

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

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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).

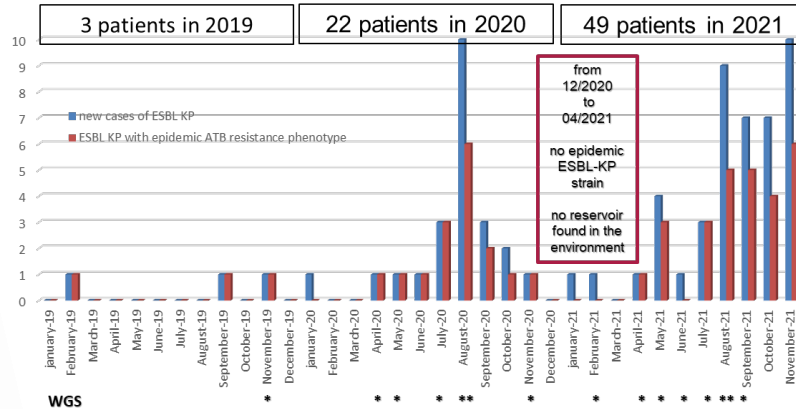


Figure 1 :Epidemic diagrams of TSBL *K. pneumoniae* from January 2019 to December 2021

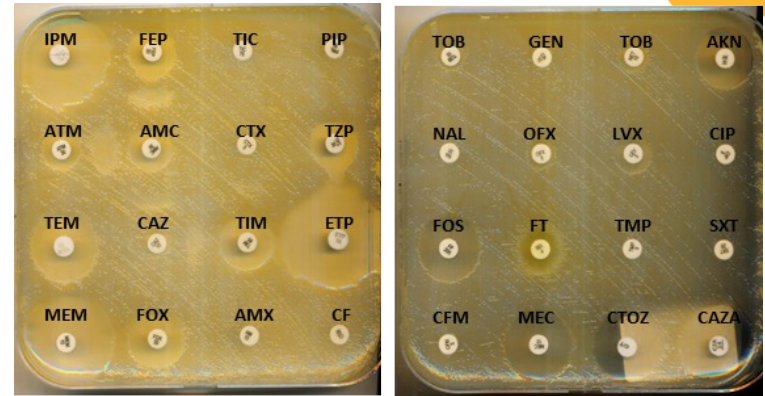


Figure 2 : Antimicrobial susceptibility pattern of the outbreak ESBL-KP

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

