Identification of a Cluster of Extended-spectrum Beta-Lactamase–Producing *Klebsiella pneumoniae* Sequence Type 101 Isolated From Food and Humans

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We report a cluster of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* sequence type 101, derived from 1 poultry and 2 clinical samples collected within the setting of a prospective study designed to determine the diversity and migration of ESBL-producing Enterobacterales between humans, foodstuffs, and wastewater.

**Key Words.** ESBL; *Klebsiella pneumoniae*; sequence type 101; poultry; One Health.

Multiple studies and outbreak reports point to health-care settings being the most important reservoir for ongoing transmission of multidrug-resistant *Klebsiella pneumoniae*, with only a few reports pointing to the food chain as another source [1, 2]. To further investigate whether such strains and their resistance genes are predominately acquired in the community or within health-care settings, we designed a prospective study to determine the genetic relatedness of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales and their mobile genetic elements recovered from clinical samples, foodstuffs, and wastewater [3]. Within this setting, we identified a mixed cluster of ESBL-producing *K. pneumoniae* isolates of the sequence type (ST) 101, derived from 1 poultry and 2 clinical samples. This high-risk ST is related to numerous hospital out-breaks [4–7], but has not been reported from food samples so far. We here report the epidemiological context and the detailed genetical analyses of this cluster; compare it to international, publicly available chromosomal and plasmid sequences; and discuss its contribution to our understanding of the epidemiology of ESBL-producing *K. pneumoniae*.

**METHODS**

Over a 12-month period (June 2017–May 2018), a prospective study [3] (ClinicalTrials.gov identifier: NCT03465683) designed to study transmission of extended-spectrum beta-lactamase–producing Enterobacteriales (ESBL-PE) was carried out in Basel, Switzerland. ESBL-PE were systematically recovered from samples collected during routine clinical care at the University Hospital Basel, while wastewater and foodstuff samples were collected monthly at predefined locations throughout the city. Whole-genome sequencing was performed on all ESBL-PE isolates collected from wastewater, foodstuff, and clinical samples (we chose 1 isolate per species from each body site per hospital stay) by Illumina NextSeq500/550, and genetic relatedness was assessed via core genome multi-locus sequence typing (cgMLST) genotyping. During the study period, 1 mixed cluster of ESBL-producing *K. pneumoniae*, isolated from 1 food sample and 2 clinical samples, was identified, applying the definition of less than 15 allelic differences in the 2358 genes analyzed. The isolates belonging to this cluster were additionally sequenced by Oxford Nanopore technologies. The chromosomes and plasmids were compared, and the resistance genes and replications were identified. The Supplementary Material further details the methodological approaches.

**RESULTS**

ESBL-producing *K. pneumoniae* ST101 was recovered from 1 chicken meat sample that originated from Switzerland and was bought from a supermarket in Basel on 23 June 2017 and from 2 rectal swab samples collected on 9 July and 13 July 2017 from 2 patients admitted to the University Hospital Basel. During the study period, 31 chicken meat samples (7 from the same brand) were collected from the same supermarket from which the contaminated chicken meat sample was bought, and none of the remaining 30 samples revealed ESBL-producing *K. pneumoniae* ST101. Phenotypic resistance profiles are shown in Supplementary Table S1. As per institutional protocol, both patients were routinely screened at hospital admission for rectal carriage of...
multidrug-resistant bacteria after being repatriated from a hospital in Thailand on 9 July 2017, where they were hospitalized for 11 days after a motorcycle accident. Both patients were treated for polytrauma with multiple fractures. No infections or microbiological results were documented on arrival, and no infections were recorded during hospitalization at our institution.

Comparison of the 3 genomes revealed 0 allelic differences in the core genome between the 2 clinical isolates and 6 allelic differences between both clinical isolates and the food isolate. A whole-genome single nucleotide polymorphism (SNP) analysis showed 0 SNPs between both clinical isolates (after filtering out low-quality variants), and found 13 SNPs scattered along the chromosome between the food and the clinical isolates. The 3 isolates have a capsule locus type KL2 and an O locus O1v2; no Klebsiella known virulence genes were identified. Whole-genome assembly based on long-read sequencing revealed a circular chromosome of about 5.1 Mb, an ESBL plasmid of 241–253 kb, and 2 small plasmids of 3 kb and 4 kb in all 3 isolates. The 3 ESBL plasmids showed at least 99.98% identity at the DNA level (Figure 1B). They share the same ESBL genes (bla_{CTX-M-15}), resistance genes to other beta-lactams (bla_{TEM-1B}), sulphonamides (sul2), quinolones (qnrS1), aminoglycosides (aph[3”]-Ib, aph[6]-Id) and plasmid replicons (IncFIBK and IncFII). Only 2 small regions (approximately 5 and 7 kb) present in the food isolate were absent in the plasmids of the clinical isolates; 1 of these regions contains the gene tetA, conferring resistance to tetracycline, and the regulatory gene tetR. Mobile genetic elements, like phages, insertion sequences, and transposons of the Tn3 family, were identified in the flanking regions, suggesting potential involvement in the mobilization of DNA fragments. In the 3 ESBL plasmids, we found additional features, like metal binding and transport genes, including whole operons for cooper resistance (copA, copB, copC, copD, cusA, cusB, cusF, and cusC), for Fe(3+) dicitrate transport (fecA, fecB, fecC, and fecD), and the ars operon (arsR, arsD, arsA, arsB, and arsC), which confers resistance to arsenicals and antimonials. Other antimicrobial-resistant genes to quinolones (oqxA and oqxB), trimethoprim (dfrA14), fosfomycin (fosA), and a bla_{SHV-28}-like gene were identified in the 3 chromosomes.

