Diagnostic Microbiology and Infectious Disease 93 (2019) 147–153

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Emergence and dissemination of colistin-resistant *Klebsiella pneumoniae* isolates expressing OXA-48 plus CTX-M-15 in patients not previously treated with colistin in a Spanish university hospital

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ARTICLE INFO

Article history:
Received 2 August 2018
Received in revised form 28 August 2018
Accepted 31 August 2018
Available online 8 September 2018

Keywords:
Klebsiella pneumoniae
Colistin resistance
OXA-48
Heteroresistance
Fitness

ABSTRACT

Dissemination of multidrug-resistant *Klebsiella pneumoniae* in the hospital environment represents a primary target of resistance containment and stewardship programs. At present, polymyxins, mostly in combination, exemplify a last-resort alternative. Colistin-resistant *K. pneumoniae* isolates harboring OXA-48 plus CTX-M-15 ($n = 21$) with the simultaneous colistin-susceptible counterparts ($n = 9$) were recovered from 14 hospitalized patients (January 2014–January 2015) admitted in different wards. In most cases, patients had not previously received colistin. Genetic relatedness experiments demonstrated that 93% (28/30) of isolates belonged to the ST11 high-risk clone. Heteroresistance and the fitness cost of colistin resistance were addressed in susceptible and resistant isolates as well as in in vitro–obtained stable mutants, and results appeared to be strain dependent. Whole genome sequencing demonstrated molecular changes in *pmrA*, *pmrB*, and *mgrB* genes. Plasmid-mediated colistin resistance genes were not found. Colistin resistance in multidrug-resistant *K. pneumoniae* isolates should be continuously monitored to detect its potential emergence, even in patients not previously exposed to colistin.

1. Introduction

Carbapenemase-producing *Klebsiella pneumoniae* strains are dispersed worldwide, and in the last decade, their prevalence has experienced an important increase in Europe, particularly in the Mediterranean area (Cantón et al., 2012; Nordmann and Poirel, 2014). While public policies try to control the spread of carbapenem resistance, susceptible isolates to last-line antibiotics are further decreasing, thus compromising patients' safety and global health (Oteo et al., 2016; Pena et al., 2014).

Limited options for carbapenemase-producing *Enterobacteriaceae* (CPE) infections usually include amikacin, tigecycline, colistin, and, frequently, fosfomycin. However, the development of colistin resistance in these isolates, found initially in KPC-producing *K. pneumoniae* isolates, is being increasingly described among isolates producing OXA-48 carbapenemase and, worryingly, in other species such as *Escherichia coli* (Cannatelli et al., 2014; Liu et al., 2015; Monaco et al., 2014). In Spain, colistin resistance has been reported previously in VIM-1–producing *K. pneumoniae* isolates belonging to the high-risk clone ST11 (Pena et al., 2014) but also in KPC-producing isolates (Cannatelli et al., 2014; Monaco et al., 2014; Oteo et al., 2016; Valentín-Martín et al., 2013).

The complexity of colistin resistance is increasing with the recent discovery of plasmid-mediated resistance in *E. coli* carrying the *mcr-1* gene (Liu et al., 2015), originally thought to be confined to China but worldwide disseminated at present. This situation has been further complicated with the appearance of new *mcr* variants (Xavier et al., 2016; Yin et al., 2017) and recognition of the difficulties of the in vitro determination of precise colistin MIC values (Vasoo, 2017).

In this study, we addressed the phenotypic and genotypic features of colistin-resistant *K. pneumoniae* isolates producing OXA-48 plus CTX-M-15 recovered at the Ramón y Cajal University Hospital, Madrid, Spain. Due to the worrying onset of this profile, isolates from both clinical samples and from rectal swabs were included. Moreover, in vitro colistin-resistant mutants derived from colistin-susceptible isolates were also studied including bioinformatics analysis of data generated by whole genome sequencing (WGS).

