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A prolonged nosocomial outbreak of ESBL *Klebsiella pneumoniae* in a neonatal intensive care unit identified by whole genome sequencing

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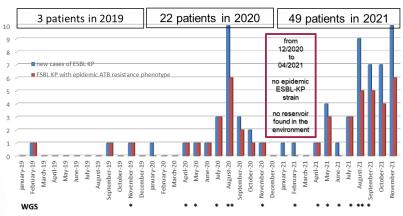


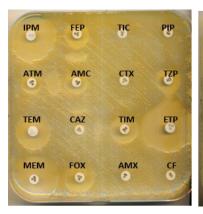
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Background:

Extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* (ESBL-KP) are a noted cause of outbreaks in neonatal intensive care units (NICU)⁽¹⁻²⁾. Rouen University Hospital NICU faced three consecutive outbreaks of ESBL-KP from January 2019 to November 2021 involving respectively 3, 22 and 49 neonates. The same antimicrobial resistance phenotype was shared between the last ESBL-KP strain isolated in November 2020 and the first two strains isolated in 2021 despite a carrier-free period among new-borns. (epidemic diagrams above).





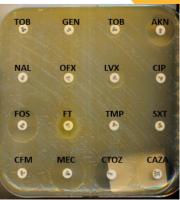


Figure 1 : Epidemic diagrams of TSBL K. pneumoniae from January 2019 to December 2021

Figure 2: Antimicrobial susceptibility pattern of the outbreak ESBL-KP

We used whole genome sequencing (WGS) to investigate these growing outbreaks despite reinforcement of hygiene practices and active screening to detect environmental reservoirs

Methods:

15 strains isolated from blood (n=2, May 2018 and last case November 2020), catheters (n=2 first cases in April and May 2021) and screening rectal swabs (n=11 from November 2019 to august 2021) were further investigated by the *Cellule Régionale d'EpidéMiologie génomique de Normandie* (CREM). First **Antimicrobial susceptibility profile** was tested using a large panel of 40 antimicrobials according to the EUCAST guidelines and breakpoints. Then **WGS** was performed on Illumina NextSeq 500 platform. **Bioinformatic analyses** were performed to define multilocus sequence type (MLST), resistome and virulence factors. For the strains which shared the same MLST type, Single Nucleotide Polymorphisms (SNPs) distance was computed by mapping the read versus a reference genome. In case of ESBL-KP ST15 the reference used was *Klebsiella pneumoniae* strain KP17-16 (CP034077.1).

Results:

The 15 multi-drug resistant ESBL-KP strains belonged to eight different ST suggesting that the three outbreaks in 2019, 2020 and 2021 were not due to a single clone. However, an epidemic strain ESBL-KP (ST15) producing CTX-M-15 has been identified from respectively three and two patients during 2020 and 2021 outbreaks. This strain carried bla genes encoding the ESBL CTX-M-15, oxacillinase OXA-1, penicillinases SHV-100 and TEM-1B. The five ST15 isolates exhibited same antibiotype susceptibility profile and virulence factors (yersiniabactin, siderophores, type 3 fimbriae). They differed from each other by a maximum of 8 SNPs. A phylogenetic analysis using 178 other KPST15 genomes from ncbi highlighted that the ESBL-KP ST15 were specific to the Rouen NICU and were not related to another public genomes.