All 3 sequences were compared to 256 K. pneumoniae ST101 genomes from 32 countries (including Switzerland), retrieved from the Genome database of the National Center for Biotechnology (NCBI) on 19 April 2020. According to cgMLST, all samples were distributed into 24 clusters, and the cluster detailed in this report remains unique, with a distance of more than 200 allelic differences to other ST101 genomes (Figure 1A; Supplementary Figure S1). The ESBL plasmids seem to be exclusive to our sequences. A search of the PLSDB database [9] revealed 5 hits (Figure 1B; Supplementary Table S2). From them, only 2 plasmids (NZ_CP025457 and NZ_CP025577) show comparable size and structure, but they lack the multidrug-resistance genes.

**DISCUSSION**

The genetically distinct chromosomes and plasmids of the food and clinical isolates reported here differ from all other

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**Figure 1.** A, Minimum spanning tree of 263 Klebsiella pneumoniae ST101 samples from 32 countries (including Switzerland), based on the allelic differences found in the 2358 genes of the K. pneumoniae sensu lato cgMLST v1.0 (Ridom SeqSphere+ v6.0.0; Ridom, Münster, Germany). The mixed cluster of the 3 isolates described in this study is encircled in blue. Isolates originating from Thailand are encircled in red. Color-filled circles denote the different cgMLST clusters. Connection lines represent allelic distance values: a continuous black line indicates less than 15, a continuous gray line indicates 16 to 30, and a discontinuous gray line indicates more than 30. B, BRIG comparison of the ESBL plasmids [8]; pKP101-F is plasmid of the food isolate; pKP101-P1 and pKP101-P2 are plasmids of the clinical isolates; NZ_CP025457, NZ_CP025577, CP042872, NZ_CP011334, and NZ_CP046945 are hits of the pKP101-F plasmid in the PLSDB database. The color intensity of the concentric rings represents the percent of identity against the reference used (pKP101-F). Replicons and resistance genes are marked in red. Abbreviations: cgMLST, core genome multi-locus sequence typing; ESBL, extended-spectrum beta-lactamase; ST, sequence type; BRIG, BLAST ring image generator; GC content, guanine-cytosine content.
Based on the SNP analysis, we speculate that both patients may have been exposed to the same contaminated food source of ESBL-producing *K. pneumoniae* in Switzerland prior to travelling to Thailand. Our study design, which does not collect detailed information on links between patient and foodstuff samples, does not allow us to conclude that this specific food isolate was the direct transmission source, but based on the number of SNPs, it is likely that the 3 isolates have a common and recent ancestor. Hence, our results suggest that foodstuffs may be an important, neglected source for the transmission of important outbreak clones to humans. Once introduced into health-care settings, further institutional spread by direct contact between health-care workers and patients or indirect contact with contaminated environments may facilitate the establishment of specific clones within health-care systems [1]. Even though no known virulence genes were found in these isolates, their multidrug resistance represents a potential risk for their carriers. Additionally, they host a set of metal resistance genes, which could favor the persistence of these microorganisms in the environment even in unfavorable conditions, facilitating their spread and eventually reaching areas of close contact with humans.

It is noteworthy that both patients were only identified as carriers, as the institutional infection prevention and control guidelines require screening of all patients repatriated from institutions abroad, in line with recommendations by public health authorities [10]. Unaware of the sequencing results, colonization with ESBL-producing *K. pneumoniae* would have been considered likely to have been acquired during the journey or the hospitalization in Thailand, based on reports of high rates of ESBL colonization in returning travelers from [11] and after hospitalization in Southeast Asia [12]. The distinct chromosomes and plasmids identified in this cluster, however, question the assumption that acquisition of these strains occurred in Thailand, a conclusion that is further supported by published *K. pneumoniae* ST101 genomes from Thailand being genetically very distant to the Swiss genomes, with 302 to 322 allelic differences. Screening policies merely based on the known epidemiology of ESBL producers may thus fail to detect potentially relevant sources, entertaining further spread.

Over the last decade, ESBL-PE, mainly *Escherichia coli*, have been increasingly identified in livestock, in the food chain, and in companion animals. However, the importance of these reservoirs in entertaining ongoing transmission in humans remains controversial [13, 14]. CTX-M-15–producing *K. pneumoniae* of other STs has been identified in companion animals [15] and retail chicken samples [16, 17], yet, so far, clustering with clinical samples has not been reported. Applying a “One Health” approach has revealed distinguishable ESBL transmission cycles in different hosts, and failed to demonstrate a close epidemiological linkage of ESBL genes and plasmid replicon types between livestock farms and people in the general population for *E. coli* [18]. Yet transmission to and from nonhuman sources may be required to maintain spread among humans [19], based on the results of a recently published modeling study revealing that human-to-human transmission within the open community alone might not be self-maintaining without transmission to and from nonhuman sources. Our findings support these results and point to the need of continuous surveillance, including detailed whole-genome sequencing studies of epidemiologically important strains to enhance our knowledge of the epidemiology of ESBL-PE and derive effective infection prevention and control measures.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

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