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https://doi.org/10.1016/j.diagmicrobio.2018.08.014
0732-8893/

2. Material and methods

2.1. Bacterial isolates

Twenty-one colistin-resistant (COL-R) carbapenemase-producing *K. pneumoniae* isolates from 14 patients were collected at our hospital from January 2014 to January 2015. Nine isolates corresponded to rectal samples, and the others were clinical isolates, mostly from urine (Table 1). Isolates were included in our study due to their antibiotic resistance pattern, as colistin resistance among carbapenemase-producing *K. pneumoniae* has never previously been observed in our hospital. This led to the inclusion of both clinical and carrier's isolates. Additionally, all other carbapenemase-producing *K. pneumoniae* isolates (all colistin susceptible) from the same patients were included in the study. Patients' demographic data, clinical origin of samples, and previous patients' exposure to colistin are shown in Table 1.

2.2. Bacterial identification and antibiotic susceptibility

Identification and initial antibiotic susceptibility testing were carried out using MicroScan Walk-Away® (Beckman Coulter, CA). Colistin (colistin sulfate, Sigma-Aldrich Inc., St. Louis, MO) MICs were confirmed by the reference broth microdilution (ISO 20776-1:2006), in cation-adjusted Mueller–Hinton broth (CAMHB), in all isolates exhibiting colistin MIC above the clinically susceptible breakpoint and in all CRE irrespective of the initial colistin susceptibility. Results were interpreted according to EUCAST 2017 criteria (version 7.1) (EUCAST, 2017) (Table 1).

Carbapenemase production and characterization were addressed by the carbapenemase-inhibitor profile using the KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica A/S, Denmark), the modified Hodge test, and the molecular eazyplex® Superbug CRE system (Amplex Biosystems GmbH, Germany) (García-Fernández et al., 2015). Final confirmation of carbapenemase/ESBL profile was assessed by PCR and sequencing using primers and conditions already published (Gijón et al., 2012).

2.3. Genetic relatedness

Pulsed-field gel electrophoresis (PFGE) was carried out using *XbaI* following the working protocol previously described (Ruiz-Garbajosa et al., 2016). Restriction patterns were analyzed and interpreted according to published criteria (Tenover et al., 1995), and the Dice coefficient was used for dendrogram construction with the Phoretrix 5.0 software (Nonlinear Dynamics Ltd.). Isolates were further characterized by multilocus sequence typing (MLST) using standard housekeeping loci (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*) according to published data (Diancourt et al., 2005) and analyzed using the Institute Pasteur database *(http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html)*.

2.4. Population analysis profile (PAP)

Possible heterogeneous resistance to colistin was assessed by PAP in 3 randomly selected colonies from colistin-susceptible isolates O28, L77, and N48, as well as in the carbapenem-susceptible *K. pneumoniae* ATCC 13883 strain following the previously reported method (El-Halfawy and Valvano, 2015). Heteroresistance was defined when a colistin-susceptible isolate, according to EUCAST clinical breakpoint, exhibited subpopulations growing in the presence of >2 mg/L of colistin. MICs of colistin from selected colonies growing in PAP experiments at concentrations higher than 2 mg/L were determined, as described above, after 2 weeks of daily serial passaging in CAMHB colistin-free medium.

Table 1
Demographic data, colistin MICs, and genetic relatedness of studied strains.

