This is the first study reporting the WGS of a previously unreported and unusual Col-R *K. pneumoniae* lacking a large region within the *mgrB* locus, thus providing detailed knowledge on the chromosomal location and genetic environment of the excised site. We are unable to define either the mechanism responsible for the deletion or the benefit for *K. pneumoniae* of having such an important deletion in a highly conserved chromosomal locus. However, the implementation of our new primers will allow determining whether more Col-R isolates contain the same or similar mechanisms of colistin resistance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2017.09.014.

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Infrequent isolation of extensively drug-resistant (XDR) *Klebsiella pneumoniae* resistant to colistin in Spain

Editor: Dr Seydina Diene

Sir,

Carbapenem co-resistance with other antimicrobial classes (e.g. fluoroquinolones or aminoglycosides) is frequently identified among Enterobacteriaceae, often limiting therapeutic options to last-resort antibiotics such as colistin or tigecycline [1]. Following the re-introduction of colistin in the mid-1990s to combat infections caused by carbapenemase-producing Gram-negative bacteria, resistance to polymyxins, particularly in carbapenem-resistant *Klebsiella pneumoniae*, has been increasing steadily worldwide and is now a major concern in Europe [2]. Similarly, co-resistance to other last-resort antibiotics such as tigecycline has been also reported [3].

We aimed to investigate the antimicrobial resistance phenotypes among carbapenem-resistant *K. pneumoniae* isolated in 2016 in a tertiary hospital in Spain and to characterise those that were extensively drug-resistant (XDR). A total of 814 *K. pneumoniae* isolates were collected in the Hospital Universitario Río Hortega (Valladolid, Castilla y León, Spain) in 2016 during internal surveillance (routine epidemiological surveillance of pharyngeal and rectal smears as well as tracheal aspirates in intensive care units, and microbiological analysis of clinical infectious samples), of which 67 isolates were confirmed as OXA-48-producers by Xpert® Carba-R (Cepheid, Sunnyvale, CA). Of the 67 isolates, 5 (7.5%) were extended-spectrum β-lactamase (ESBL)-producers and were resistant to colistin and quinolones, and 4 isolates (6.0%) were also resistant to tigecycline (Table 1). Interestingly, four of the five XDR isolates were collected in a narrow time frame of within 1 week in May 2016.

Antimicrobial susceptibility testing was performed and minimum inhibitory concentrations (MICs) was determined for the five XDR isolates. All were resistant to penicillins, third-generation cephalosporins, carbapenems, quinolones, aminoglycosides and colistin, and four of them were also resistant to tigecycline (Table 1). The colistin MIC was >16 μg/mL for all isolates.

The five XDR isolates were analysed by whole-genome sequencing in order to genotype and elucidate their resistome and other

Table 1
Antimicrobial resistance phenotypes and genes (resistome) of five *Klebsiella pneumoniae* isolates of human origin, Spain, May 2016.

	K0	K1	K2	K3	K5
Origin	Rectal smear, surveillance	Tracheal aspirate	Rectal smear, surveillance	Surgical drain, surveillance	Tracheal aspirate
Sex/age (years)	F/79	M/76	M/71	M/84	M/72
MLST [a]	ST16	ST11	ST11	ST11	ST11
Genome length (bp)	5 456 795	5 478 125	5 508 494	5 482 446	5 468 281
Minimum inhibitory concentration (μg/mL) [resistance phenotype]					
AMC	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]
Ampicillin	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]
Aztreonam	32 [R]	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]
TZP	≥128 [R]	≥128 [R]	≥128 [R]	≥128 [R]	≥128 [R]
Imipenem	2 [S]	4 [I]	8 [I]	≥16 [R]	2 [S]
Meropenem	4 [I]	4 [I]	ND	≥16 [R]	2 [S]
Ertapenem	4 [R]	≥8 [R]	4 [R]	≥8 [R]	≥8 [R]
Cefuroxime axetil	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]
Cefoxitin	8 [R]	ND	≥64 [R]	≥64 [R]	≥64 [R]
Cefotaxime	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]
Ceftazidime	16 [R]	≥64 [R]	≥64 [R]	≥64 [R]	≥64 [R]
Cefepime	2 [I]	16 [R]	8 [R]	≥64 [R]	16 [R]
Gentamicin	≤1 [S]	≥16 [R]	4 [S]	≥16 [R]	≥16 [R]
Tobramycin	≥16 [R]	≥16 [R]	≥16 [R]	≥16 [R]	≥16 [R]
Amikacin	≤2 [S]	8 [S]	8 [S]	16 [S]	8 [S]
Minocycline	≥16 [R]	≥16 [R]	4 [S]	≥16 [R]	≥16 [R]
Tigecycline	4 [R]	4 [R]	1 [S]	4 [R]	≥8 [R]
Colistin	≥16 [R]	≥16 [R]	≥16 [R]	≥16 [R]	≥16 [R]
Doripenem	2 [I]	≥8 [R]	≥8 [R]	≥8 [R]	2 [I]
SXT	≥320 [R]	≤20 [S]	≥320 [R]	≤20 [S]	≤20 [S]
Fosfomycin	≤16 [S]	≥256 [R]	≥256 [R]	≥256 [R]	≥256 [R]
Ciprofloxacin	≥4 [R]	≥4 [R]	≥4 [R]	≥4 [R]	≥4 [R]
Nalidixic acid	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]	≥32 [R]
Levofloxacin	≥8 [R]	≥8 [R]	≥8 [R]	≥8 [R]	≥8 [R]
No. of resistance genes	14	11	17	12	10
Common resistome	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, **$bla_{OXA\text{-}48}$**, *catB4*, *fosA*, *oqxA*, *oqxB*				
Other resistome	*aac(6′)-Ib*-cr, *aadA2*, *aph(3″)-Ia*, $bla_{SHV\text{-}11}$, *dfrA12*, *mph*(A), *sul1*	*aac(3)-IIa*, *aac(6′)-Ib*-cr, $bla_{SHV\text{-}11}$, *qnrB1*	*aadA2*, $bla_{SHV\text{-}11}$, **$bla_{VIM\text{-}1}$**, *catA1*, *catB2*, *dfrA12*, *dfrB1*, *mph*(E), *msr*(E), *sul1*	*aac(3)-IIa*, *aac(6′)-Ib*-cr, *aph(3″)-Ia*, $bla_{SHV\text{-}11}$, *qnrB1*	*aac(3)-IIa*, *aac(6′)-Ib*-cr, $bla_{SHV\text{-}11}$

