



**HAL**  
open science

# **ESBL-producing *Klebsiella pneumoniae* in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission**

Pierre Edwige L Fils, Pascal Cholley, Houssein Gbaguidi-Haore, Didier Hocquet,  
Marlène Sauget, Xavier Bertrand

## **► To cite this version:**

Pierre Edwige L Fils, Pascal Cholley, Houssein Gbaguidi-Haore, Didier Hocquet, Marlène Sauget, et al.. ESBL-producing *Klebsiella pneumoniae* in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission. PLoS ONE, 2021, 16 (3), pp.e0247875. <10.1371/journal.pone.0247875>. <hal-03356812>

**HAL Id: hal-03356812**

**<https://hal.science/hal-03356812v1>**

Submitted on 6 Jun 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

## RESEARCH ARTICLE

# ESBL-producing *Klebsiella pneumoniae* in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission

Pierre Edwige L. Fils<sup>1</sup>, Pascal Cholley<sup>1,2</sup>, Houssein Gbaguidi-Haore<sup>1,2</sup>, Didier Hocquet<sup>1,2,3</sup>, Marlène Sauget<sup>1,2,3\*</sup>, Xavier Bertrand<sup>1,2</sup>

**1** Hygiène Hospitalière, Centre Hospitalier Universitaire, Besançon, France, **2** UMR-CNRS 6249 Chrono-environnement, Université de Bourgogne Franche-Comté, Besançon, France, **3** Centre de Ressources Biologiques—Filière Microbiologique de Besançon, Centre Hospitalier Régional Universitaire, Besançon, France

\* [msauget@chu-besancon.fr](mailto:msauget@chu-besancon.fr)



## OPEN ACCESS

**Citation:** Fils PEL, Cholley P, Gbaguidi-Haore H, Hocquet D, Sauget M, Bertrand X (2021) ESBL-producing *Klebsiella pneumoniae* in a University hospital: Molecular features, diffusion of epidemic clones and evaluation of cross-transmission. PLoS ONE 16(3): e0247875. <https://doi.org/10.1371/journal.pone.0247875>

**Editor:** Grzegorz Woźniakowski, University of Nicolaus Copernicus in Torun, POLAND

**Received:** October 16, 2020

**Accepted:** February 16, 2021

**Published:** March 24, 2021

**Peer Review History:** PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0247875>

**Copyright:** © 2021 Fils et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper.

## Abstract

The worldwide spread of *Klebsiella pneumoniae* producing extended-spectrum  $\beta$ -lactamase (ESBL-Kp) is a significant threat. Specifically, various pandemic clones of ESBL-Kp are involved in hospital outbreaks and caused serious infections. In that context, we assessed the phenotypic and molecular features of a collection of ESBL-Kp isolates in a French university hospital and evaluated the occurrence of potential cross-transmissions. Over a 2-year period (2017–2018), 204 non-duplicate isolates of ESBL-Kp were isolated from clinical ( $n = 118$ , 57.8%) or screening ( $n = 86$ , 42.2%) sample cultures. These isolates were predominantly resistant to cotrimoxazole (88.8%) and ofloxacin (82.8%) but remained susceptible to imipenem (99.3%) and amikacin (93.8%). CTX-M-15 was the most frequent ESBL identified (83.6%). Multilocus sequence typing and pulse-field gel electrophoresis analysis showed an important genetic variability with 41 sequence types (ST) and 50 pulsotypes identified, and the over representation of the international epidemic clones ST307 and ST405. An epidemiological link attesting probable cross-transmission has been identified for 16 patients clustered in 4 groups during the study period. In conclusion, we showed here the dissemination of pandemic clones of ESBL-Kp in our hospital on a background of clonal diversity.

## Introduction

Over the four past decades, the worldwide spread of extended-spectrum  $\beta$ -lactamases *Enterobacteriales* has become a significant threat [1–3]. The recent emergence of carbapenem-resistant *Enterobacteriales* has further restricted antimicrobial treatment options and has amplified the threat to public health [4]. Carbapenem-resistant and ESBL-producing *Enterobacteriales* are in the WHO priority pathogens list for research and development of new antibiotics [5].