Patient	Age[a], gender	Ward(s)	Antibiotic treatment	Sample (no.)	Date of isolation dd/mm/year	CST MIC (mg/L)[b][c]	MLST
1	50, male	General Surgery	AMC, TZP, AMK	Rectal (O53)	07/07/2014	16	ST11
2	96, male	Traumatology	AMC, TZP	Urine (O63)	09/07/2014	32	ST11
				Urine (O28)	18/06/2014	1	ST11
3	89, male	Internal Medicine	AMC, AMK	Urine (O67)	11/07/2014	32	ST11
				Rectal (U35)	05/01/2015	1	ST11
				Urine (L77)	17/01/2014	0.5	ST11
				Urine (N78)	07/04/2014	16	ST11
				Urine (NN8)	11/04/2014	32	ST11
				Urine (NN79)	30/05/2014	16	ST11
4	79, female	Nephrology	LZD, AMK, CST[c], CZA	Urine (O52)	04/07/2014	32	ST11
				Urine (P71)	20/08/2014	16	ST11
				Urine (R80)	15/10/2014	32	ST11
				Urine (U31)	05/01/2015	0.5	ST11
5	74, male	Internal Medicine	AMC, MEM	Rectal (P78)	27/08/2014	16	ST11
6	63, female	Oncology	AMC, ERY	Rectal (Q11)	04/09/2014	16	ST11
				Rectal (S29)	23/10/2014	16	ST11
7	67, female	Thoracic Surgery	AMC, CLI, CRO, CXM	Wound (U17)	22/12/2014	2	ST54
				Drainage (U46)	07/01/2015	16	ST11
				Urine (M57)	19/02/2014	1	ST11
				Urine (N48)	24/03/2014	0.25	ST11
8	58, male	Urology	CST[d], AMK	Urine (U8)	23/12/2014	16	ST11
				Urine (V12)	26/01/2015	1	ST11
				Rectal (NN76)	26/05/2014	1	ST11
9	72, male	Neurosurgery	TZP, MEM, AMK, LZD	Sputum (Q32)	05/09/2014	32	ST11
10	67, male	Gastroenterology	MEM, VAN, LZD, CIP	Rectal (S47)	30/10/2014	8	ST11
11	91, female	Gastroenterology	AMC, CIP	Rectal (NN54)	14/05/2014	32	ST11
				Urine (S55)	04/11/2014	32	ST11
12	94, female	Internal Medicine	AMC, FOF	Rectal (U10)	23/12/2014	16	ST11
13	74, male	Urology	CIP, TZP	Rectal (U39)	05/01/2014	16	ST11
14	52, male	Gastroenterology	CIP, TZP, IPM, MEM, ERY, LZD	Rectal (V1)	16/01/2015	4	ST1427

AMC = amoxicillin-clavulanic acid; AMK = amikacin; CIP = ciprofloxacin; CLI = clindamycin; CRO = ceftriaxone; CST = colistin; CXM = cefuroxime; CZA = ceftazidime-avibactam; ERY = erythromycin; FOF = fosfomycin; IPM = imipenem; LZD = linezolid; MEM = meropenem; TZP = piperacillin-tazobactam; VAN = vancomycin.

[a] Expressed in years.

[b] Standard broth microdilution with EUCAST interpretation (S: ≤2 mg/L, R: >2 mg/L).

[c] Colistin was used as clinical treatment.

[d] Colistin was used as prophylaxis.

2.5. Molecular characterization of colistin resistance and WGS

Colistin-susceptible O28, L77, N48, and NN76 and the corresponding colistin-resistant isolates (O67, O52, U8, and Q32, respectively) as well as ATCC 13883 control strain were chosen for WGS, as well as stably colistin-resistant colonies from L77-, N48-, and ATCC 13883–resistant subpopulations recovered from PAP experiments. The WGS was performed using the Illumina MiSeq platform (2×300 bp) at a mean read depth of 158×. Each genome was assembled with VelvetOptimiser 2.2.5 (Zerbino and Birney, 2008) and SPAdes v3.9.0 (Bankevich et al., 2012). We used Prokka 1.12-beta (Seemann, 2014) to annotate the N48 assembled genome, which was selected as reference, and BWA v0.7.5 (Li, 2013) and Samtools v0.1.18 (Li et al., 2009) for mapping each isolate against N48. Variants were detected by VarScan v2.2 (Koboldt et al., 2012) applying a strong filtering. Each mutation or indel was annotated using SnpEff v4 (Cingolani et al., 2012); ISMapper software was used for detection of insertion sequences (Hawkey et al., 2015).

Independently, nonsynonymous point mutations in genes *pmrB*, *pmrA,* and *mgrB* observed in whole genome reads were confirmed by PCR and Sanger sequencing using primers and conditions previously described (Cannatelli et al., 2014; Xavier et al., 2016). Finally, the presence of plasmid *mcr-1* was screened with eazyplex® *mcr-1* provided by Amplex Diagnostics GmbH and following manufacturer's instructions.

