Surveillance and outbreak report

National survey of colistin resistance among carbapenemase-producing Enterobacteriaceae and outbreak caused by colistin-resistant OXA-48-producing Klebsiella pneumoniae, France, 2014

A Jayol¹, L Poirel¹, L Dortet²,³,⁴, P Nordmann¹,⁵
1. Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
2. Associated National Reference Centre for Antibiotic Resistance, Le Kremlin-Bicêtre, France
3. Faculty of Medicine, South-Paris University, Le Kremlin-Bicêtre, France
4. Bacteriology-Hygiene unit, Hospital Bicêtre, Assistance Publique /Hôpitaux de Paris, Le Kremlin-Bicêtre, France
5. University of Lausanne and University Hospital Center, Lausanne, Switzerland

Correspondence: Laurent Poirel (laurent.poirel@unifr.ch)

Citation style for this article:
Jayol A, Poirel L, Dortet L, Nordmann P. National survey of colistin resistance among carbapenemase-producing Enterobacteriaceae and outbreak caused by colistin-resistant OXA-48-producing Klebsiella pneumoniae, France, 2014. Euro Surveill. 2016;21(37):pii=30339. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.37.30339

Article submitted on 30 October 2015 / accepted on 04 April 2016 / published on 15 September 2016

From January 2014 to December 2014, 972 consecutive non-replicate carbapenemase-producing Enterobacteriaceae isolates from colonised or infected patients were collected at the Associated French National Reference Centre as part of the French national survey on antimicrobial resistance. It included 577 Klebsiella spp. (59%), 236 Escherichia coli (24%), 108 Enterobacter spp. (11%), 50 Citrobacter spp. (5%), and a single Salmonella spp. isolate (0.1%). Of 561 K. pneumoniae isolates, 35 were found to be resistant to colistin (6.2%). PFGE analysis revealed a clonal outbreak involving 15 K. pneumoniae isolates belonging to sequence type ST11, recovered in a single hospital in the Picardie region in northern France. Those clonally related isolates showed variable levels of resistance to colistin, ranging from 4 to 64 mg/L. They harboured the blaOXA-48 carbapenemase gene and the blaCTX-M-15 extended-spectrum beta-lactamase gene. Among the 91 Enterobacter cloacae isolates, seven were resistant to colistin and produced different types of carbapenemases. Surprisingly, none of the E. coli and Citrobacter spp. isolates showed resistance to colistin. This national survey including carbapenemase-producing isolates recovered in 2014 reported a high rate of colistin resistance in K. pneumoniae and E. cloacae (6.2% and 7.7%, respectively) in France.

Introduction
Carbapenemase-producing Enterobacteriaceae (CPE) resistant to colistin are increasingly reported. They represent an additional link in the development of pan-drug resistance. However, the epidemiology of colistin resistance among enterobacterial isolates is currently almost unknown in most parts of the world. In Italy, an increase in carbapenemase-producing Enterobacteriaceae has been noted in the past years, but the situation remains unknown in France [1]. The lack of information about the prevalence of colistin resistance among multidrug-resistant enterobacterial isolates derives from several reasons: (i) so far, there has been limited interest in that field, (ii) methods used for determination of colistin susceptibility are not adequate, and (iii) the lack of well-defined breakpoints does not allow precise determination of prevalence. However, the recent identification of a plasmid-borne polymyxin resistance determinant (MCR-1) raised a very serious concern in that resistance to colistin might widely disseminate [2].

The aim of this study was to evaluate retrospectively the prevalence of colistin resistance among a collection of CPE strains recovered in France during a period of one year and to analyse the phenotypic, genotypic features and clonality of the colistin-resistant isolates.