**Funding:** This work was supported by a grant from the University of Franche-Comté to P.E.L.F.

**Competing interests:** The authors have declared that no competing interests exist.

Among *Enterobacterales*, *K. pneumoniae* producing extended-spectrum  $\beta$ -lactamases (ESBL-Kp) is an important nosocomial pathogen with the potential to cause serious infectious diseases such as bacteremia and pneumonia [6–8]. Recently multi-drug-resistant *K. pneumoniae* emergence has led to incurable infections [9–12]. Several class of ESBLs have been described in *K. pneumoniae*. ESBLs that derived from penicillinases TEM and SHV emerged in the 1980s and CTX-M type enzymes such as CTX-M-15 have arisen during the 2000s [13]. This change reflects an important capacity for gene transfer, possibly between epidemic clones [14–16]. The pandemic clones ST258, ST11, ST15, and ST147 spread since two decades and recently, the CTX-M-15-producing ST307 clone emerged globally [17]. Most hospital outbreaks are due to this multidrug-resistant *K. pneumoniae* clones [17–19]. The emergence of carbapenem-resistant strains further complicates the management of these infections [4, 10, 20].

In France, *K. pneumoniae* is the 5<sup>th</sup> most prevalent pathogen responsible for healthcare-associated infection [21]. In the last 15 years, the proportion of *K. pneumoniae* isolates from bloodstream infections resistant to third-generation cephalosporins increased from 4% (in 2005) to 29% (in 2017) with more than 80% of this resistance being due to ESBL production [22]. On the other hand the proportion of carbapenem-resistant *K. pneumoniae* was to 1% in 2019 [23]. Thus, in view of the wide diffusion of epidemic clones, our objective was to describe the phenotypic and molecular characteristics of the ESBL-Kp isolated among patients hospitalized in the Besançon University hospital in 2017 and 2018. Secondly, we have evaluated the cross-transmission of ESBL-Kp major clonal groups within our hospital.

## Materials and methods

### Setting, study period and patients included

We conducted a retrospective cohort study for a 2-year period (from January 2017 to December 2018) in the Besançon University hospital, a 1400-bed hospital with approximately 50,000 admissions and 320,000 patient-days annually. Over the study period, each hospitalized patient with at least one clinical isolate or one screening isolate positive with ESBL-Kp was included. Day care admissions and consultations were excluded. Screening is only carried out in specific hospitalization ward with particular risk, including medical and surgical intensive care units, haematology, neurosurgery and nephrology units. During the study period 10,637 ESBL screenings were performed for 4,238 patients.

### Bacterial isolates

All the isolates were identified as *K. pneumoniae* by MALDI-TOF MS Microflex LT (Bruker Daltonik GmbH, Bremen, Germany) according to the manufacturer's recommendations and routinely tested for ESBL production using the synergy test [24]. ESBL-Kp isolates were stored at  $-80^{\circ}\text{C}$  at the Centre de Ressources Biologiques Filière Microbiologique, Besançon (CRB-FMB, Biobanque BB-0033-00090). For patients with multiple positive samples, we retained for further analysis only the first isolate of ESBL-Kp.

### Antibiotic susceptibility testing

The activity of 13 antibiotics (amoxicillin, amoxicillin/clavulanic acid, ticarcillin, ticarcillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, cefoxitin, imipenem, ertapenem, ofloxacin, amikacin, and the combination sulfamethoxazole/trimethoprim) was assessed according to EUCAST recommendations [24].

## Molecular genotyping

To identify the genes encoding ESBL, the DNA of all the isolates were screened as described before [25]. For the identification of carbapenemases, all isolates non-susceptible to ertapenem (*i.e.* zone diameter  $\geq 25$  mm around a 10- $\mu$ g ertapenem disk) were tested by PCR for the presence of *bla*<sub>OXA-48</sub>, *bla*<sub>KPC</sub>, and *bla*<sub>NDM</sub> genes [26]. The sequence type (ST) of isolates was determined by multilocus sequence typing (MLST) according to Diancourt scheme [27]. The clonality of all ESBL-Kp isolates was investigated by pulsed-field gel electrophoresis (PFGE) following *Xba*I digestion and pulsotypes (PTs) were defined according to international recommendations [28].