2.6. Growth curve assays

To examine the in vitro fitness cost of the acquisition of colistin resistance, growth kinetics under noncompetitive conditions were performed with the Bioscreen C system (Thermo Lab Systems, Vantaa, Finland) for selected colistin-resistant isolates (O67, O52, U8, and Q32) and colistin-susceptible (O28, L77, N48, and NN76), and colistin-resistant subpopulations recovered from PAP experiments with L77 (ML77) and N48 (MN48) isolates. Experiments were carried out in parallel in Mueller–Hinton broth (MHB) and in CAMHB to control for a possible bias induced by differences in the concentration of divalent cations. Briefly, strains were first grown overnight (18 h) at 37 °C in MHB and CAMHB. Overnight cultures were diluted 1:1000 into fresh broth, approximately 10^5 UFC/mL, and 300 μL of this bacterial suspension was transferred into a 100-well microplate. Optical density (OD) was measured at 600 nm every 15 min along 20 h. In order to assure culture optical homogeneity, the plates were gently agitated for 10 s before each OD measurement. For each strain, 5 biological replicates were assayed in duplicate in each experiment (10 readings per strain per experiment). Two independent experiments were performed for all strains analyzed. A blank well was included in each experiment to subtract the background OD of broth media. Growth rates (GRs), lag times, correlation coefficients (*r*s), and relative growth rates (RGRs) were calculated in the exponential phase using the software *GrowthRates 2.1* (Hall et al., 2014).

3. Results

3.1. K. pneumoniae isolates and patients' characteristics

A total of 30 multidrug-resistant *K. pneumoniae* isolates, all of them harboring $bla_{\text{OXA-48}}$ plus $bla_{\text{CTX-M-15}}$ (21 colistin resistant and 9 colistin susceptible), were recovered from 14 patients from January 2014 to January 2015 (Table 1). The colistin-susceptible isolates were recovered in 5 of these 14 patients with their colistin-resistant counterparts. Patients, with a median age of 73 years, were admitted in different wards across the hospital. Most of them had significant underlying diseases and were hospitalized more than once, usually through the Emergency Unit. Among these patients and throughout the study period, colistin exposure could only be confirmed in 2 patients, either as treatment (patient 4) or prophylaxis (patient 8). Other antibiotics prescribed in these patients are detailed in Table 1. Beta-lactam plus beta-lactam inhibitors combinations or carbapenems were used in 12 and 4 patients, respectively, whereas 3 of them received both. Colistin MICs for colistin-resistant isolates ranged between 4 and 32 mg/L according to both susceptibility-testing methods used. The corresponding range for colistin-susceptible isolates was 0.25–2 mg/L.

3.2. Population structure

Twenty-eight isolates belonged to the MLST sequence type ST11, the most frequent OXA-48–producing ST recovered in our hospital, and were grouped in the same PFGE pattern (Dice's coefficient > 0.82) (Table 1, Fig. 1). Interestingly, there were 2 isolates that belonged to different ST: 1 colistin-susceptible isolate recovered from a wound in patient 7 that was ascribed to ST54 and 1 colistin-resistant isolate recovered from a rectal swab at the end of the study period corresponding to ST1427 from patient 14. These 2 STs are not associated with ST11.

3.3. Heteroresistance RESULTS

Heteroresistance was considered as a possible cause of the rise of colistin resistance. However, PAP results showed heteroresistance in the case of colistin-susceptible isolates (O28, L77, and N48 from patients 3, 4, and 8, respectively), with colonies growing in colistin concentrations higher than 2 mg/L (Fig. 2). Furthermore, resistant subpopulations, able to grow at concentrations up to 16 mg/L of colistin, were found in ATCC13883 strain, similar to previous reports (Poudyal et al., 2008). Stable resistant subpopulations, recovered from clinical isolates L77 and N48 and the ATCC control strain, had MICs ranging between 8 and 16 mg/L, and resistance was found to be stable after daily passaging in colistin-free medium for 2 weeks. No stable colistin-resistant colonies from the O28 strain were obtained.

