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Long-Term Care Facility (LTCF) Residents Colonized With Multidrug-Resistant (MDR) *Klebsiella pneumoniae* Lineages Frequently Causing Infections in Portuguese Clinical Institutions

To the Editor—The recent increase of extended-spectrum β-lactamase (ESBL)–producing *Klebsiella pneumoniae* (*Kp*) and the emergence of carbapenemase-producing *Kp* in Portuguese clinical settings parallels epidemiological trends described in other countries.[1–4] Moreover, *Kp* isolates causing hospital infection often correspond to the patient's own colonizing strains, stressing the need to survey fecal carriage of multidrug-resistant (MDR) *Kp* in patients from different clinical settings.[5] Long-term care facilities (LTCFs) are fundamental institutions in contemporary healthcare services, mainly assisting elderly people who, due to frequent hospitalizations, recurrent antibiotic consumption, and communal living, are at a high risk of infection by MDR bacteria.[6] Different studies among European LTCFs residents have reported high rates of colonization by CTX-M-15-producing *Escherichia coli* (*Ec*) B2-ST131, but little is known regarding the prevalence and diversity of other MDR *Enterobacteriaceae* species (and particularly *Kp*) colonizing LTCFs residents.[6,7] The aim of this study was to assess the fecal carriage rate and epidemiological features of non-*Ec Enterobacteriaceae* isolates resistant to extended-spectrum β-lactams among Portuguese LTCF residents.

Fecal samples from residents (n = 47) at LTCF 1 (25 beds; n = 25 samples) and LTCF 2 (40 beds; n = 22 samples) in northern Portugal were collected during July 2015 and January 2016, respectively. Demographic and clinical characteristics of the residents are summarized in Online Supplemental Table S1. Rectal swabs were suspended in 2 mL saline solution, and 0.2 mL were seeded on chromID ESBL (bioMèrieux, Marcy-l'Étoile, France) and chromID Carba SMART plates (bioMèrieux, Marcy-l'Étoile, France) directly and after a preenrichment step in 10 mL of trypticase soy broth containing a 10-μg meropenem disk, and incubation at 37°C for 18 hours for the screening of ESBL and/or plasmid-mediated AmpC and carbapenemase producers at 37°C for 24–48 hours, respectively.[8,9] All presumptive non-*Ec Enterobacteriaceae* (representative morphotypes per plate) were selected for further characterization. ESBL/plasmid–mediated AmpC or carbapenemase production was confirmed by phenotypic or Blue-Carba tests, respectively, polymerase chain reaction (PCR), and sequencing.[9] Susceptibility testing to non-β-lactams antibiotics was performed by the disk diffusion[10] and bacterial identification was confirmed by species-specific PCR, and/or matrix-assisted laser-desorption/ionization–time-of-flight mass spectroscopy. Clonal relatedness among *Kp* isolates was evaluated by Fourier transform infrared (FTIR) spectroscopy, multilocus sequence typing (MLST), and *wzi* capsular typing.[11–13]

Air samples (250 L) from different common indoor (3 from LTCF 1 and 1 from LTCF 2) and outdoor spaces were also collected using an MAS100 (Merck Millipore, Germany) air sampler to assess microbiological air quality. The different culture medium plates (ie, plate count agar [PCA], chromID ESBL, and chromID Carba SMART) used were incubated at 25°C for 72 hours (PCA, for fungi quantification) or at 37°C for 48 hours (PCA and selective medium, for bacteria quantification). Microbiological air quality (expressed in CFU/m^3) was categorized according to the Portuguese law.[14]

A high proportion of fecal samples (19 of 47; 40.4%) was positive for non-*Ec Enterobacteriaceae* producing ESBL (29.8%) or plasmid-mediated AmpC (10.6%). Despite some epidemiological differences, similar colonization rates were observed in both institutions: 44% in LTCF 1 and 36% in LTCF 2 (Table 1). Notably, in 53% of the samples, we also identified ESBL-producing *Ec,* leading to an overall rate of fecal carriage with ESBL producers of 81% (data not shown). ESBL carriage was significantly associated with the gender, length of stay, and residents of shared rooms, whereas plasmid-mediated AmpC carriage was only significantly associated with consumption of β-lactams in the previous 3 months (Online Supplemental Table S1). Air quality was within the established standards only at LTCF 1, although in both institutions no growth was detected on selective media. The colonization rates by ESBL-producing non-*Ec Enterobacteriaceae* (29.8%) were significantly higher than those observed in these species among LTCFs and nursing home residents years ago in Portugal in 2008–2012 (~6%) or in LTCFs in other European countries in 2012–2013 (~8%).[6] Despite the low sample size, this extraordinary increase (~5-fold) is worrisome in this at-risk population; it is probably influenced by the recent global expansion of MDR *Kp* isolates in Portuguese clinical institutions.[4] Carbapenemase-producing *Enterobacteriaceae* were not

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TABLE 1. Epidemiological Data of ESBL- or Plasmid-Mediated AmpC-Producing Non-*E. coli* Isolates Identified in Residents From Portuguese Long-Term Care Facilities (LTCFs)