## Patient data collection

To evaluate the cross-transmission of epidemic clones, we consulted the medical records of patients carrying isolates displaying the major PTs of ST405 and ST307 clonal groups. We collected retrospectively from January 2017 to December 2018 the following data for each patient: (i) data about the current hospitalization (dates of admission and discharge, length of stay, hospitalization ward), (ii) date and type of sample positive with ESBL-Kp, and (iii) patient outcome. Cross-transmission was considered as probable between two patients if they were hospitalized in the same department over the same period of time.

## Ethics statement

The French regulation allows the study of bacterial strain along with their associated data after information of the patient. Approval of ethical committee was not required in that particular case. The patients whose anonymized data (age; risk factors) were given the following information: "Use of samples and microorganisms for research purposes: samples (blood samples, biopsies, surgical specimens) can be taken to establish a diagnosis and to adapt your treatment or that of your child. Some of these samples or the microorganisms they contain may be stored for diagnostic or research purposes. Your samples are anonymized. The medical data associated with the samples and the microorganisms are collected in a computer file authorized by the CNIL, Commission Nationale de l'Informatique et des Libertés (French Data Protection Authority). You have the right to access and rectify the data entered. You may at any time reconsider your decision without any consequences for your care (or that of your child) and oppose the use of biopsies and operating documents for research by contacting the Franche-Comté Regional Tumorothèque (Tel. +33 3 81 66 89 66) or oppose the use of blood samples or microorganisms for research by contacting the Centre de Ressource Biologique-Filière Microbiologique (Tel. +33 3 70 63 21 34).

## Results

Over the two-year survey, 204 non-duplicate ESBL-Kp isolates were retrieved from clinical ( $n = 118$ , 57.8%) or screening ( $n = 86$ , 42.2%) sample cultures. Among these, 146 (72%) isolates were available for further analysis. ESBL-Kp isolates came from rectal swab or feces ( $n = 59$ ; 40.4%), urines ( $n = 54$ ; 37.0%), blood ( $n = 9$ ; 6.2%) and other samples ( $n = 24$ ; 16.4%). The isolates were found mainly in the intensive care units ( $n = 49$ ; 34%), followed by haematology ( $n = 25$ ; 17%), emergency ( $n = 17$ ; 12%), surgery ( $n = 11$ ; 8%) and hepatology units ( $n = 7$ ; 5%).

Our isolates were predominantly resistant to cefotaxime (98.0%), cefepime (90.4%), ofloxacin (82.8%) and to the combination sulfamethoxazole/trimetoprim (88.8%). However, a small

proportion of ESBL-Kp were resistant to carbapenems (0.7% and 6.8% for imipenem and ertapenem, respectively) and to amikacin (6.2%).

A large majority of the 146 ESBL-Kp isolates available for further analysis produced ESBLs of the CTX-M group, with 122 producing CTX-M-15 (83.6%), 5 producing CTX-M-14 (3.4%), 4 producing CTX-M-3 (2.7%), and 1 producing CTX-M-9 (0.7%). We found only 10 and 4 isolates producing SHV (6.9%) and TEM-like ESBLs (2.7%), respectively (Table 1). Among the 10 isolates non-susceptible to carbapenems, only one harbored a carbapenemase-encoding gene (*bla*<sub>OXA-48</sub>).

Our ESBL-Kp collection was distributed in 41 STs among which ST405 (36 isolates) and ST307 (29 isolates) predominated. Others STs including more than 5 isolates were ST336 (10 isolates), ST15 (9 isolates), ST13 (5 isolates) (Table 1). PFGE analysis distributed 146 isolates in 50 PTs (from PT1 to PT50). The three predominant PTs (PT28, PT26, and PT38) regrouped isolates (35, 26, and 10) from three major STs (ST405, ST307, and ST336), respectively (Table 1).