3.4. Molecular characterization of the arn/pmrHFIJKLM operon

Amplification and sequencing of the *arn/pmrHFIJKLM* operon from resistant isolates O67, O52, U8, and Q32 recovered from patients 3, 4, 8, and 9 gave various results. No mutations in *pmrB*, *pmrA*, and *mgrB* genes, when compared with the wild genotype of susceptible isolates, were observed in O67 and O52. Moreover, no other previously reported mechanisms responsible for colistin resistance could be found by WGS, including those involving *phoP/Q* and *crrAB* genes. The isolate Q32 presented the substitution T140P in PmrB, and isolate U8 showed the change G53S in PmrA (Table 2). Other observed changes were the presence of the insertion sequence IS*Kpn18* in the intergenic region between the hypothetical protein KPNJ2_RS10015-coding gene and *mgrB* for isolate ML77, and the disruption of *mgrB* for isolates MN48 and Q32 by IS*Kpn14*. The latter was the insertion sequence most frequently detected in this dataset, but its position has changed in the tested isolates when compared with that of the susceptible isolate. No other main changes responsible for colistin resistance, including modifications of lipopolysaccharide composition, were detected by WGS among resistant isolates that could explain their resistance phenotype, including the ML77 and MN48 colistin-resistant variants (Olaitan et al., 2014, b). Moreover, *mcr-1*, *-2*, *-3*, *-4,* and *-5* genes were not detected in any of the clinical isolates, and genome reads did not show differences in plasmids' content between colistin-susceptible and colistin-resistant isolates.

3.5. Growth rate analysis

Fitness cost of colistin resistance was found not to be consistent among the isolates tested. Relative growth rates, lag times, and statistical signification in both MH and CAMHB are shown in Table 2 and Fig. 3, the latter showing a box-and-whisker plot comparing growth rates among the isolates. Statistically significant changes in fitness were observed for O28 (colistin susceptible) and O67 (colistin resistant) (patient 3) in both MH and CAMHB. Also significant were the results

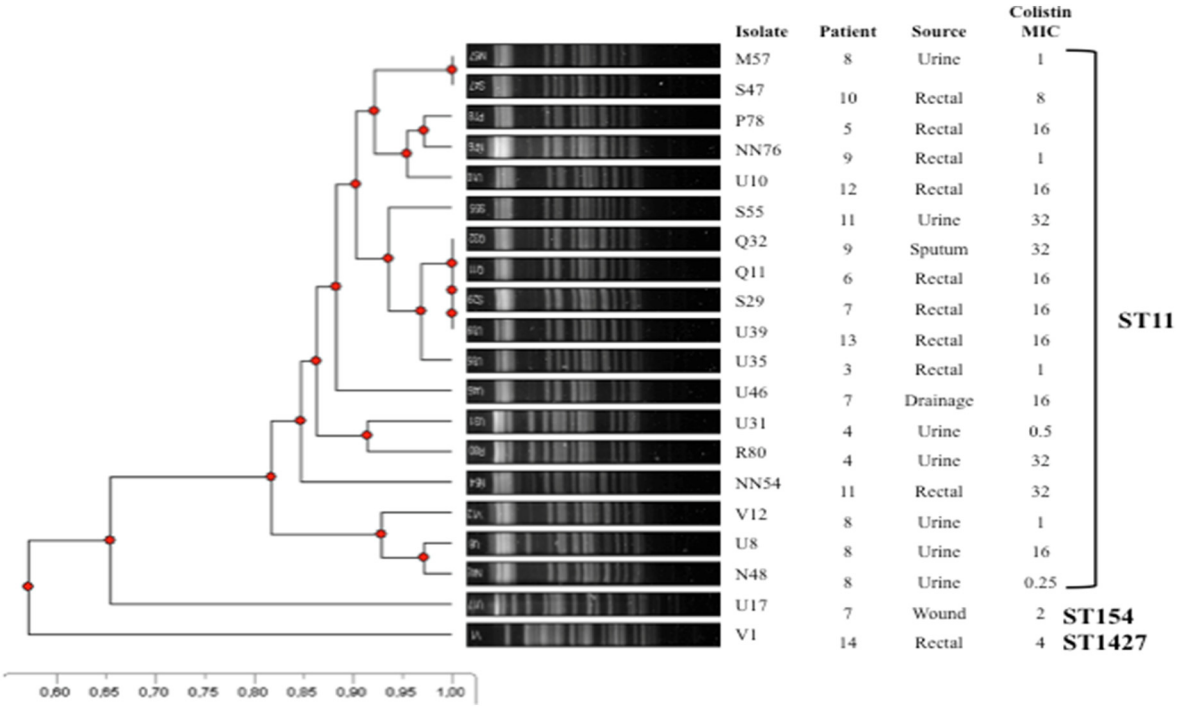

Fig. 1. Genotypic analysis of *K. pneumoniae* isolates representing all PFGE patterns and STs found in the studied population. Dendrogram analysis of *XbaI*-PFGE profiles is shown. Isolate and patient number, source, colistin MIC, and MLST sequence type are shown. ST = sequence type.

