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Case report

Highlighting extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* outbreak by routine genomic typing

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ABSTRACT

Whole genome sequencing has become the gold standard for any microbiological investigations. Taking the opportunity to doing it prospectively and routinely allowed to detect undeclared outbreaks. Thanks to that, we investigated and ended a rare epidemic extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* ST584 strain on two intensive care units over a 4-month period.

1. Introduction

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen and a natural inhabitant of the human intestinal tract. This species can also be found on mucosal surfaces in mammals, as well as in water, sewage, soil, and plants [1–3]. Amongst various mechanisms of antibiotic resistance, *K. pneumoniae* can acquire enzymes such as extended-spectrum beta-lactamases (ESBL) which reduce the number of therapeutic options available leading to the prescription of last line antibiotics: carbapenems [4]. Consequently, carbapenem resistant strains have emerged which can cause severe infections among immunocompetent patients [5]. Recent antibiotic use, catheter carriage, mechanical ventilation and prolonged hospitalization are risk factors for antibiotic resistant *K. pneumoniae* infections [6].

K. pneumoniae is responsible for nosocomial infections and hospital outbreaks due to cross transmission from patient to patient, from environmental reservoirs to patients or both intricate mechanisms [7,8]. Surveillance of ESBL-producing Enterobacteriaceae (ESBL-E) is recommended in epidemic circumstances in intensive care units (ICUs) in France [9]. Here we describe the investigation and corrective measures for the first reported hospital outbreak due to an ESBL-producing *K. pneumoniae* ST584 strain.

Abbreviations: ESBL, Extended-spectrum β-lactamases.

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2. Materials and methods

2.1. Setting

The outbreak occurred in a regional cardio-thoracic (CT) ICU (22 beds) and conventional CT ward (22 beds) at Caen University Hospital, a 1410-beds teaching hospital in Normandy, France. During their medical care, patients follow the same pathway: they are initially hospitalized on the CT conventional ward before surgery, following which they are transferred to the CT-ICU. They return to the CT conventional ward once they are stabilized. The same team of health care workers (HCWs) are dispatched in the two units. All patients hospitalized in the hospital's ICUs have a weekly ESBL-Enterobacteriaceae (ESBL-E) carriage screening using rectal swabs. For each patient, the first ESBL-E isolated from screening or clinical samples is tested for antimicrobial susceptibility testing (AST) and DNA extracted to whole genome sequencing (WGS) in order to monitor the circulation of ESBL-E populations and to confirm human to human transmission.

On the 2nd of November 2020 the infection control team (ICT) was notified of a high rate of carriage and infections of ESBL-producing *K. pneumoniae* in the CT intensive care ward which lead to the initiation of an investigation.

2.2. Interventions

For each patient with an ESBL-*K. pneumoniae* carriage or infection the ICT checked the correct application of contact precautions and analyzed their medical files to study the spatiotemporal link between cases. The ICT questioned HCWs from these wards on their