Overall, we have identified two major clonal groups: (i) ST405 with the majority of isolates, (35 out of 36) that clustered in the PT28, (ii) ST307 with the majority of isolates (26 out of 29) that clustered in the PT26. All isolates of these dominant PTs carried *bla*<sub>CTX-M-15</sub> except one ST307 that harboured *bla*<sub>CTX-M-3</sub> (Table 1). Fig 1 represents the timeline of hospital stay of the patients positive with ESBL-Kp of ST405 and ST307 major PTs to evidence probable cross-transmission. For ST307, we identified three patient clusters over the study period: (i) one in hepatology implying three patients, (ii) one in the medical intensive care unit with 3 patients, and (iii) one in the surgical intensive care unit implying 5 patients (Fig 1A). Besides, five out of the eight patients (P12, P14, P16, P17, P19) positive with ESBL-Kp ST405 had an obvious epidemiological link in the haematology unit (Fig 1B).

**Table 1. Characteristics of ESBL-producing *K. pneumoniae* isolates in the University hospital of Besançon (eastern France), 2017–2018 (n = 146 non-duplicate isolates).**

Sequence type	No of isolates	Pulsotype (no of isolates)	ESBL-encoding gene (no of isolates)	Proportion susceptible to the main antibiotics* (%)								
				P	PI	ESC	FOX	CPE	OFLO	AN	SXT	
ST405	36	PT28 (35)	<i>bla</i> <sub>CTX-M-15</sub>	}	0	6	0	92	99	14	97	6
		PT1 (1)	<i>bla</i> <sub>CTX-M-15</sub>									
ST307	29	PT26 (26)	<i>bla</i> <sub>CTX-M-3</sub> (1), <i>bla</i> <sub>CTX-M-15</sub> (25)	}	0	0	0	77	95	0	100	4
		PT33 (2)	<i>bla</i> <sub>CTX-M-15</sub>									
		PT13 (1)	<i>bla</i> <sub>CTX-M-15</sub>									
ST336	10	PT38 (10)	<i>bla</i> <sub>CTX-M-15</sub> (10)		0	0	0	78	94	0	80	0
ST15	9	PT5 (4)	<i>bla</i> <sub>CTX-M-15</sub> (4)	}	0	26	0	71	89	0	100	44
		PT15 (2)	<i>bla</i> <sub>CTX-M-15</sub> (2)									
		PT32 (2)	<i>bla</i> <sub>CTX-M-15</sub> (2)									
		PT45 (1)	<i>bla</i> <sub>CTX-M-15</sub> (1)									
ST13	5	PT8 (5)	<i>bla</i> <sub>CTX-M-15</sub> (5)		0	7	0	40	100	20	100	0
Other STs (36)	57	39 PTs	<i>bla</i> <sub>CTX-M-3</sub> (3), <i>bla</i> <sub>CTX-M-9</sub> (1), <i>bla</i> <sub>CTX-M-14</sub> (5), <i>bla</i> <sub>CTX-M-15</sub> (34), <i>bla</i> <sub>SHV-2</sub> (8), <i>bla</i> <sub>TEM-like</sub> (4), <i>bla</i> <sub>SHV-11</sub> (1), <i>bla</i> <sub>SHV-12</sub> (1)		0	29	15	66	96	34	89	18

\*P: penicillins (amoxicillin, ticarcillin), PI: penicillins with  $\beta$ -lactamase inhibitors (amoxicillin/clavulanic acid, piperacillin/tazobactam, ticarcillin/clavulanic acid), ESC: extended-spectrum cephalosporins (cefepime, cefotaxime), FOX: ceftioxin, CPE: carbapenems (ertapenem, imipenem), OFLO: ofloxacin, AN: amikacin, SXT: sulfamethoxazole/trimethoprim.