obtained in both media for the 2 isolates of patient 9, Q32 (colistin resistant) with a mutated *pmrB* but with a higher growth rate than NN76 (colistin susceptible), lacking mutations but exhibiting decreased fitness. However, in isolates L77 (colistin susceptible) and O52/ML77 (colistin resistant) from patient 4, a decrease in fitness was only statistically significant in growth rates carried out in CAMHB. In the case of N48 and U8 (patient 8), no differences were observed regardless the experimental conditions, even though the latter had a mutated *pmrA*.

Finally, the growth rate analysis for resistant subpopulations showed a decreased fitness for the L77 in vitro derived mutant (ML77) in CAMHB that was statistically significant but not when grown in MH. No changes could be observed for the N48 in vitro derived mutant (MN48) compared to its susceptible counterpart.

4. Discussion

Resistance to colistin in OXA-48, CTX-M-15 producing *K. pneumoniae* isolates emerged in a relatively brief time in our hospital, where colistin is increasingly being used due to our endemic carbapenemase situation (Hernández-García et al., 2018). Our results show that the emergence of this resistance is associated with the predominantly circulating ST11, a high-risk *K. pneumoniae* clone responsible for the dissemination of ESBLs and carbapenemases in different countries (Cannatelli et al., 2014; Oteo et al., 2016; Pena et al., 2014). The dissemination of this clone, along with the absence of direct colistin selective pressure in the majority of patients, generated an unpredictable penetration of this resistance trait in our center. This situation was previously noted (Rojas et al.,

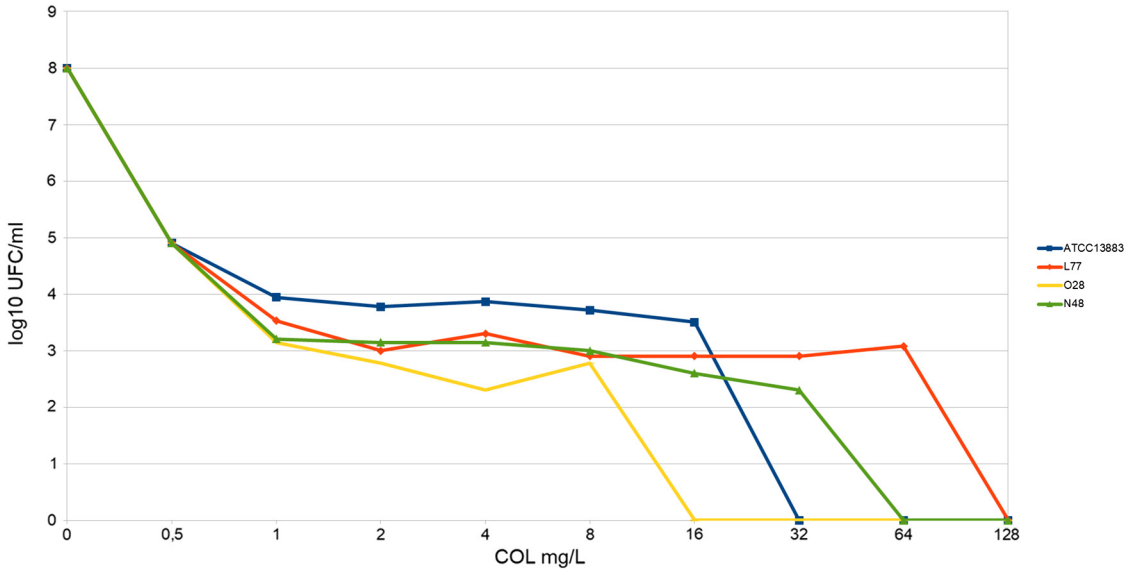

Fig. 2. PAP of 3 selected colistin-susceptible clinical isolates and *K. pneumoniae* 13883 strain.

