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Short Communication

Genomic comparison of carbapenem-resistant Enterobacteriaceae from humans and gulls in Alaska

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ABSTRACT

Objectives: Wildlife may harbour clinically important antimicrobial-resistant bacteria, but the role of wildlife in the epidemiology of antimicrobial-resistant bacterial infections in humans is largely unknown. In this study, we aimed to assess dissemination of the bla_{KPC} carbapenemase gene among humans and gulls in Alaska.
Methods: We performed whole-genome sequencing to determine the genetic context of bla_{KPC} in bacterial isolates from all four human carbapenemase-producing Enterobacteriaceae (CPE) infections reported in Alaska between 2013–2018 and to compare the sequences with seven previously reported CPE isolates from gull faeces within the same region and time period.
Results: Genomic analysis of CPE isolates suggested independent acquisition events among humans with no evidence for direct transmission of bla_{KPC} between people and gulls. However, some isolates shared conserved genetic elements surrounding bla_{KPC}, suggesting possible exchange between species.
Conclusion: Our results highlight the genomic plasticity associated with bla_{KPC} and demonstrate that sampling of wildlife may be useful for identifying clinically relevant antimicrobial resistance not observed through local passive surveillance in humans.

1. Introduction

Carbapenems are considered to be antibiotics of last resort for treating multidrug-resistant bacterial pathogens, and resistance to these important antibiotics has become a surveillance target in humans and the environment in recent years. The first *Klebsiella pneumoniae* carbapenemase (KPC), an enzyme conferring carbapenem resistance, was identified in 1996 in a hospital in the eastern USA [1]. The bla_{KPC} gene has since spread globally within healthcare facilities, less commonly at the community level and, rarer still, to wildlife [2,3]. This gene has been identified in different Gram-negative bacterial species and is highly mobile. Horizontal gene transfer of bla_{KPC} between bacterial strains, species and even genera can occur through a variety of mechanisms, including transfer of conjugative plasmids. Specifically, the gene is commonly located on an ~10-kb Tn*4401* transposon, which itself is capable of transposition into different genomic regions [4].

A previous investigation reported the detection of seven carbapenem-resistant *Escherichia coli* isolates from gull (*Larus* spp.) faeces collected in Alaska during 2016, including four harbouring the carbapenemase gene bla_{OXA-48} and three harbouring bla_{KPC-2} [2]. Four human clinical cases of carbapenemase-producing Enterobacteriaceae (CPE) were also reported in Alaska between 2013–2018, all attributed to *K. pneumoniae* and reported to harbour bla_{KPC} [5]. In the current study, we performed whole-genome sequencing on bacterial isolates from these four human CPE isolates and compared the genomic data with the three bla_{KPC-2}-positive *E. coli* isolates from gulls to assess dissemination of the bla_{KPC} gene within Alaska.

2. Methods

Whole-genome sequencing was performed using Illumina HiSeq (Illumina Inc., San Diego, CA, USA) and PacBio Sequel (Pacific Biosciences, Menlo Park, CA, USA) platforms on four CPE isolates from humans isolated between 2013–2018 and three CPE isolates from gulls isolated in 2016. Briefly, draft genomes were assembled from short- and long-read sequences. Antimicrobial

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Table 1
Genetic context of bla_{KPC} in Enterobacteriaceae isolates from humans and gulls in Alaska.

ID	Year	Species	Host	bla_{KPC} type	Plasmid type	Plasmid size (bp)	Transposon	Flanking sequences
A1_180 (CP040384)	2016	*Escherichia coli*	Gull	KPC-2	IncC	118,204	Tn*4401a*	TCAAT . . . TCAAT
A1_181 (CP040068)	2016	*E. coli*	Gull	KPC-2	IncC	210,031	Tn*4401a*	TCAAT . . . TCAAT
A1_182	2016	*E. coli*	Gull	KPC-2	IncC	210,041	Tn*4401a*	TCAAT . . . TCAAT
KPC1709400028	2017	*Klebsiella pneumoniae*	Human	KPC-3	$IncFII_{K2}$	124,760	Tn*4401a*[a]	TCAAT . . . TGATC . . . TGATC . . . TCAAT[a]
KPC1422300207	2014	*K. pneumoniae*	Human	KPC-3	$IncFII_{\gamma}$	68,168	Tn*4401d*	AGAGA . . . AGAAC
KPC1808800061	2018	*K. pneumoniae*	Human	KPC-3	$IncFII_{\gamma}$	69,370	Tn*4401d*	AGAGA . . . AGAAC
KPC1715200133	2017	*K. pneumoniae*	Human	KPC-2	NA	NA	Tn*4401a*	GTCCT . . . GTCCT

NA, not applicable.
[a] Inverted duplicated transposon.

resistance genes, multilocus sequence types and plasmid types were identified in silico, and core genome single nucleotide polymorphisms (SNPs) were used for phylogenetic inference. See Supplemental material for detailed methodology. All genomic data supporting the findings of this investigation are publicly available in the Sequence Read Archive (BioProject **PRJNA622828**).

3. Results and discussion

All four human *K. pneumoniae* isolates were sequence type 258 (ST258), a widespread epidemic clone [6], and harboured either bla_{KPC-2} or bla_{KPC-3}. The bla_{KPC} gene was located within Tn*4401* transposons, which were found in different genetic backgrounds. Isolates KPC1808800061 and KPC142200207 harboured an $IncFII_{\gamma}$ plasmid in which bla_{KPC-3} was located within a Tn*4401d* transposon (Table 1). Isolate KPC1709400028 harboured an $IncFII_{K2}$ plasmid with two inverted identical Tn*4401a* transposons, each harbouring bla_{KPC-3} (Table 1). This plasmid shared >99% DNA sequence similarity to the widespread plasmid pKpQIL [7]. Isolate KPC1715200133 harboured bla_{KPC-2} within a chromosomally-encoded Tn*4401a* transposon that was inserted into a putative guanine deaminase gene (Table 1). In addition to the different genetic backgrounds of bla_{KPC} and different target site duplication flanking regions, the four human ST258 isolates had different antimicrobial resistance gene profiles (Fig. 1).