<https://doi.org/10.1371/journal.pone.0247875.t001>



our collection mostly harbored *bla*<sub>CTX-M-15</sub>, they were ofloxacin and sulfamethoxazole/trimethoprim resistant (100% and 96%, respectively) but not to amikacin (0%). The only OXA-48 carbapenemase-producing ST307 isolate that we have identified belonged to one of the sporadic PT. The early identification of this carbapenemase-producing isolate triggered specific infection control measures that presumably avoid its spread.

The clustering of CTX-M-15 producing ST405 isolates into a dominant PT was also in favor of its intra-hospital diffusion. Other groups reported hospital epidemics with ESBL-Kp ST405 producing CTX-M-15 [8, 36, 37]. Although this clone has been reported to produce both CTX-M-15 and a carbapenemase, all but one ST405 isolate of our collection were susceptible to carbapenems [38–43]. Similarly, the global ST15 clone often produce both an ESBL and a carbapenemase [18, 19, 44, 45] while all ST15 isolates of our collection were susceptible to imipenem [8]. The overall proportion of antibiotic resistant isolates in the present collection is consistent with the literature and suspected outbreak isolates remained susceptible to ceftioxin, carbapenems and amikacin (Table 1) [46].

This study has some limitations. First, the ESBL-Kp were collected in a single hospital in France. A multi-centric study could determine whether the epidemic clones identified here (*i.e.* ST307, ST405) also spread in other French hospitals. Although the typing methods (*i.e.* MLST, PFGE) used here were appropriate to investigate local outbreaks of bacterial pathogens, the whole genome sequencing would have (*i*) provided more detailed information on ESBL-Kp phylogeny and (*ii*) allowed the identification of virulence genes and the genetic environment of the ESBL-encoding genes.

## Conclusion

We documented in a University Hospital (Eastern France) the dissemination of two epidemic clones of ESBL-kp (ST405, ST307) on a background of clonal diversity. Implementation of additional infection control measures for patients positive with ESBL-Kp is fully justified. Further studies are needed to identify the genetic and biological features that favor the spread of these multidrug resistant epidemic clones.