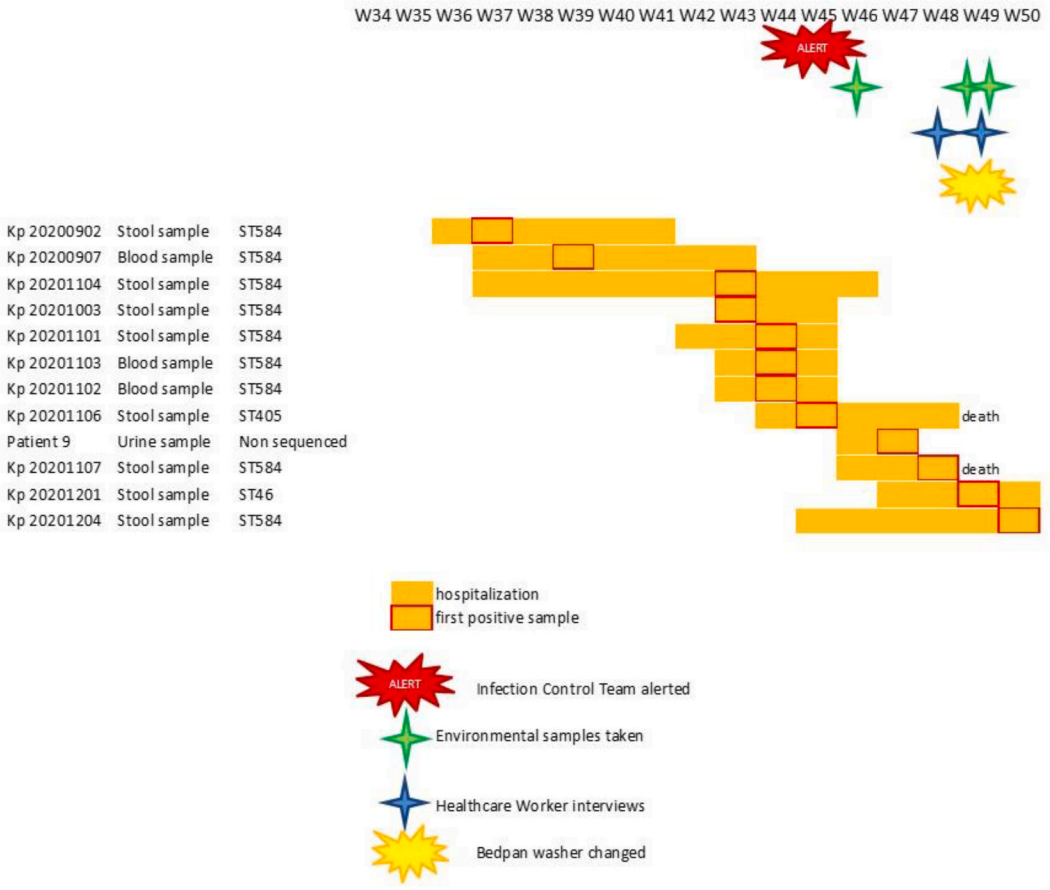

Fig. 1. Chronological description of the ESBL-Kp epidemic in 2020 in Caen University teaching hospital, France.
Chronological description of the ESBL-Kp outbreak in 2020 on two ICU in Caen University Hospital, Caen, France. Twelve outbreak-related cases of ESBL-Kp were identified from September 1st, 2020 and December 6th, 2020. The provenance of the first positive ESBL-Kp sample is described (stool, blood or urine), as well as the Sequence Type (ST) for each case. The urine sample for Patient 9 was no longer cultivable and was not analyzed. Two of the twelve affected patients (Kp 20201106 and Kp 20201107) died in the hospital of non-infectious causes. The length of hospitalization for each case is represented (orange shading), with the week of their first positive ESBL-Kp sample outlined in red. Information on the dates of interventions by the Infection Control Team are presented on the figure, with the initial alert given on the 2nd November 2020, environmental samples performed on week 46, 48 and 49, HCW interviews on week 48 and 49 and change of the defective bedpan washer week 49.

hygiene practices and cleaning procedures for shared medical material. Simultaneously, environmental samples were taken from surfaces and medical material to identify potential indirect cross transmission as well as from sink drain traps and liquids on the wards to look for a potential environmental reservoir.

2.2.1. Microbiology

WGS has been in place in routine in Caen University Hospital since 2019. All the first isolated ESBL-E strains isolated in ICUs of our hospital were tested for AST by disk diffusion according the EUCAST-CASFM guidelines (https://www.sfm-microbiologie.org/) and sequenced after conventional DNA extraction using the Nextseq 500 Illumina technology. Libraries were prepared using the Illumina Nextera Kit generating 150 bp paired-end reads. Quality assessment was performed using fastqc software. Genome were *de novo* assembled using Spades version 3.12. Quality of the assembly was checked using quast software. Several typing methods were applied on the genomes. Multi-Locus Sequence Type (ST) determination based on bigsdb Pasteur database (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Presence of acquired antibiotics genes was assessed using resfinder tool (http://genomicepidemiology.org). A variant calling was performed, on the genomes of the strains which shared the same ST in our database containing nine ESBL-producing *K. pneumoniae* genomes that have been sequenced since the 1st January 2019, using both Burrows-Wheeler Aligner and FreeBayes and the following reference genome ASM393747v1. A Single Nucleotide Polymorphism (SNP) distance matrix was computed. Outbreak cases were defined as a patient hospitalized either on the CT surgery ward or in the CT ICU with a positive sample for ESBL-producing *K. pneumoniae* which belonged to the same ST and displayed less than 10 SNP of divergence. All the sequenced genomes are available on the following Bioprojet (PRJNA811107).