Genes encoding carbapenemases are marked in bold.
AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole; R, resistant; I, intermediate; S, susceptible; ND, not determined.
[a] Multilocus sequence typing (MLST) according to PubMLST.org.

virulence determinants present as previously described [4]. An average of 0.7 M reads per isolate aligned to 151–181 contigs, assembled using SPAdes v.3.9.0, to yield draft genomes of ca. 5.5M bp. This information has been deposited at DDBJ/ENA/GenBank under the BioProject ID **PRJNA414210**. Genome annotation was performed using Prokka and the resistome of the draft genome was analysed by blastn searches against the ResFinder database [3,5]. Although the isolates were collected in a short timeframe, pangenome comparison demonstrated that they were not phylogenetically related, as four of the isolates belonged to sequence type 11 (ST11) and one to ST16. ST11 is an important hyperepidemic nosocomial human clone distributed worldwide, particularly in Spain, and is one of the most common worldwide STs associated with colistin-resistant *K. pneumoniae*.

The resistomes of the isolates are summarised in Table 1. The $bla_{OXA–48}$ carbapenemase gene and the $bla_{CTX\text{-}M\text{-}15}$ ESBL gene were present in all five isolates. The $bla_{OXA\text{-}1}$ and $bla_{SHV\text{-}11}$ broad-spectrum β-lactamase genes as well as genes encoding resistance to phenicols, fosfomycin and fluoroquinolones were also present in all of the isolates. Interestingly, the $bla_{VIM\text{-}1}$ carbapenemase gene was also found in one isolate (K2), indicating that this isolate had genes from two distinct carbapenemases families, i.e. class B ($bla_{VIM\text{-}1}$) and D class ($bla_{OXA–48}$). Transferable *mcr* genes coding for colistin resistance were absent. Inactivation by truncation of the *mgrB* gene, a negative regulator of the PhoPQ signalling system identified as a source of colistin resistance, was observed in isolates K2 (truncated with insertion sequence IS*5*) and K3 (incomplete). Mutations in genes involved in lipid A modifications (the two-component systems PhoPQ and PmrAB) were analysed by blastn and multiple mutations were observed. The mutation profile was common for K1, K2, K3 and K5, giving amino acid substitutions in various genes contributing to colistin resistance: *crrB* (L296Q); *eptA* (V39L, S260T, A279G); *phoP* (L26Q, R114A, N191D); *phoQ* (A21T, D146I, D150G, D434G); *pmrB* (L344P); and *pmrK* (H156H, N441D). Isolate K0 also included a mutation in the *pmrJ* gene (L94I).

The presence of putative plasmids was evaluated by blastn searches against the PlasmidFinder database. Isolates K1, K3 and K5 had IncR_1 and IncFIB(K)_1_Kpn3 plasmids, whilst K0 and K2 exhibited IncL/M(pOXA-48)_1_pOXA-48 and IncFIB(K)_1_Kpn3 plasmids. The $bla_{OXA\text{-}48}$ and $bla_{VIM\text{-}1}$ genes were found on plasmids, whilst the $bla_{SHV\text{-}11}$ gene was in the chromosome. The presence of virulence factors was analysed against the Virulence Factors Database (VFDB). The ferric yersiniabactin uptake receptor (*fyuA*), iron regulatory protein (*irp1-2*) and yersiniabactin siderophore system (*ybtAEPQSTU*) genes were found in all isolates except for K0.