Methods
Carbapenemase-producing Enterobacteriaceae isolates
From January to December 2014, 972 consecutive non-duplicate isolates of carbapenemase-producing Enterobacteriaceae were isolated in private laboratories and hospitals in France either by screening for colonisation or by analysing clinical samples in the context of infections. They were recovered from rectal swabs or stools (n = 625), urine samples (n = 250), respiratory
tract samples (n = 35), blood samples (n = 22), wounds (n = 24), catheter (n = 7), vaginal swabs (n = 3) and other specimens (n = 6). Those isolates were sent to the Associated French National Reference Centre for characterisation of resistance mechanisms to carbapenems as part of the French antibiotic resistance survey. The 972 carbapenemase-producing Enterobacteriaceae isolates included 577 isolates of Klebsiella spp. (59%), 236 isolates of Escherichia coli (24%), 108 isolates of Enterobacter spp. (11%), 50 isolates of Citrobacter spp. (5%), and a single isolate of Salmonella spp. (0.1%). Species that are naturally resistant to colistin (Proteus spp., Morganella morgani, Providencia spp., and Serratia spp.) had been excluded before the initiation of this study. Only a single isolate per patient was included in the study. All isolates were identified using the Microflex bench-top MALDI-TOF mass spectrometer (Bruker, Champs-sur-Marne, France).

**Antimicrobial susceptibility testing**

Minimum inhibitory concentrations (MIC) of colistin (CS) were determined using broth microdilution method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) [3]. As recommended, E. coli ATCC 25922 was used as quality control strain.

For the colistin-resistant isolates, susceptibility to other classes of antibiotics was also tested. Susceptibility to imipenem, ertapenem, and tigecycline was tested by broth microdilution method according to CLSI guidelines, whereas susceptibility to the other antibiotics was tested by the standardised agar disk diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4]. The antibiotics tested using disk diffusion method were: amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), cefepime (FEP), temocillin (TEM), ciprofloxacin (CIP), gentamicin (GM), amikacin

---

**Figure 1**

Geographic distribution of colistin-resistant Enterobacteriaceae isolates, France, January–December 2014 (n = 43)

### Easb: Enterobacter asburiae; Ecl: Enterobacter cloacae; Kpn: Klebsiella pneumoniae.
(AK), trimethoprim-sulfamethoxazole (SXT) and fosfomycin (FOS).

The MIC results for colistin and the disk diffusion diameters were interpreted according to susceptibility breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4].

Molecular characterisation

The \textit{mgrB} genes of \textit{K. pneumoniae} and \textit{Enterobacter} spp. isolates were amplified using specific primers (Table 1), knowing that the MgrB protein is a negative regulator of the PhoPQ two-component system and that alterations in the \textit{mgrB} gene are commonly involved in acquisition of colistin resistance in \textit{K. pneumoniae} [5-7]. The plasmid-mediated \textit{mcr}-1 gene encoding colistin resistance was sought as described previously [2]. Detection of extended-spectrum beta-lactamases (ESBL) and carbapenemases genes was performed with specific primers as described previously [8]. Both strands of the amplification products obtained were sequenced with an ABI 3100 sequencer (Applied Biosystems, Foster City, US). The nucleotide and deduced protein sequences were analysed at the National Centre for Biotechnology Information website (www.ncbi.nlm.nih.gov) by the Basic Local Alignment Search Tool (BLAST) programme.

Genotyping

Genotyping was performed to evaluate the clonal relationship of the colistin-resistant \textit{K. pneumoniae} and \textit{E. cloacae} isolates by pulsed-field gel electrophoresis (PFGE) with \textit{XbaI}-digested genomic DNA and interpreted according to Tenover criteria [9]. Multilocus sequence typing (MLST) for \textit{K. pneumoniae} was performed using the simplified protocol at the Institut Pasteur website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html) [10].

Results

\textit{Klebsiella pneumoniae}

Of 561 \textit{K. pneumoniae} isolates, 35 were found to be resistant to colistin (6%). Fifteen of the 35 colistin-resistant \textit{K. pneumoniae} isolates were recovered from a single hospital in the Picardie region, northern France (Figure 1). We could not obtain the exact dates of their isolations due to the retrospective nature of the study. These isolates had mostly been recovered from rectal swab specimens, but also from a catheter, a urinary sample, a wound exudate and a respiratory specimen (isolates 1 to 15, Table 2). PFGE analysis revealed that the 15 isolates were clonally related (Figure 2, Table 2). The clone was of the ST11 type, and was susceptible only to cefoxitin, amikacin and fosfomycin (Table 2). A single isolate among these 15 was susceptible to tigecycline. The 15 isolates harboured both the \textit{blaOXA-48} carbapenemase gene, and the \textit{blaCTX-M-15} extended-spectrum beta-lactamase (ESBL) gene, and the MICs for colistin ranged from 4 to 64 mg/L (Table 2).