3. Results

3.1. Description of outbreak

Between 1st September 2020 and 2nd November 2020, eight cases of ESBL-producing *K. pneumoniae* were recorded on the two wards, leading to the initial alert on the 2nd of November 2020. Four more patients were tested positive between the 3rd of November 2020 and the 6th of December 2020. A total of 12 patients either carried (n = 8) or were infected (n = 4) by an ESBL-*K. pneumoniae*. All patients recovered except for two of them who died in the hospital of non-infectious causes (Fig. 1).

Nine of the eleven strains sequenced belonged to the same ST584 (6 from stool and 3 from blood samples) while the two remaining ones isolated from stool samples belonged to other *Kp* populations, ST46 and ST405 respectively (Fig. 1). The ST584 strains harbored the same resistome: *blaCTX-M-15 and blaTEM-1B, aph(3″)-Ib* and *aph* [6]*-Id, sul2* and *dfrA14, tet(A)*, *qnrB1,* conferring resistance to penicillins, third-generation cephalosporins, aminoglycosides, co-trimoxazole, tetracycline and decreased susceptibility to quinolones, respectively. This resistance was confirmed by disk-diffusion method according to the referent guidelines. Variant calling analyses revealed that the maximum genomic distance between the strains was 9 SNP, suggesting the dissemination of a unique epidemic strain during a four-month period in two unique but linked wards. Furthermore, no other ESBL-producing *K. pneumoniae* ST584 have been isolated from ICU patients in our hospital since 2019.

Alongside patient sample analysis, 35 environmental samples were taken from medical devices (patient armchairs, patient lifts, ultrasound probes, ECG machines), 10 from surfaces such as computer keyboards, 8 from liquids (hand soap, ultrasound gel etc.), as well as 16 samples from sink drain traps on the wards. All samples were analyzed for microbiology using conventional culture procedures. Of these 69 samples, none were positive for Enterobacteriaceae. Moreover, interviews of personnel did not identify any errors in hygiene practice such as hand hygiene, hygiene and maintenance of shared medical equipment and correct use of PPE. A dysfunctional bedpan washer was present on the CT ICU and was highly suspected as a reservoir as no further strain of ESBL-*K. pneumoniae* ST584 appeared after it was it was changed at the end of November 2020; however, no samples were taken from it before its removal. As genomic typing routine did not reveal any additional ESBL-E belonging to this epidemic strain in all of the ICUs in the establishment since December 2020, this led to two conclusions: (i) the bedpan washer was probably the source of the outbreak, (ii) the *K. pneumoniae* ST584 outbreak could be considered resolved.

4. Discussion and conclusion

To our knowledge, this outbreak is due to a rarely described *K. pneumoniae* ST584 strain. It has been found in animals, which also produced the CTX-*M*-15 enzyme, and in a human hospital acquisition which produced a KPC-2-encoded carbapenemase [10,11]. None of the environmental samples were positive for Enterobacteriaceae. However, the implication of the bedpan washer in the episode was suspected. The dynamic of the outbreak has been still unknown, it could be direct transmission from the potential environmental reservoirs to patients, or cross-transmission from HCWs to patients or lastly an association of these mechanisms.

Thanks to the systematic prospective genomic program in place since 2019, the ICT were able to confirm the authenticity of the outbreak as well as the end of the episode in December 2020 with no additional *K. pneumoniae* ST584 strains found. WGS provides the ability to rapidly identify cases, control outbreaks and identify new strains of MDR Enterobacteriaceae. This active surveillance system appears to be essential in the battle against antibiotic resistance and human cross transmissions.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data associated with this study has been deposited at PRJNA under the accession number 811107.

Additional information

No additional information is available for this paper.

Consent

This study has been conducted in compliance with the Helsinki Declaration (ethical principles for medical research involving human subjects) and in accordance with the guidelines of research board of our teaching hospital, Caen, France. Ethic committee of CHU Caen Normandie reviewed and approved the study. It was a non-interventional study: specimens used in this study were part of the routine patient management without any additional sampling. Furthermore, informed consent was obtained from all subjects and/or their legal guardian(s). Data has been analyzed and presented anonymously.

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None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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