This research contributes to a better understanding of the epidemiology of multidrug-resistant *K. pneumoniae* in clinical settings. The discovery during routine hospital surveillance of several isolates carrying a multiresistant phenotype for most β-lactam antibiotics, quinolones, carbapenems and colistin represents a serious concern that must be taken into consideration before the use of antimicrobial therapies and should be further investigated in other clinical settings.

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Methicillin-sensitive *Staphylococcus aureus* and emerging dominant sequence type 188 *Staphylococcus aureus* in severe community-associated infections

Editor: S. Dancer

Sir,

In the past decades, the role of methicillin-sensitive *Staphylococcus aureus* (MSSA) in severe community–associated infections has been less investigated than that of methicillin-resistant *S. aureus* (MRSA). MSSA can cause almost two times the bacteraemia of MRSA [1]. However, to our knowledge, the popular perspective is that MSSA usually causes endemic infections both in community and healthcare settings and no special MSSA lineage has been identified.

From 2013 to 2016, we recovered 97 *S. aureus* isolates from patients with invasive community–associated *S. aureus* infections in three 1000-bed hospitals. A total of 71 of the isolates were from adults (median age: 48 years; interquartile range [IQR]: 36–64 years) and 26 were from children (median age: 1.5 years; IQR: 8 months–3 years). Infections were caused more commonly by MSSA (73, 75%) than by MRSA (24, 25%). Among 97 *S. aureus* isolates, 16 STs were identified by multilocus sequence typing (MLST) (Table 1). The five most prevalent STs were ST188 (18 isolates), ST5 (7 isolates), ST965 (5 isolates), ST59 (6 isolates), and ST1281 (4 isolates). We also randomly selected 100 invasive hospital-associated *S. aureus* isolates for comparison, among which two ST188 isolates were identified.

All 20 ST188 *S. aureus* isolates belonged to *spa* type t189 and one indistinguishable pulsed-field gel electrophoresis (PFGE) group. Nineteen were identified to be MSSA and were resistant only to penicillin and susceptible to other β-lactams, tetracycline, ciprofloxacin, gentamicin, erythromycin, clindamycin, tigecycline, linezolid, trimethoprim–sulfamethoxazole and vancomycin. The remaining hospital-acquired ST188 isolate was MRSA. Detection of staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*), toxic shock **s**yndrome toxin (*tst*), exfoliative toxins (*eta* and *etb*), and Panton-Valentine leukocidin (*pvl*) demonstrated that 20 ST188 *S. aureus* isolates carried low rates for nine virulence genes: only two isolates carrying *seb*, one isolate carrying *tst*, and one isolate carrying *sec* and *tst*. Clinical data revealed that 14 of 20 ST188 *S. aureus* infections could be diagnosed as sepsis. Seven patients were aged from several days to 8 months, one patient was aged 12 years, and 12 patients were aged over 60 years.

Compared with 100 *S. aureus* control isolates from invasive hospital-associated infections, MSSA accounted for a much greater proportion of invasive community-associated infections than MRSA ($P < 0.001$) (Table 1). Taking 10% as an arbitrary dominance borderline, there was a dominant ST188 lineage solely among CA-MSSA. There were also dominant ST59 and ST5 among CA–MRSA, as well as dominant ST239, ST5 and ST59 among HA-MRSA (Table 1).

To investigate the possible sources of ST188, we searched for ST188 *S. aureus* registered on http://saureus.mlst.net/ and found 37 strains, 14 of which are from bovine with mastitis and one from Rhesus Macaque. A meta-analysis search on PubMed identified six studies reporting ST188 *S. aureus* strains, five of which are CA-MSSA and one HA-MRSA [2,3]. We also investigated genetic distribution of *S. aureus* from local food sources, mostly isolated from meat and dairy products, and identified this dominant clone [4].

In contrast to the widely accepted viewpoint that MSSA infections are endemic, our findings show that a great proportion of invasive CA-MSSA infections are due to a dominant ST188 *S. aureus*. From the noted information, we conclude that ST188 *S. aureus* may originate from animal sources and can cause severe infections in vulnerable people. Although these strains carry low rates of usually detected virulence genes, they are still highly virulent for very young

Table 1
Dominant *S. aureus* lineages from invasive community-associated and hospital-associated *S. aureus* infections.

Characteristic	Community-associated		Hospital-associated	
	MRSA	MSSA	MRSA	MSSA
Number of isolates	24	73	66	34
Number of STs	6	16	7	14
Dominant STs (prevalence >10%)	ST59 (25%), ST5 (21%)	ST188 (25%)	ST239 (35%), ST5 (24%), ST59 (12%)	Not found

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*.