The other 20 colistin-resistant \textit{K. pneumoniae} strains were mostly recovered from the regions Ile-de-France (n = 6) and Provence-Alpes-Côte d’Azur (n = 10) (Figure 1). These strains presented high MIC values for colistin ranging from 16 to >128 mg/L (isolates 16 to 35, Table 2). They produced either the carbapenemases \textit{OXA-48} (15/20), KPC-2 (3/20), NDM-1 (1/20), or both \textit{OXA-48} and NDM-1 together (1/20) (Table 2). Overall, 14 of the 20 isolates produced the ESBL CTX-M-15. PFGE analysis identified 18 clonal patterns among the 20 isolates (n = 3 for clone M) (Figure 2, Table 2), and MLST assigned the isolates to eight sequence types (STs) (Table 2).

Sequencing of the \textit{mgrB} gene of those \textit{K. pneumoniae} isolates revealed various \textit{mgrB} alterations and none
of the strains harboured the plasmid-encoded mcr-1 gene.

Antimicrobial susceptibility data for the colistin-resistant K. pneumoniae isolates not involved in the outbreak revealed that most isolates (19/20) were non-susceptible to third- and fourth-generation cephalosporins (Figure 3A). They were also frequently resistant to ciprofloxacin (19/20), trimethoprim-sulfamethoxazole (15/20) and tigecycline (14/20). They were less often resistant to gentamicin and cefoxitin (13/20 and 11/20, respectively). Amikacin and fosfomycin remained the most active agents against colistin-resistant K. pneumoniae (16/20 and 15/20 were susceptible, respectively) (Figure 3A).

Enterobacter spp.
Among the 91 Enterobacter cloacae isolates, seven were resistant to colistin (7.7%). They showed high MIC values for colistin (ranging from 16 to >128 mg/L) (isolates 36 to 42, Table 2). They produced the carbapenemases OXA-48 (4/7), VIM-1 (1/7), IMP-1 (1/7), or both OXA-48 and VIM-1 together (1/7) (Table 2). In total, three of seven strains were CTX-M producers, with two isolates producing CTX-M-15 and a single isolate producing CTX-M-2. The colistin-resistant E. cloacae isolates were recovered in different geographical regions in France (Figure 1, Table 2), and results of the PFGE analysis revealed that they were not clonally related (data not shown).

The single carbapenem-resistant E. asburiae strain was resistant to colistin. It had an MIC of colistin above 128 mg/L and produced the VIM-1 carbapenemase (isolate 43, Table 2).
All *Enterobacter* spp. isolates had a wild-type *mgrB* gene, leaving unexplained the colistin resistance mechanism (*E. cloacae* and *E. asburiae*) (Table 2).

Of the eight colistin-resistant *Enterobacter* spp. isolates, four were non-susceptible to cephalosporin and tigecycline, and three were non-susceptible to ciprofloxacin, trimethoprim-sulfamethoxazole and gentamicin (Figure 3B). Amikacin and fosfomycin were the most active agents against colistin-resistant *E. cloacae* (all seven isolates were susceptible) (Figure 3B).

**Other species**

None of the *E. coli* (n = 236) and *Citrobacter* spp. (n = 50) isolates were resistant to colistin.

**Discussion**

We describe here a clonal outbreak involving 15 *K. pneumoniae* isolates recovered from a single hospital in the Picardie region in northern France. This outbreak was caused by a colistin-resistant OXA-48 and CTX-M-15-producing *K. pneumoniae* of ST11 type that was susceptible only to cefoxitin, amikacin and fosfomycin. Surprisingly, those clonally related isolates had variable MIC values for colistin ranging from 4 to 64 mg/L. An ST11 clone co-producing OXA-48 and CTX-M-15 was responsible for a large outbreak involving 44 patients in a hospital in Madrid, Spain, from 2009 to 2014 but only 3.4% of the isolates were resistant to colistin [11].