In comparison, the three bla_{KPC-2} positive *E. coli* isolates from gulls in Alaska harboured bla_{KPC-2} within Tn*4401a* transposons on IncC plasmids (Table 1). The 9906-bp Tn*4401a* transposons had identical sequences to the transposons from two human isolates, with the exception of a single SNP differentiating bla_{KPC-2} from bla_{KPC-3}. Furthermore, one *K. pneumoniae* plasmid from a human-origin isolate shared a >20-kbp DNA segment with an *E. coli* plasmid isolated from a gull (Fig. 2). Thus, comparisons among KPC-positive CPE isolates originating from humans and gulls in Alaska revealed varying levels of genetic similarity at discrete genetic loci.

The finding of KPC genes within a highly conserved Tn*4401a* transposon in different plasmid types and bacterial genera from different hosts highlights the genomic plasticity associated with this resistance gene. Sheppard et al. previously illustrated the dissemination of bla_{KPC} within a single healthcare facility over a period of 5 years and analogised it to a nested Russian doll-like mobility [8]. Our results demonstrate comparable nested genetic levels of bla_{KPC} diversity between bacterial isolates from humans and gulls in Alaska, although without direct epidemiological links between hosts.

The diversity of antimicrobial resistance gene profiles and genetic backgrounds of the KPC gene among human-origin multidrug-resistant *K. pneumoniae* isolates in Alaska is congruent with independent acquisition of CPE, which likely occurred outside of Alaska given that all patients had previously been hospitalised outside of the state prior to diagnosis [5]. Similarly, given the

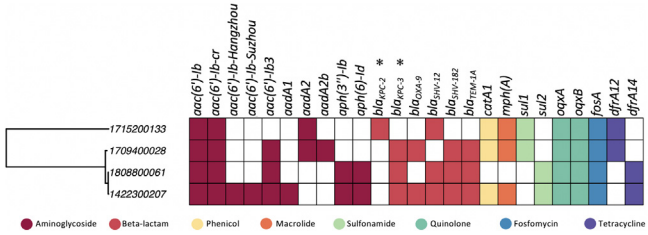

Fig. 1. Core genome midpoint-rooted maximum-likelihood phylogenetic tree of four carbapenem-resistant *Klebsiella pneumoniae* human isolates harbouring a bla_{KPC} carbapenemase gene (*). Shaded squares in the matrix to the right indicates presence of antimicrobial resistance genes, in which different colours represent different antibiotic classes.

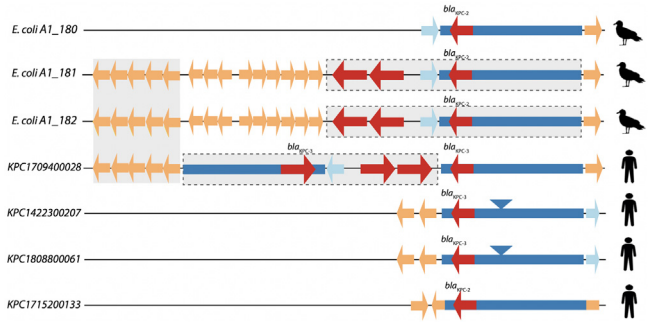

Fig. 2. Genetic background of bla_{KPC} in three *Escherichia coli* isolates from gulls and four *Klebsiella pneumoniae* isolates from humans sampled in Alaska. Dotted boxes indicate duplicated inverted sequence and shaded regions indicate identical nucleotide identity (with the exception of a single single nucleotide polymorphism differentiating bla_{KPC-2} from bla_{KPC-3}). Tn*4401* transposons (dark blue) with triangles indicating deletions, antimicrobial resistance genes (red), hypothetical proteins (orange) and transposon elements (light blue) are indicated.

migratory connectivity of birds from Southcentral Alaska with areas within the Pacific Northwest and California and the tendency of gulls to inhabit landfills during the non-breeding season [9], gulls may have acquired bla_{KPC} outside of Alaska and maintained it within individuals or within the gull population throughout the breeding and post-breeding period (i.e. 2–4 months). This scenario seems plausible given that colistin-resistant *E. coli* was detected in the environment of experimentally challenged gulls 29 days after they were inoculated with *mcr-1*-positive *E. coli* [10]. Alternatively, humans and/or gulls may have acquired CPE through direct or indirect routes from local healthy carriers (see Ref. [11]) or out-of-state visitors, given incomplete information on community carriage of antimicrobial resistance within Southcentral Alaska and the limited scope of research and surveillance for CPE within this region.

We find it striking that only four CPE isolates have been reported in humans in Alaska over a 6-year period and seven gull

faecal samples were positive for CPE in a single year. We recognise that the number of isolates compared in this study is low, however we compared all isolates reported in a state with minimal agriculture, relatively low human population density, and where gulls are presumed to acquire antimicrobial-resistant bacteria from human sources. Wildlife may serve as indicators of clinically important antimicrobial resistance in the environment [12] and may be associated with human-acquired antimicrobial-resistant bacterial infections [13]. Through the application of a One Health genomic approach that elucidated the genetic context of bla_{KPC} at multiple genetic levels, we demonstrate that sampling wildlife can provide information about critically important antibiotic resistance not identified through passive surveillance at a similar spatiotemporal scale in the human health sector.

Ethical approval

Not required.

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Competing interests

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2021.02.028.

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