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Original article

Bloodstream infections in patients with rectal colonization by *Klebsiella pneumoniae* producing different type of carbapenemases: a prospective, cohort study (CHIMERA study)

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ABSTRACT

Objective: To investigate the hypothesis that intestinal colonization by different types of carbapenemase-resistant *Klebsiella pneumoniae* (CR-Kp) leads to different risks for bloodstream infections (BSI) caused by the same colonizing organism.

Methods: Prospective observational study including consecutive CR-Kp rectal carriers admitted to the Pisa University Hospital (December 2018 to December 2019). Patients underwent rectal swabbing with molecular testing for the different carbapenemases at hospital admission and during hospitalization. Rectal carriers were classified as: NDM, KPC, VIM and OXA-48. The primary end point was the rate of BSI by the same colonizing organism in each study group. A multivariate logistic regression analysis was performed to identify factors independently associated with the risk for BSI by the colonizing organism.

Results: Of 677 rectal carriers, 382/677 (56.4%) were colonized by NDM, 247/677 (36.5%) by KPC, 39/677 (5.8%) by VIM and 9/677 (1.3%) by OXA-48. Dissemination of NDM-Kp was mostly sustained by ST147, while KPC-Kp belonged to ST512. A higher rate of BSI was documented in NDM rectal carriers compared with KPC rectal carriers (59/382, 15.4% versus 20/247, 8.1%, p 0.004). Incidence rates of BSI per 100 patients/month were significantly higher in the NDM group (22.33, 95% CI 17.26–28.88) than in the KPC group (9.56, 95% CI 6.17–14.82). On multivariate analysis, multi-site extraintestinal colonization, solid organ transplantation, invasive procedures, intravascular device, admission to intensive care unit, cephalosporin, fluoroquinolones and NDM rectal colonization (OR 3.27, 95% CI 1.73–6.18, $p < 0.001$) were independently associated with BSI.

Conclusions: NDM-Kp was associated with increased risk of BSI compared with KPC-Kp. This finding seems to be strongly related to the high-risk clone ST147. **Marco Falcone, Clin Microbiol Infect 2021;▪:1**

Introduction

Carbapenem-resistant *Enterobacterales* (CRE) represent a threat for public health, being among the top multidrug-resistant pathogens on WHO's priority list [1–4]. Intestinal colonization by CRE is a risk factor for developing subsequent infection [5,6]. In patients

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colonized by *Klebsiella pneumoniae* carbapenemase (KPC) -producing *Klebsiella pneumoniae* (KPC-Kp), the rate of bloodstream infections (BSI) ranges from 8% to 23% [6,7], and specific factors for development of subsequent BSI have been identified [7,8]. However, metallo-β-lactamases and other carbapenemases are spreading worldwide [9–11], and each class of enzymes confers variable susceptibility profiles and implies different treatment strategies [12–14].

The aim of our study was to assess the rate of subsequent BSI caused by the same colonizing strain in patients with positive rectal swab for carbapenem-resistant *K. pneumoniae* (CR-Kp) and different molecular determinants of resistance.

Materials and methods

Study design and patient population

CHIMERA is a single-centre, longitudinal, prospective, observational study involving consecutive adult patients with rectal colonization by CR-Kp admitted to the 1100-bed University Hospital of Pisa from December 2018 to December 2019. During the study period, patients were screened for intestinal colonization with CRE on arrival. The screening was performed in patients hospitalized in medical/surgical/intensive care unit (ICU) wards, with the exclusion of patients with a provision of hospital stay <48 hours (e.g. patients undergoing minor surgery). After initial screening, all hospitalized patients were monitored through periodical rectal swabs on a regular basis (once a week in non-ICU wards and twice a week in ICU). The study included patients who tested positive on hospital admission (prevalent cases) and those who became positive during the hospital stay (incident cases). Patients with rectal colonization by more than one carbapenemase were excluded.