Several outbreaks of colistin-resistant KPC-producing *K. pneumoniae* (mainly attributed to the international epidemic clone type ST258) have been reported across Europe, in Greece [12,13], Hungary [14], Italy [15-17] and the Netherlands [18]. A single outbreak of colistin-resistant VIM-1-producing *K. pneumoniae* has also been described in Spain [19].

We report also 20 colistin-resistant *K. pneumoniae* strains recovered from the regions Ille-de-France and Provence-Alpes-Côte d’Azur. These strains belonged to 10 sequence types (n = 2 ST147, n = 3 ST258, n = 6 ST101, n = 3 ST307) and PFGE analysis identified 18 patterns among the 20 isolates. All three KPC-producing *K. pneumoniae* isolates belonged to ST258, the most common clone for KPC-producing isolates [20]. The OXA-48-producing *K. pneumoniae* isolates belonged to nine sequence types with six strains that were ST101, the most common clone identified among OXA-48-positive *K. pneumoniae* [21].

The rates of colistin resistance among the carbapenemase-producing isolates were 7.7% for *Enterobacter* spp. and 3.6% for *K. pneumoniae* isolates (excluding the isolates responsible for the outbreak in the Picardie region). The resistance rate observed among the carbapenemase-producing *K. pneumoniae* isolates was much lower than the high rates reported in the neighbouring countries of southern Europe such as Spain (20%) [19] and Italy (43%) [1].

None of the 236 carbapenemase-producing *E. coli* isolates were colistin-resistant or carried the *mcr-1* gene. This is surprising considering that a recent report of the French antimicrobial resistance Resapath surveillance network identified the plasmid-borne *mcr-1* gene in 21% of ESBL-producing *E. coli* isolates recovered from faeces of veal calves in France between 2005 and mid-2014 [22]. The plasmid-borne *mcr-1* colistin resistance gene has also been found in many neighbouring countries of France, for example among ESBL-producing *Enterobacteriaceae* isolates recovered from river water and imported vegetable samples in Switzerland [23], in *E. coli* isolates recovered from calves and piglets in Belgium [24], in swine and human wound infections in Germany [25], and in food and human bloodstream infections in Denmark [26]. The *mcr-1* gene was also detected in *Salmonella enterica* from food samples in Portugal [27] and France [28]. An *E. coli* isolate co-harboring the *blaVIM-1* carbapenemase gene and the *mcr-1* gene was described in Switzerland [29] and an isolate co-producing NDM-9 and MCR-1 was reported from China [30]. We believe that the plasmid carrying the *mcr-1* gene might be currently more prevalent among ESBL-producing isolates than among carbapenemase-producing isolates in human samples, which would explain why we did not identify this gene in our collection of carbapenemase-producing isolates.

Amikacin and fosfomycin were most effective against the colistin and carbapenem-resistant *K. pneumoniae* (susceptibility rates of 80% and 75%, respectively) and

---

**Table 1**

| Oligonucleotides   | Sequence (5’-3’)                          | Reference |
|--------------------|-------------------------------------------|-----------|
| Kpn mgrB ext F     | TTA AGA AGG CCG TGC TAT CC               | [12]      |
| Kpn mgrB ext R     | AAG GGC TTC ATT CTA CCA CC               | [13]      |
| Kpn mgrB int F     | CGG TGG GTT TTA CTG ATA GTC              | This study|
| Kpn mgrB int R     | GAA CAT CCT GGT CGC ACA TT               | This study|
| Ent mgrB ext F     | CGG TTT ACT CTA TGA AAC AAG TGC         | This study|
| Ent mgrB ext R     | GCG AAG GAG GGA AAT CAC CT              | This study|