Resident(s)	Gender/Age	Date of LTCF Admission	Location of Previous Internment	Strain	Room Type	Species (No.)	ESBL or Plasmid-Mediated AmpC	ST	FTIR Clusters	*wzi*	Resistance to Non-β-Lactam[a]
LTCF 1 (July 2015)											
LT1–LT6	F(4)/74–85 M(2)/60–71	14.10.2014–24.06.2015	H1, H2, H3, LTCF1, LTCF2, LTCF3	LT1.1–LT6.1	S (3), I (3)	*Kp* (6)	CTX-M-15	ST15	A	*wzi*19	(AMK), CIP, CLO, (GEN), KAN, NAL, NET, STR, SUL, SXT, (TET), TMP,TOB
LT7	F/78	02.06.2015	LTCF3	LT7.1	I	*Kp*	CTX-M-15	ST15	A	*wzi*19	CIP, CLO, GEN, KAN, NAL, NET, STR, SUL, SXT, TMP, TOB
				LT7.2		*Kp*	CTX-M-15	ST15	B	*wzi*24	CIP, GEN, KAN, NAL, STR, SUL, SXT, TET, TMP
LT8	F/90	08.07.2915	H4	LT8.1	S	*Kp*	CTX-M-15	ST15	B1	*wzi*24-like	AMK, CIP, CLO, GEN, KAN, NAL, NET, STR, SUL, SXT, TMP, TOB
LT9	M/75	15.06.2015	LTCF3	LT9.1	I	*Kp*	CTX-M-15	ST15	B	*wzi*24	CIP, GEN, KAN, NAL, NET, STR, SUL, SXT, TET, TMP, TOB
				LT9.2		*Ea*	CTX-M-15	NA	NA	NA	AMK, CIP, GEN, KAN, NAL, NET, STR, SUL, SXT, TET, TMP, TOB
LT10	M/61	06.02.2015	H3	LT10.1	I	*Kp*	CTX-M-15	ST262[b]	C	*wzi*143	CIP, GEN, KAN, NAL, NET, STR, SUL, SXT, TET, TMP, TOB
LT11	F/76	27.05.2015	H5	LT11.1	I	*Kp*	DHA-1	ST11	D	*wzi*75	CIP, CLO, KAN, NAL, STR, SUL, TOB
LTCF 2 (January 2016)											
LT21-22	M(2)/53–79	05.05.2015 11.04.2013	LTCF4, H2	LT21.1–LT22.1	S (1), I (1)	*Kp* (2)	CTX-M-15	ST348	H	*wzi*94	CIP, (CLO), (GEN), (KAN), NAL, (NET), STR, SUL, SXT, TMP, (TET), (TOB)
LT23	M/80	12.07.2013	H(NI)	LT23.1	S	*Kp*	CTX-M-15	ST15	B	*wzi*24	CIP, KAN, NAL, NET, SUL, SXT, STR, TMP, TOB
LT24	M/93	07.08.2015	H6	LT24.1	I	*Ea*	CTX-M-15	NA	NA	NA	AMK, GEN, KAN, NET, TET, TOB
LT25	F/84	08.07.2015	LTCF5	LT25.1	S	*Kp*	DHA-1	NI	E	*wzi*193-like	CIP, KAN, NAL, SUL, STX, TMP, TOB
LT26	F/84	21.09.2015	LTCF4	LT26.1	S	*Kp*	DHA-1	ST252	F	*wzi*81	CIP, KAN, NAL, SUL, TOB
LT27	F/81	15.10.2015	LTCF6	LT27.1	S	*Kp*	DHA-1	ST661	G	*wzi*373	CIP, KAN, NAL, SUK, TET, TOB
LT28	F/48	21.08.2012	LTCF7	LT28.1	I	*Ent.*	DHA-1	NA	NA	NA	CIP, KAN, SUL, STR
				LT28.2		*Pm*	DHA-1	NA	NA	NA	CIP, KAN, NAL, SUL, TOB

NA, not applicable; NI, not identified; F, female; M, male; H, hospital; LTCF, long-term care facility; S, shared room; I, individual room; *Kp, K. pneumoniae*; *Ea, E. aerogenes*; *Ent., Enterobacter* spp.; *Pm, Proteus mirabilis*; ST, sequence type; AMK, amikacin; CIP, ciprofloxacin; CLO, chloramphenicol; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NET, netilmicin; STR, streptomycin; SUL, sulphonamides; SXT, sulfamethoxazole; TET, tetracycline; TMP, trimethoprim; TOB, tobramycin.

[a]Variability among isolates is shown in parenthesis.