## Author Contributions

**Conceptualization:** Xavier Bertrand.

**Formal analysis:** Pierre Edwige L. Fils, Marlène Sauget.

**Funding acquisition:** Xavier Bertrand.

**Investigation:** Pierre Edwige L. Fils, Pascal Cholley.

**Methodology:** Houssein Gbaguidi-Haore.

**Project administration:** Xavier Bertrand.

**Resources:** Xavier Bertrand.

**Supervision:** Xavier Bertrand.

**Validation:** Marlène Sauget.

**Writing – original draft:** Marlène Sauget.

**Writing – review & editing:** Didier Hocquet, Xavier Bertrand.

## References

1. Paterson DL, Bonomo RA. Extended-spectrum  $\beta$ -lactamases: a clinical update. *Clin Microbiol Rev.* 2005; 18:657–86. <https://doi.org/10.1128/CMR.18.4.657-686.2005> PMID: 16223952
2. Pitout JD, Laupland KB. Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis.* 2008; 8:159–66. [https://doi.org/10.1016/S1473-3099\(08\)70041-0](https://doi.org/10.1016/S1473-3099(08)70041-0) PMID: 18291338
3. Tal Jasper R, Coyle JR, Katz DE, Marchaim D. The complex epidemiology of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae. *Future Microbiol.* 2015; 10:819–39. <https://doi.org/10.2217/fmb.15.16> PMID: 26000652
4. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother.* 2015; 59:5873–84. <https://doi.org/10.1128/AAC.01019-15> PMID: 26169401
5. World Health Organisation. News release. 2017 [cited 22 September 2020]; Available from: <https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>.
6. Podschun R, Ullmann U. *Klebsiella spp.* as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998; 11:589–603. PMID: 9767057
7. Gupta A, Ampofo K, Rubenstein D, Saiman L. Extended spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* infections: a review of the literature. *J Perinatol.* 2003; 23:439–43. <https://doi.org/10.1038/sj.jp.7210973> PMID: 13679928
8. Becker L, Fuchs S, Pfeifer Y, Semmler T, Eckmanns T, Korr G, et al. Whole genome sequence analysis of CTX-M-15 producing *Klebsiella* isolates allowed dissecting a polyclonal outbreak scenario. *Front Microbiol.* 2018; 9:322. <https://doi.org/10.3389/fmicb.2018.00322> PMID: 29527200
9. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis.* 2009; 9:228–36. [https://doi.org/10.1016/S1473-3099\(09\)70054-4](https://doi.org/10.1016/S1473-3099(09)70054-4) PMID: 19324295
10. Bowers JR, Kitchel B, Driebe EM, MacCannell DR, Roe C, Lemmer D, et al. Genomic analysis of the emergence and rapid global dissemination of the clonal group 258 *Klebsiella pneumoniae* pandemic. *PLoS One.* 2015; 10:e0133727. <https://doi.org/10.1371/journal.pone.0133727> PMID: 26196384
11. Paczosa MK, Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol Mol Biol Rev.* 2016; 80:629–61. <https://doi.org/10.1128/MMBR.00078-15> PMID: 27307579
12. Dong N, Zhang R, Liu L, Li R, Lin D, Chan EW, et al. Genome analysis of clinical multilocus sequence Type 11 *Klebsiella pneumoniae* from China. *Microb Genom.* 2018;4. <https://doi.org/10.1099/mgen.0.000149> PMID: 29424684
13. Liu H, Wang Y, Wang G, Xing Q, Shao L, Dong X, et al. The prevalence of *Escherichia coli* strains with extended spectrum  $\beta$ -lactamases isolated in China. *Front Microbiol.* 2015; 6:335. <https://doi.org/10.3389/fmicb.2015.00335> PMID: 25954262
14. Canton R, Gonzalez-Alba JM, Galan JC. CTX-M enzymes: origin and diffusion. *Front Microbiol.* 2012; 3:110. <https://doi.org/10.3389/fmicb.2012.00110> PMID: 22485109
15. Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev.* 2011; 35:736–55. <https://doi.org/10.1111/j.1574-6976.2011.00268.x> PMID: 21303394
16. Elhani D, Bakir L, Aouni M. The changing epidemiology of extended spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae*. *Ann Biol Clin.* 2011; 69:523–9. <https://doi.org/10.1684/abc.2011.0610> PMID: 22008131
17. Wyres KL, Hawkey J, Hetland MAK, Fostervold A, Wick RR, Judd LM, et al. Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. *J Antimicrob Chemother.* 2019; 74:577–81. <https://doi.org/10.1093/jac/dky492> PMID: 30517666
18. Lee MY, Ko KS, Kang CI, Chung DR, Peck KR, Song JH. High prevalence of CTX-M-15-producing *Klebsiella pneumoniae* isolates in Asian countries: diverse clones and clonal dissemination. *Int J Antimicrob Agents.* 2011; 38:160–3. <https://doi.org/10.1016/j.ijantimicag.2011.03.020> PMID: 21605960
19. Rodrigues C, Machado E, Ramos H, Peixe L, Novais A. Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFIIK). *Int J Med Microbiol.* 2014; 304:1100–8. <https://doi.org/10.1016/j.ijmm.2014.08.003> PMID: 25190354
20. Bonura C, Giuffrè M, Aleo A, Fasciana T, Di Bernardo F, Stampone T, et al. An update of the evolving epidemic of blaKPC carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014: emergence of multiple non-ST258 clones. *PLoS One.* 2015; 10:e0132936. <https://doi.org/10.1371/journal.pone.0132936> PMID: 26177547