---

www.eurosurveillance.org
### Table 2a

Characteristics of the colistin-resistant *Klebsiella pneumoniae* and *Enterobacter* spp. clinical isolates, France, January–December 2014 (n = 43)

| Isolate | Site of isolation | Origin | MIC CS<sup>a</sup> | mgrB genotype | Carbapenemase | Associated beta-lactamase | Co-resistances<sup>b</sup> | ST | PFGE |
|---------|------------------|--------|-------------------|---------------|---------------|--------------------------|--------------------------|----|------|
| 1       | ?                | Picardie | 32                | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT               | 11 | A    |
| 2       | Catheter         | Picardie | 8                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 3       | Rectal swab      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 4       | Rectal swab      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 5       | Rectal swab      | Picardie | 64                | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 6       | Urine            | Picardie | 32                | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 7       | Rectal swab      | Picardie | 64                | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 8       | Wound            | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 9       | Respiratory      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 10      | Rectal swab      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 11      | Rectal swab      | Picardie | 8                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 12      | Rectal swab      | Picardie | 64                | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 13      | Rectal swab      | Picardie | 8                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 14      | Rectal swab      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 15      | Rectal swab      | Picardie | 4                 | mgrB WT       | OXA-48        | CTX-M-15                 | CIP GM SXT TIG           | 11 | A    |
| 16      | Rectal swab      | Nord-Pas-de-Calais | 128 | IS<sup>a</sup>R in promoter region (between nt −45 and −46) | OXA-48 | CTX-M-15 | CIP GM SXT TIG | 147 | B |
| 17      | Rectal swab      | Ile-de-France| 128 | mgrB WT | NDM | CTX-M-15 | CIP GM SXT TIG | 147 | C |
| 18      | Rectal swab      | PACA | >128 | IS<sup>a</sup>X<sub>202</sub>-like in coding region (between nt +74 and +75) | KPC | - | CIP AK SXT TIG | 258 | D |
| 19      | Rectal swab      | Rhône-Alpes | 128 | MgrB truncated (27 amino acids) | KPC | - | CIP AK SXT TIG | 258 | E |
| 20      | Blood            | PACA | 16               | Full gene deletion<sup>c</sup> | KPC | - | CIP AK SXT TIG | 258 | F |
| 21      | Rectal swab      | Lorraine | 64             | Single nucleotide deletion (nt 74) | OXA-48 | CTX-M-15 | CIP GM | 101 | G |
| 22      | Rectal swab      | Ile-de-France | 32            | Single nucleotide deletion (nt 23) | OXA-48 | CTX-M-15 | CIP GM | 101 | H |
| 23      | Rectal swab      | Ile-de-France | 64            | IS<sup>a</sup>R in promoter region (between nt −36 and −37) | OXA-48 + NDM | CTX-M-15 | CIP GM | 101 | I |
| 24      | Urine            | PACA | 64               | IS<sup>a</sup>R in promoter region (between nt −45 and −46) | OXA-48 | CTX-M-15 | CIP GM SXT TIG | 101 | J |
| 25      | Abcess           | Ile-de-France | 32            | MgrB M27K | OXA-48 | CTX-M-15 | CIP GM | 101 | K |
| 26      | Urine            | Pays de la Loire | 128          | Duplication of 19 nucleotides | OXA-48 | CTX-M-15 | CIP SXT FOS | 101 | L |
| 27      | Urine            | PACA | 64               | IS<sup>a</sup>R in coding region (between nt +21 and +22) | OXA-48 | CTX-M-15 | CIP GM SXT TIG | 307 | M |
| 28      | Rectal swab      | PACA | 64               | mgrB WT | OXA-48 | CTX-M-15 | CIP GM SXT TIG FOS | 307 | M |
| 29      | Rectal swab      | PACA | 64               | IS<sup>a</sup>-like in coding region (between nt +74 and +75) | OXA-48 | CTX-M-15 | CIP GM SXT TIG | 307 | M |
| 30      | Urine            | PACA | >128             | Full gene deletion<sup>c</sup> | OXA-48 | - | CIP GM AK SXT TIG FOS | 611 | N |
| 31      | Rectal swab      | Ile-de-France | 32            | IS<sup>a</sup>X<sub>14</sub>-like in promoter region (between nt −45 and −46) | OXA-48 | - | CIP GM SXT TIG | 23 | O |

Isolates 1-35: *Klebsiella pneumoniae*; 36–42: *Enterobacter cloacae*; 43: *Enterobacter asburiae*.