| Patient | Clinical | Days before | Date of | | Sequence | Number | | Susceptibility profile to |
|------------------|---|-------------------------------------|--|---|--------------------------------------|----------|--|---|
| n° | sample | acquisition (n) | isolation | Acquisition unit (date) | type MLST | of SNP | Beta-lactamase genes | antibiotics |
| 3 4 7 9 | stool stool blood catether catether | 8 13 6 4 5 | 20/04/2020 20/07/2020 14/11/2020 26/04/2021 01/05/2021 | NICU room 408 NICU room 410 NICU room 412 NICU room 411 NICU room 410/412 | ST15 ST15 ST15 ST15 ST15 | 1 to 8 | blaCTX-M-15_blaOXA-1_blaSHV-100_blaTEM-1B blaCTX-M-15_blaOXA-1_blaSHV-100_blaTEM-1B blaCTX-M-15_blaOXA-1_blaSHV-100_blaTEM-1B blaCTX-M-15_blaOXA-1_blaSHV-100_blaTEM-1B blaCTX-M-15_blaCTX-M-27_blaDHA-1_blaOXA-1_blaSHV-100_blaTEM-1B | cefoxitin, CTOZ, CAZA, carbapenems, fosfomycin, amikacin, tetracycline and chloramphenicol |
| 1 2 8 | blood stool stool | imported case 2 imported case | 10/05/2018 25/11/2019 23/02/2021 | PICU PICU PICU room 403 | ST280 ST323 ST405 | | blaCTX-M-15_blaSHV-5_blaTEM-1B blaCTX-M-15_blaOXA-1_blaSHV-99_blaTEM-1B blaCTX-M-3_blaOXA-1_blaSHV-76_blaTEM-1B | same profile as ST15 strains same profile as ST15 strains same profile as ST15 strains |
| 12 13 15 | stool stool | 34 16 23 | 02/08/2021 29/08/2021 12/09/2021 | NNCU room 334 NNCU room 333 NNCU room 339 | ST584 ST584 ST584 | 45 to 56 | blaCTX-M-15_blaSHV-38_blaTEM-1B blaCTX-M-15_blaSHV-38_blaTEM-1B blaCTX-M-15_blaSHV-38_blaTEM-1B | PIP/TAZ, cefoxitin, CTOZ, CAZA, carbapenems, aminoglycosides |
| 6 | stool | 9 | 17/08/2020 | NNCU room 334 | ST35 | | blaSHV-2_blaSHV-33 | PIP/TAZ, cefoxitin, CTOZ, CAZA, carbapenems, aminoglycosides, cotrimoxazole |
| 5 | stool | 25 | 16/08/2020 | NNCU room 432/431 | ST607 | 0 | blaCTX-M-15_blaSHV-78 | PIP/TAZ, cefoxitin, CAZA, CTOZ, |
| 11 | stool | 21 | 07/06/2021 | NNCU room 431 | ST607 | 0 | blaCTX-M-15_blaSHV-78 | carbapenems, aminoglycosides, fluoroquinolones, cotrimoxazole, |
| 14 | stool | 13 | 29/08/2021 | NICU room 410 | ST661 | | blaCTX-M-15_blaSHV-27 | same profile as ST607 strains |

NICU: neonatal intensive care unit; NNCU: neonatal care unit; PICU: pediatric intensive care unit; PIP/TAZ: piperacillin+tazobactam; CTOZ: ceftolozane+tazobactam; CAZA: ceftazidime+avibactam;





Figure 3: Four epidemic ESBL-KP ST15 isolated in the teaching hospital of Rouen and the 80 most related ST15 genomes form ncbi. Analyzes showned the the strains from Rouen belonged to their own cluster Cross—transmission of the epidemic strain ST15 occurred between seven neonates in NICU and nine patients in neonatal ward between during the 2020 outbreak. Screening for environmental reservoir showed the presence of ESBL-KP strains in trap sinks but they didn't have any ST type common with strains isolated from clinical samples. Contaminated trap sinks were systematically removed during 2020. Re-emergence of this ST15 epidemic strain in April 2021 remains unclear as no carrier among patients nor environmental reservoir were identified since November 2020.

Excepted the ST15 epidemic clone, **7** other ST ESBL-KP have been identified in our study. Three and two strains belonged to the ST584 and ST607 respectively. Except for two strains, all the ESBL-KP carried the ESBL *blaCTX-M-15* gene; the two others carried *blaCTX-M-3* (KP ST405) and one *blaSHV-2* (KP ST35). ESBL-KP strains belonging to ST607, 584, 323 and 280 harboured only type three fimbriae as virulence factors whereas the other strains had more than ten virulence encoding genes: yersiniabactin, siderophores, or capsule.

The two ESBL-KP **ST607** strains have been isolated from patients hospitalized in the same room **one year apart** and were strictly identical (0 SNP) suggesting a possible acquisition from an **quiescent strain living in an environmental reservoir**. To note, ST607 type is not frequently involved in NICU outbreaks but a ST607 ESBL-KP CTX-M-15 producing strain responsible for a nosocomial outbreak from July to September 2013 has been reported in a NICU in Amiens located 125 km far from Rouen⁽³⁾.

Conclusion:

WGS is a powerful tool to investigate outbreaks. Genomic analysis showed that an unique epidemic strain was responsible of two of these three outbreaks and was specific to this NICU. This ESBL CTX-M-15 producing KP belonged to ST15, an ST type frequently involved in ESBL-KP outbreaks within NICUs. Persistence of this strain remains unknown despite the lack of a common patient or environmental reservoir identified between the two episodes.

Bibliography:

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