21. Santé publique France. Enquête nationale de prévalence des infections nosocomiales et des traitements anti-infectieux en établissements de santé, mai-juin 2017. 2019 [cited 22 september 2020]; Available from: <https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-associees-aux-soins-et-resistance-aux-antibiotiques/infections-associees-aux-soins/documents/enquetes-etudes/enquete-nationale-de-prevalence-des-infections-nosocomiales-et-des-traitements-anti-infectieux-en-etablissements-de-sante-mai-juin-2017>.
22. Santé publique France. EARS-Net France, 2002–2017. 2019 [cited 22 september 2020]; Available from: <https://www.santepubliquefrance.fr/maladies-et-traumatismes/infections-associees-aux-soins-et-resistance-aux-antibiotiques/resistance-aux-antibiotiques/documents/ears-net-france-2002-2017-contribution-de-la-france-au-reseau-europeen-de-surveillance-de-la-resistance-bacterienne-aux-antibiotiques>.
23. European Centre for Disease Prevention and Control. Surveillance Atlas of Infectious Diseases. [cited 08 January 2021]; Available from: <https://www.ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc>.
24. EUCAST. European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. July 2017
25. Brechet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P, et al. Wastewater treatment plants release large amounts of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* into the environment. *Clin Infect Dis* 2014; 58:1658–65. <https://doi.org/10.1093/cid/ciu190> PMID: 24795329
26. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 10.0, valid from 2020-01-0. [cited 08 January 2021]; Available from: [https://eucast.org/clinical\\_breakpoints/](https://eucast.org/clinical_breakpoints/).
27. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol*. 2005; 43:4178–82. <https://doi.org/10.1128/JCM.43.8.4178-4182.2005> PMID: 16081970
28. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*. 1995; 33:2233–9. <https://doi.org/10.1128/JCM.33.9.2233-2239.1995> PMID: 7494007
29. Boo NY, Ng SF, Lim VK. A case-control study of risk factors associated with rectal colonization of extended-spectrum  $\beta$ -lactamase producing *Klebsiella* sp. in newborn infants. *J Hosp Infect*. 2005; 61:68–74. <https://doi.org/10.1016/j.jhin.2005.01.025> PMID: 15953660
30. Calbo E, Garau J. The changing epidemiology of hospital outbreaks due to ESBL-producing *Klebsiella pneumoniae*: the CTX-M-15 type consolidation. *Future Microbiol*. 2015; 10:1063–75. <https://doi.org/10.2217/fmb.15.22> PMID: 26059626
31. Hendrik TC, Voor In 't Holt AF, Vos MC. Clinical and molecular epidemiology of Extended-Spectrum  $\beta$ -Lactamase-producing *Klebsiella* spp.: a systematic review and meta-analyses. *PLoS One*. 2015; 10: e0140754. <https://doi.org/10.1371/journal.pone.0140754> PMID: 26485570
32. Long SW, Olsen RJ, Eagar TN, Beres SB, Zhao P, Davis JJ, et al. Population genomic analysis of 1,777 Extended-Spectrum  $\beta$ -Lactamase-producing *Klebsiella pneumoniae* isolates, Houston, Texas: unexpected abundance of clonal group 307. *MBio*. 2017;8. <https://doi.org/10.1128/mBio.00489-17> PMID: 28512093
33. Novovic K, Trudic A, Brkic S, Vasiljevic Z, Kojic M, Medic D, et al. Molecular epidemiology of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* in Serbia from 2013 to 2016. *Antimicrob Agents Chemother*. 2017;61. <https://doi.org/10.1128/AAC.02550-16> PMID: 28242665
34. Park DJ, Yu JK, Park KG, Park YJ. Genotypes of ciprofloxacin-resistant *Klebsiella pneumoniae* in Korea and their characteristics according to the genetic lineages. *Microb Drug Resist*. 2015; 21:622–30. <https://doi.org/10.1089/mdr.2015.0001> PMID: 26207318
35. Geraci DM, Bonura C, Giuffre M, Saporito L, Graziano G, Aleo A, et al. Is the monoclonal spread of the ST258, KPC-3-producing clone being replaced in southern Italy by the dissemination of multiple clones of carbapenem-nonsusceptible, KPC-3-producing *Klebsiella pneumoniae*? *Clin Microbiol Infect*. 2015; 21:e15–7. <https://doi.org/10.1016/j.cmi.2014.08.022> PMID: 25658574
36. Machuca J, Lopez-Cerero L, Fernandez-Cuenca F, Gracia-Ahufinger I, Ruiz-Carrascoso G, Rodriguez-Lopez F, et al. Characterization of an outbreak due to CTX-M-15-producing *Klebsiella pneumoniae* lacking the blaOXA-48 gene belonging to clone ST405 in a neonatal unit in southern Spain. *J Antimicrob Chemother*. 2016; 71:2353–5. <https://doi.org/10.1093/jac/dkw137> PMID: 27118773
37. Xercavins M, Jimenez E, Padilla E, Riera M, Freixas N, Boix-Palop L, et al. High clonal diversity of ESBL-producing *Klebsiella pneumoniae* isolates from clinical samples in a non-outbreak situation. A cohort study. *Antimicrob Resist Infect Control*. 2020; 9:5. <https://doi.org/10.1186/s13756-019-0661-9> PMID: 31911833