AK: amikacin; CIP: ciprofloxacin; CS: colistin; FOS: fosfomycin; GM: gentamicin; MIC: minimum inhibitory concentration; NA: not applicable; nt: nucleotide; PACA: Provence-Alpes-Côte-d’Azur; PFGE: pulsed-field gel electrophoresis; ST: sequence type; SXT: trimethoprim-sulfamethoxazole; TIG: tigecycline; WT: wildtype.

<sup>a</sup> MIC of colistin determined by broth microdilution method.

<sup>b</sup> Resistant or intermediate susceptibility to antibiotic.

<sup>c</sup> Full gene deletion: no PCR product was detected with external or internal primers.
The rate of tigecycline non-susceptibility was high (70% for *K. pneumoniae* and 50% for *Enterobacter* spp.), probably because of a strong selective pressure by this last-line antibiotic.

**Conclusion**

This national survey on carbapenemase-producing isolates recovered in 2014 discovered a high rate of colistin resistance in *K. pneumoniae* and *E. cloacae* (6.2% and 7.7%, respectively) in France. These resistance rates remain much lower than those observed in other European countries such as Greece, Italy and Spain. No plasmid-encoded *mcr-1* gene was identified here. Therefore it seems that it is still possible to control the spread of those multidrug-resistant isolates based on accurate identification of colistin resistance and isolation of plasmid-encoded MCR-1 producers.

**Conflict of interest**

None declared.

**Authors’ contributions**

AJ, LP, and PN contributed to the design of the study. AJ performed the experiments. AJ, LP, LD, and PN analysed the data. AJ, LP, and PN contributed to the writing of the manuscript.

**References**

1. Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, et al. Colistin resistance superimposed on carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014. Euro Surveill. 2014;19(42). DOI: 10.2807/1560-7917.ES2014.19.42.20939 PMID: 25358041

2. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a rapidly evolving problem. Lancet Infect Dis. 2016;16(2):161-8. DOI: 10.1016/S1473-3099(15)00424-7 PMID: 26603172

3. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 9th ed. CLSI document M07-A9. Wayne: CLSI; 2015.

4. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoints tables for interpretation of MICs and zone diameters, Version 5.0. Vaxjö: EUCAST. 2015.

5. Cannatelli A, Giani T, D’Andrea MM, Di Pilato V, Arena F, Conte V, et al. MgrB inactivation is a common mechanism of colistin resistance. Clin Infect Dis. 2015;60(2):161-8. DOI: 10.1093/cid/ciu773 PMID: 25990707

**Table 2b**

Characteristics of the colistin-resistant *Klebsiella pneumoniae* and *Enterobacter* spp. clinical isolates, France, January–December 2014 (n = 43)

| Isolate | Site of isolation | Origin | MIC (CS)* | mgrB genotype | Carbapenemase | Associated beta-lactamase | Co-resistancesb | ST | PFGE |
|---------|------------------|--------|-----------|---------------|---------------|--------------------------|-----------------|----|------|
| 32      | Rectal swab      | PACA   | 32        | IS*12-like in coding region (between nt +36 and +37) | OXA-48 | CTX-M-15 | CIP GM SXT TIG | 20 | P    |
| 33      | Respiratory      | Ile-de-France | 32    | IS*1R in promoter region (between nt −61 and −62) | OXA-48 | CTX-M-15 | CIP SXT TIG FOS | Q  |
| 34      | Blood            | PACA   | 112       | mgrB WT       | OXA-48 | CTX-M-15 | CIP SXT TIG | 39 | R    |
| 35      | Rectal swab      | PACA   | 32        | mgrB truncated (32 amino acids) | OXA-48 | -       | FOS | 13 | S    |
| 36      | Rectal swab      | Nord-Pas-de-Calais | 64     | mgrB WT       | OXA-48+ VIM | CTX-M-15 | CIP GM SXT | NA  | T    |
| 37      | Stools           | Languedoc-Roussillon | 64   | mgrB WT       | OXA-48 | CTX-M-15 | GM AK | NA  | U    |
| 38      | Respiratory      | Ile-de-France | 32    | mgrB WT       | VIM | -       | SXT TIG | NA  | V    |
| 39      | Rectal swab      | PACA   | 112       | mgrB WT       | OXA-48 | -       | CIP GM SXT TIG | NA  | W    |
| 40      | Rectal swab      | Ile-de-France | 16     | mgrB WT       | OXA-48 | -       | FOS | NA  | X    |
| 41      | Rectal swab      | PACA   | 112       | mgrB WT       | IMP | PACA | CTX-M-2 | No  | Y    |
| 42      | Respiratory      | Rhône-Alpes | 112     | mgrB WT       | OXA-48 | -       | TIG | NA  | Z    |
| 43      | Urine            | Nord-Pas-de-Calais | 112   | mgrB WT       | VIM-1 | -       | CIP TIG | NA  | α    |