[b]Single locus variant (SLV) of ST405.

detected even though they have been increasingly identified among clinical isolates in our country.[2,3]

CTX-M-15 (16 isolates; 14 samples) and DHA-1 (6 isolates; 5 samples) were, respectively, the only ESBL and plasmid-mediated AmpC variants identified (Table 1). In LTCF 1, CTX-M-15 was predominant (n = 12 isolates in 91% of the samples), especially among *Kp* ST15 lineages (7 *wzi*19-K19, 3 *wzi*24-K24). A DHA-1-producing ST11 (*wzi*75) *Kp* isolate was sporadically detected (Table 1). Both ST15 *Kp* clonal lineages (*wzi*19, *wzi*24) or ST11-*wzi*75 have been frequently identified among isolates producing CTX-M-15, KPC-3 or DHA-1 in Portuguese clinical institutions.[1–3] Conversely, in LTCF 2, CTX-M-15 (4 isolates in 50% of the samples) was detected in 2 *Kp* clones (2 ST348-*wzi*94 and 1 ST15-*wzi*24) and 1 *Enterobacter aerogenes*, whereas DHA-1 (n = 5 isolates in 50% of the samples) was identified in diverse species and *Kp* lineages (Table 1). Notably, the cocolonization by isolates from the same or different species harboring the same enzyme suggests in vivo transfer of *bla* gene.

Our study reveals alarming (and increased) colonization rates by MDR non-*Ec Enterobacteriaceae* among LTCFs residents. These rates represent a challenge for these institutions in terms of antibiotic stewardship and infection prevention and control, and they highlight the risk of further dissemination to healthcare professionals, residents' families, and the community in general. Most of the *Kp* lineages (ST11 [*wzi*75], ST15 [*wzi*19/*wzi*24], and ST348 [*wzi*94]) identified are the same lineages involved in infections in clinical institutions from the same region, evidencing a higher risk of infection among this vulnerable population. The variability observed on the acquired antibiotic resistance genes among colonization/infection isolates in our settings suggests an open genome of such lineages for plasmid exchange.

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SUPPLEMENTARY MATERIAL

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Room Occupancy-Associated Transmission of MDRO, *Clostridium difficile*, or Norovirus: Results From a Room Surveillance Project

To the Editor—Mitchell et al[1] conducted a systematic review and meta-analysis to determine the risk of pathogen acquisition for patients associated with prior room occupancy. Overall, pooled acquisition odds ratio was 2.14 (95% confidence interval [CI], 1.65–2.77) for study pathogens: methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant enterococci (VRE); *Clostridium difficile*; *Acinetobacter* spp; extended-spectrum β-lactamase–producing coliforms; *Pseudomonas* spp. When comparing data between gram-positive and gram-negative organisms, the pooled acquisition odds ratios were 2.65 (95% CI, 2.02–3.47) for gram-negative organisms and 1.89 (95% CI, 1.62–2.21) for gram-positive organisms. Most of the included studies were performed in areas highly endemic for multidrug-resistant organisms (MDROs).

In January 2016, we initiated a prospective room surveillance project to determine whether the room is a risk factor for transmission to future occupants in a tertiary-care center with a low endemic MDRO burden. In addition, we considered rooms occupied by patients with symptomatic *C. difficile* or norovirus infection with a presumed high level of environmental contamination, for which a virucidal and sporicidal disinfectant was used for terminal cleaning and disinfection. Only routine infection control quality assurance or mandatory reportable surveillance data were used for this study, which was conducted according to German Federal Infection Prevention Law (IfSG) and German Federal Data protection law (Bundesdatenschutzgesetz).

All positive microbiological results of MDROs, *Clostridium difficile*, and norovirus cases were reported to the infection control registry. A likely case or room occupancy–associated transmission was defined as an occurrence of the same organism in a patient in the same room in the 6 weeks following discharge of a patient with known colonization or infection. For this report, we analyzed our experience during the first year of surveillance:

We identified 130 MDRO-cases: 95 MRSA, 9 MDR *Escherichia coli*, 9 MDR *Pseudomonas aeruginosa*, 5 MDR *Klebsiella pneumoniae*, 5 MDR *Serratia marcescens*, 2 MDR *Enterobacter cloacae*, 2 VRE, 1 MDR *Acinetobacter baumannii*, and 1 MDR *Citrobacter freundii*. Among these, 20 cases were classified as nosocomial according to current German surveillance definitions (www.nrz-hygiene.de) and 19 of these were not related to prior room occupancy.

A single case of MDR *Klebsiella pneumoniae* met inclusion criteria and was related to a small outbreak in the neonatal intensive care unit with transmission by a suctioning device.

We reported 7 cases of norovirus and 39 patients with *C. difficile* to the registry. A single norovirus case was nosocomial and 7 *C. difficile* cases were nosocomial, but none was related to prior room occupancy.

In contrast to previous studies,[2] we could not demonstrate an increased risk for newly admitted patients to a room that had been occupied by a patient with an MDRO, *C. difficile*, or norovirus. Thus, we conclude that routine terminal cleaning and disinfection of all reachable surfaces in the room is sufficient to prevent the spread of these organisms, even in cases with a presumed high level of environmental contamination (eg, vomiting and diarrhea). The only case primarily linked to a room was falsely positive; it was part of a point-source outbreak not related to room cleaning or disinfection.

Our study is limited by the low endemic rate of MDRO and the lack of compliance checks of terminal cleaning procedures,[3,4] but it reflects realistic daily practice in a large tertiary-care center in Germany. Enhanced cleaning and disinfections strategies[5] may therefore only be necessary if the room is identified as a risk factor for transmission within an institution by surveillance such as ours.

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