Table 2
Fitness cost of colistin resistance in colistin-resistant clinical strains and in the in vitro derived mutants compared with colistin-susceptible strains.

Patient	Strain	CST MIC mg/L	CST resistance genotype	MH						CAMHB					
				GR (min^{-1})	Lag time (min)	*r*	RGR	Fitness cost (%)	*P* value	GR (min^{-1})	Lag time (min)	*r*	RGR	Fitness cost (%)	*P* value
3	O28 (WT)	1	-	0.173	172.005	0.923				0.171	167.220	0.926			
	O67 (CCR)	32	-	0.162	209.085	0.928	0.935	6.50	0.000	0.162	215.095	0.926	0.952	4.85	0.000
	L77 (WT)	0.5	-	0.163	197.960	0.937				0.167	211.725	0.928			
4	ML77 (CM)	16	ISKpn18 insertion between KPNJ2_02042 and *mgrB*	0.168	213.830	0.921	1.025	−2.47	1.000	0.160	225.490	0.921	0.954	4.62	0.002
	O52 (CCR)	32	-	0.159	233.125	0.923	0.973	2.66	0.059	0.159	238.285	0.930	0.952	4.75	0.000
	N48 (WT)	0.25	-	0.168	177.515	0.925				0.168	173.895	0.918			
8	MN48 (CM)	16	ISKpn14 disruption of *mgrB*	0.168	179.065	0.927	1.002	−0.20	0.636	0.168	179.695	0.920	0.996	0.43	1.000
	U8 (CCR)	16	PmrA G53S	0.171	190.430	0.921	1.019	−1.94	0.999	0.168	184.520	0.923	0.999	0.08	1.000
	NN76 (WT)	1	-	0.168	224.233	0.917				0.155	218.736	0.876			
9	Q32 (CCR)	32	PmrB T140P ISKpn14 disruption of *mrgB*	0.173	191.225	0.922	1.031	−3.11	0.000	0.168	188.550	0.935	1.083	−8.31	0.000

CST = colistin; MH = Mueller–Hinton; CAMHB = cation-adjusted Mueller–Hinton broth; WT = wild type; CCR = clinical colistin-resistant; CM = colistin-resistant mutant.
Growth rate (GR), lag time, regression coefficient (*r*), relative growth rate (RGR), fitness cost (percentage) and statistical significance (*P* value) in both MH and CAMHB.

2017) and demonstrated that only 22% of the patients infected and/or colonized with colistin- and carbapenem-resistant *K. pneumoniae* isolates had been previously exposed to colistin.

In our study, previous exposure to colistin could only account for the potential emergence of this resistance in two patients, one as treatment and the other as prophylaxis. This situation suggests that the emergence of colistin resistance may depend not only on direct colistin selective pressure but also on other factors, including those affecting infection control and the use of other agents acting as selectors, like chlorhexidine, as recently published (Wand et al., 2017). In the other 12 patients, 11 with infections and/or colonization with the colistin-resistant ST11 clone, exposition to colistin or any relation at admission or with hospital stay was not demonstrated, and hidden routes of transmission cannot be ruled out. Furthermore, the emergence of a different colistin-resistant clone, ST1427, in one patient indicates further emergence of colistin resistance, which continues nowadays with sporadic findings of these colistin-resistant isolates. ST1427 is also considered a high-risk clone and has been associated with ESBLs and carbapenemases dissemination (Zhou et al., 2015).

Strikingly, already reported colistin heteroresistance (Jayol et al., 2015; Meletis et al., 2011; Poudyal et al., 2008) was also detected in our isolates even in well-confirmed susceptible isolates. These results, taken together with the apparent lack of accuracy of certain methods for colistin MIC determination, illustrate the difficulties when testing colistin susceptibility in routine clinical laboratories (Hindler and Humphries, 2013; Landman et al., 2013; Vasoo, 2017). A further step towards resistance characterization is the fitness analysis, using growth rates as a surrogate marker of possible changes in bacterial physiology. However, although the results obtained with our set of strains do not allow drawing general conclusions on the fitness cost of colistin resistance, they illustrate the subjacent heterogeneity behind this resistance trait and the possibility of potential bias if the experimental conditions are not carefully controlled (Groisman et al., 2013; Kubicek-Sutherland et al., 2015; Wright et al., 2015). In our study, we demonstrate that changes in fitness were strain dependent and were affected by the medium used to perform the experiment.