Isolates 1-35: *Klebsiella pneumoniae*; 36–42: *Enterobacter cloacae*; 43: *Enterobacter asburiae*.

AK: amikacin; CIP: ciprofloxacin; CS: colistin; FOS: fosfomycin; GM: gentamicin; MIC: minimum inhibitory concentration; NA: not applicable; nt: nucleotide; PACA: Provence-Alpes-Côte-d’Azur; PFGE: pulsed-field gel electrophoresis; ST: sequence type; SXT: trimethoprim-sulfamethoxazole; TIG: tigecycline; WT: wildtype.

a MIC of colistin determined by broth microdilution method.

b Resistant or intermediate susceptibility to antibiotic.

*E. cloacae* isolates (susceptibility rates of 87%). The rate of tigecycline non-susceptibility was high (70% for *K. pneumoniae* and 50% for *Enterobacter* spp.), probably because of a strong selective pressure by this last-line antibiotic.

E. cloacae isolates (susceptibility rates of 87%). The rate of tigecycline non-susceptibility was high (70% for *K. pneumoniae* and 50% for *Enterobacter* spp.), probably because of a strong selective pressure by this last-line antibiotic.
resistance in KPC-producing Klebsiella pneumoniae of clinical origin. Antimicrob Agents Chemother. 2014;58(10):5696-703. DOI: 10.1128/AAC.03110-14 PMID: 25025853.

6. Cheng YH, Lin TL, Pan YJ, Wang YP, Lin YT, Wang JT. Colistin resistance mechanisms in Klebsiella pneumoniae strains from Taiwan. Antimicrob Agents Chemother. 2015;59(5):2909-13. DOI: 10.1128/AAC.06763-14 PMID: 25691664.

7. Poirel L, Jayał A, Bontron S, Villeugs MV, Ozdamar M, Törgüklü S., et al. The mcr gene as a key target for acquired resistance to colistin in Klebsiella pneumoniae. J Antimicrob Chemother. 2015;70(1):75-80. DOI: 10.1093/jkdc/jku323 PMID: 25190723.

8. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791-8. DOI: 10.3201/eid1710.110655 PMID: 22000347.

9. Tenover FC, Arbet RD, Goering RV, Mickelsen PA, Murray BE, et al. Co-occurrence of extended spectrum beta lactamase-producers and MCR-1 encoding genes on plasmids. Lancet Infect Dis. 2016;16(3):281-2. DOI: 10.1016/S1473-3099(16)00007-4 PMID: 26774244.

10. Nordmann P, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, et al. Co-occurrence of extended spectrum beta lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis. 2016;16(3):282-3. DOI: 10.1016/S1473-3099(16)00009-8 PMID: 26774242.

11. Falgenhauer L, Waeszsda SE, Yao Y, Imizaligotu C, Kásbohrer A, Roessler U, et al. Colistin resistance gene mcr-1 in extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacteriaceae. Lancet Infect Dis. 2016;16(3):283-4. DOI: 10.1016/S1473-3099(16)00010-2 PMID: 26774247.

License and copyright

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY 4.0) Licence. You may share and adapt the material, but must give appropriate credit to the source, provide a link to the licence, and indicate if changes were made.

This article is copyright of the authors, 2016.