38. Glupczynski Y, Huang TD, Bouchahrour W, Rezende de Castro R, Bauraing C, Gerard M, et al. Rapid emergence and spread of OXA-48-producing carbapenem-resistant Enterobacteriaceae isolates in Belgian hospitals. *Int J Antimicrob Agents*. 2012; 39:168–72. <https://doi.org/10.1016/j.ijantimicag.2011.10.005> PMID: 22115539
39. Gharout-Sait A, Alsharapy SA, Brasme L, Touati A, Kermas R, Bakour S, et al. Enterobacteriaceae isolates carrying the New Delhi metallo-beta-lactamase gene in Yemen. *J Med Microbiol*. 2014; 63:1316–23. <https://doi.org/10.1099/jmm.0.073767-0> PMID: 25009193
40. Liapis E, Pantel A, Robert J, Nicolas-Chanoine MH, Cavaille L, van der Mee-Marquet N, et al. Molecular epidemiology of OXA-48-producing *Klebsiella pneumoniae* in France. *Clin Microbiol Infect*. 2014; 20: O1121–3. <https://doi.org/10.1111/1469-0691.12727> PMID: 24942039
41. Del Franco M, Paone L, Novati R, Giacomazzi CG, Bagattini M, Galotto C, et al. Molecular epidemiology of carbapenem resistant Enterobacteriaceae in Valle d'Aosta region, Italy, shows the emergence of KPC-2 producing *Klebsiella pneumoniae* clonal complex 101 (ST101 and ST1789). *BMC Microbiol*. 2015; 15:260. <https://doi.org/10.1186/s12866-015-0597-z> PMID: 26552763
42. Palacios-Baena ZR, Oteo J, Conejo C, Larrosa MN, Bou G, Fernandez-Martinez M, et al. Comprehensive clinical and epidemiological assessment of colonisation and infection due to carbapenemase-producing Enterobacteriaceae in Spain. *J Infect*. 2016; 72:152–60. <https://doi.org/10.1016/j.jinf.2015.10.008> PMID: 26546855
43. Ruiz-Garbajosa P, Hernandez-Garcia M, Beatobe L, Tato M, Mendez MI, Grandal M, et al. A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother*. 2016; 71:348–52. <https://doi.org/10.1093/jac/dkv355> PMID: 26518051
44. Damjanova I, Toth A, Paszti J, Hajbel-Vekony G, Jakab M, Berta J, et al. Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type  $\beta$ -lactamase-producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005—the new 'MRSA's'? *J Antimicrob Chemother*. 2008; 62:978–85. <https://doi.org/10.1093/jac/dkn287> PMID: 18667450
45. Breurec S, Guessennd N, Timinouni M, Le TA, Cao V, Ngandjio A, et al. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect*. 2013; 19:349–55. <https://doi.org/10.1111/j.1469-0691.2012.03805.x> PMID: 22390772
46. Zhou K, Lokate M, Deurenberg RH, Arends J, Lo-Ten Foe J, Grundmann H, et al. Characterization of a CTX-M-15 producing *Klebsiella pneumoniae* outbreak strain assigned to a novel Sequence Type (1427). *Front Microbiol*. 2015; 6:1250. <https://doi.org/10.3389/fmicb.2015.01250> PMID: 26617589