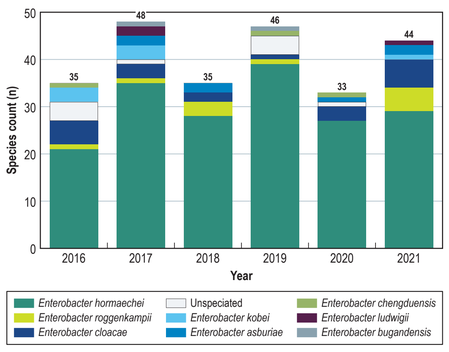


Figure 2. Select carbapenemase distribution among CREnt stratified by geographic region and year.

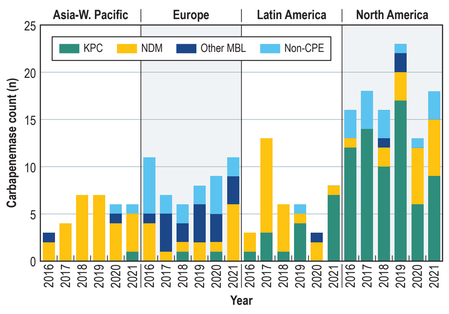
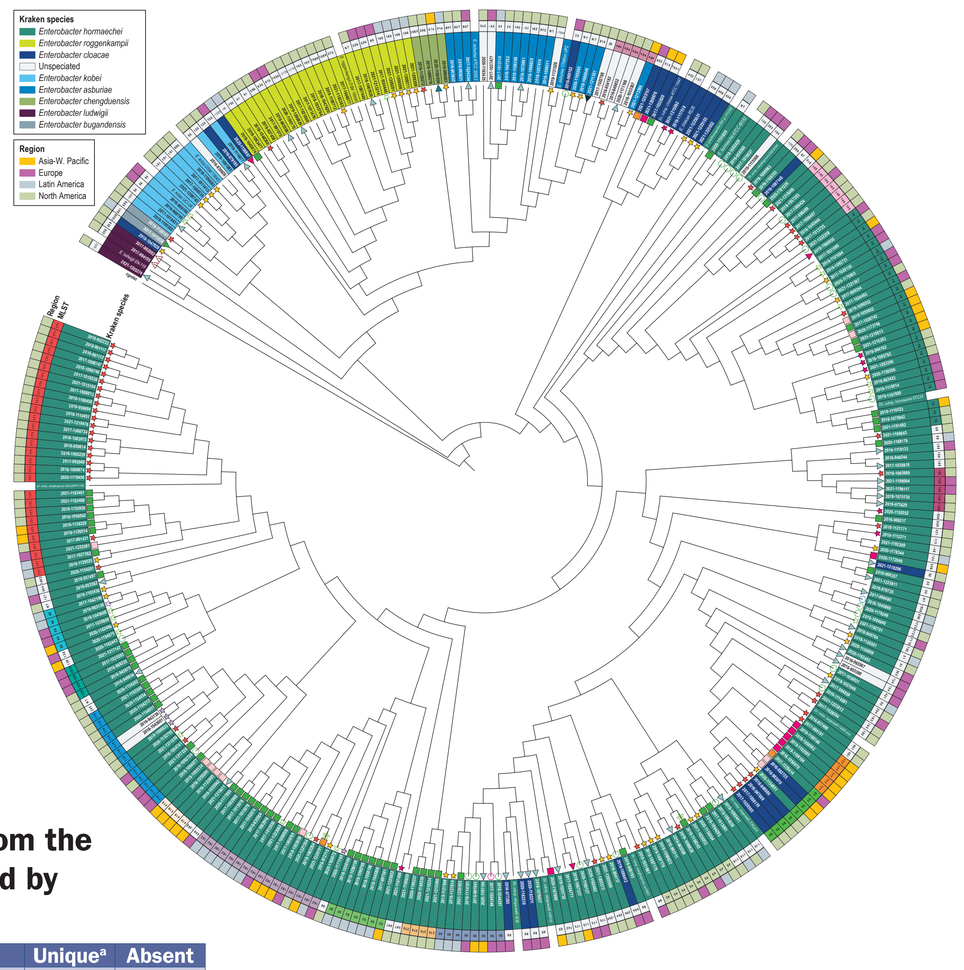


Figure 3. cgMLST phylogeny of *Enterobacter* spp. collected through the Sentry Antimicrobial Surveillance Program 2016–2021.



The tree is rooted at the cgMLST scheme created as outlined in Materials and Methods. Concentric datasets were applied for all isolates included from the Sentry program and are order from innermost to outermost (coloration and symbols correspond to individual legends): carbapenem resistance mechanism (NDM, squares: NDM-1, green; NDM-4, magenta; NDM-5, gold; NDM-7, rose; KPC, stars: KPC-2, yellow; KPC-3, orange; KPC-4, magenta; KPC-6, lavender; VIM, open circles: VIM-1, green; VIM-4, magenta; VIM-23, blue; non-CPE, filled grey triangle; IMI, right-facing filled triangle: IMI-1, green; IMI-4, magenta; IMI-12, black; NMC-A, open triangles; IMP, left-facing filled triangles: IMP-1, green; IMP-8, grey-blue; IMP-12, black; OXA, open stars: OXA-48, green; OXA-163, purple); non-CREnt isolates and reference strains have no symbol; Kraken2 species ID; standard 7-gene MLST; Country. Reference sequences included for species group identification: *E. ludwigii* EN-119 (GCA_001750725.1), *E. kobei* UCI24 (GCA_000534275.1), *E. kobei* DSM 13645 (GCA_001729765.1), *E. roggenkampii* DSM16690 (GCA_001729805.1), *E. asburiae* ATCC 35953 (GCA_001521715.1), *E. asburiae* 17Nkhm-UP2 (GCA_007035805.1), *E. cloacae* subsp. *cloacae* ATCC 13047 (GCA_000025565.1), *E. cloacae* RS35 (GCA_023702375.1), *E. quasihormaechei* D41-sc-1712200 (GCA_013376835.1), *E. hormaechei* ATCC 49162 (ATCC), *E. hormaechei* subsp. *xiangfangensis* DSM 46348 (GCA_019048625.1), *E. hormaechei* subsp. *hormaechei* ECC33 (GCA_025266795.1), *E. hormaechei* subsp. *steigerwaltii* 14269-yvys (GCA_023023665.1), *E. hormaechei* subsp. *steigerwaltii* JT.WLB1A (GCA_025244885.1), *E. hormaechei* subsp. *xiangfangensis* va18651 (GCA_024585325.1), *E. hormaechei* subsp. *steigerwaltii* VKH10 (GCA_024218835.1), *E. hormaechei* subsp. *steigerwaltii* 4236 (GCA_025311595.1), *E. hormaechei* subsp. *xiangfangensis* OSUCZKPC4-140 (GCA_022376815.1)

Table 2. Genes unique to and absent from the *E. hormaechei* cgMLST scheme, grouped by functional COG category

COG Functional Category (Category Codes)	Unique[a]	Absent
Nutrient transport and metabolism (E, F, G, H, I, P, Q)	24	9
Intracellular trafficking, secretion, and vesicular transport (U)	14	0
Function unknown (S)	13	3
Regulation (K, O, T)	9	7
Intracellular processes (C, J, L)	9	4
Cell cycle processes (D, M)	7	3
General function prediction only (R)	5	4
Extracellular processes (N, V, W)	5	1
Grand Total	86	31

[a] Only COGs with a confidence of >95% were included.

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