On the other hand, WGS revealed different events that are presumably implicated in the colistin-resistant phenotype, including mutations

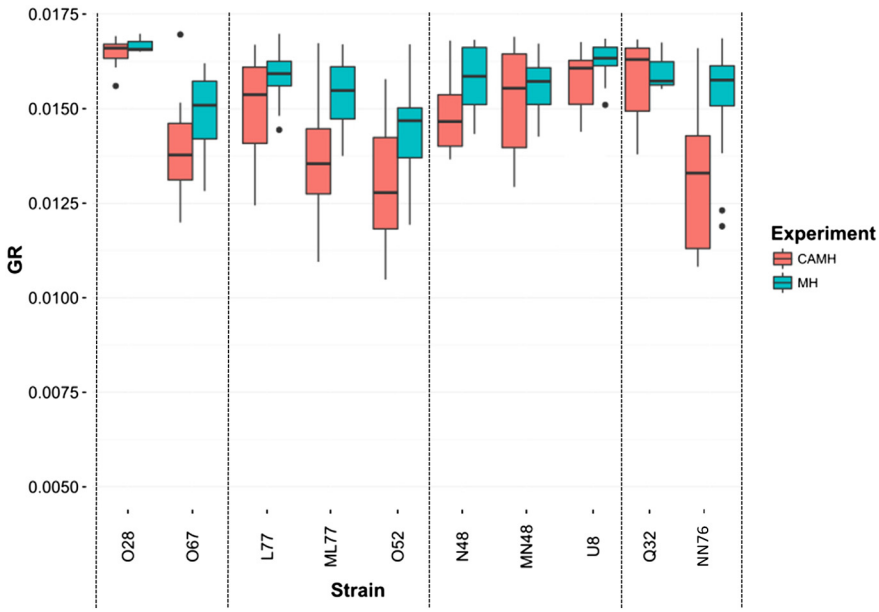

Fig. 3. Box and whiskers plot representing growth rates of *K. pneumoniae* strains subjected to growth curve assays.

and/or disruption in *pmrA* or *mgrB* genes. Moreover, an intergenic insertion close to *mgrB* gene was also detected. Its relevance remains to be elucidated but adds to the growing amount of variations found in *mgrB* from colistin-resistant isolates (Olaitan et al., 2014, b; Wright et al., 2015). Despite these findings, no shared changes that could indicate a clonal expansion of colistin resistance in our hospital were present. This lies in accordance with the considerable number of genomic changes that have demonstrated to cause a decrease in colistin susceptibility by complementation experiments (Cannatelli et al., 2014; Jayol et al., 2014; Wright et al., 2015). To note that WGS was not conclusive in some of the isolates and even, all *mcr* genes described allowing the plasmid-mediated horizontal spread of colistin resistance (Xavier et al., 2016; Yin et al., 2017), were absent in our isolates.

In summary, our study illustrates the appearance and successful spread of resistance to colistin in CPE. For CPE isolates, almost countless infection control measures are established, but these may still be insufficient to eliminate them when they reach an endemic situation facilitating the acquisition of additional resistance traits, such as colistin resistance. Furthermore, the need for surveillance and for accuracy methods in detecting reduced susceptibility to colistin appears indisputable. Molecular detection of specific genetic mechanisms as *mcr* variants or point mutations will not cover the whole mechanisms involved in colistin resistance and is beyond the scope of routine testing workload. However, careful evaluation of colistin susceptibility should be warranted at clinical laboratories even in patients not exposed to colistin, as this antimicrobial is still one of the first options, mainly in combination, for the treatment of CPE.

Acknowledgments

The contents and scientific background of this work were supported by Plan Nacional de I + D + i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0011) cofinanced by European Development Regional Fund "A way to achieve Europe" (ERDF), Operative program Intelligent Growth 2014–2020. The authors acknowledge Dr. Rosa del Campo for scientific advice in genetic relatedness analysis.

Competing interests

The authors have no conflict of interests.

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