Patients with CR-Kp rectal colonization were prospectively followed up during hospitalization for subsequent infection by the same colonizing strain. All episodes of CR-Kp infections were recorded and patients were followed up until 30 days or death (see Supplememtary material, Fig. S1).

The study was approved by the local ethics committee (IRB no. 61185). Informed consent was obtained from all participants at hospital admission. In the case of patients with cognitive disturbances or altered consciousness, informed consent was obtained from legally authorized representatives.

Outcomes and exposure

The primary study outcome was the rate of BSI subsequent to colonization caused by the same colonizing strain. The association and strain concordance between the intestinal strain and the disease strain was ascertained by: (a) same microbial identification, (b) same molecular mechanism of carbapenem-resistance and (c) same susceptibility profile. The exposure variable was the type of carbapenemase produced by the colonizing organism grouped into four groups: KPC, NDM, VIM and OXA-48.

Independent variables and definitions

The case report form is provided in the Supplementary material (Table S1). Data about sites of colonization beyond stool were collected. Samples positive for CR-Kp, including urine, respiratory samples and skin cultures, obtained from patients without signs of infection and who had not received a specific antibiotic treatment, were considered as extraintestinal CR-Kp colonization. Screening other sites of colonization was performed once weekly in ICU and according to clinicians' decision in other wards.

Primary BSI and central-line-associated BSI were defined according to CDC/NHSN criteria [15]. Early-onset bacteraemia was defined as the occurrence of BSI within 7 days from the detection of rectal CR-Kp. Infectious episodes were classified according to CDC/NHSN definitions [16]. Pneumonia was defined as health-care-associated, hospital-acquired or ventilator-associated [17].

The Charlson Co-morbidity Index was calculated. Immunosuppressive therapy was defined as the receipt of immunosuppressants or prednisone at a dosage of 20 mg/day (or equivalent) for at least 2 weeks within the preceding 30 days [18]. Invasive procedures performed during hospitalization included intra-abdominal procedures (endoscopic retrograde cholangiopancreatography, percutaneous biliary drainage, biliary stenting, ureteral stenting or other urological tract procedures, intra-abdominal drain placement, gastro-oesophageal or rectosigmoid colon endoscopy, hysteroscopy), bronchoscopy, angiography and escharotomy. Previous antibiotics included: old β-lactam/β-lactamase inhibitors (amoxicillin/clavulanate, ampicillin/sulbactam or piperacillin/tazobactam), carbapenems, cephalosporins, fluoroquinolones, and other antibiotic therapies.

Microbiology

Rectal swabs were processed using three sequential steps: (a) molecular detection of *bla* genes involved in carbapenem resistance, (b) culture and pathogen identification, and (c) antibiotic susceptibility testing. Molecular screening was performed using the GeneXpert® System (Xpert-CARBA test; Cepheid, Sunnyvale, CA, USA). This real-time PCR test identifies the following genes: bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{IMP} and bla_{OXA-48}. Species identification was performed using matrix-assisted laser desorption/ionization time-of flight mass spectrometry (Bruker Daltonics GmbH, Bremen, Germany). Antimicrobial susceptibility tests were performed with the SensiTitre™ system (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. MICs were classified according to EUCAST breakpoints [19].

Isolates were subjected to whole-genome sequencing with an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) and a paired-end approach (2 × 300 bp). Multilocus sequence typing was performed according to the Institute Pasteur scheme (https://bigsdb.pasteur.fr/). The clonality of all CR-Kp isolates was investigated by pulsed-field gel electrophoresis.

Statistical analysis

Sample size estimation was performed on the basis of a previous study estimating that the risk of developing BSI in KPC-Kp-colonized patients was 7.8% [6]. Group sample sizes of 244 per group would achieve 90% power to detect an odds ratio of the group proportions of 2.5. The proportion in the NDM-Kp group was assumed to be 0.0780 under the null hypothesis and 0.1447 under the alternative hypothesis. The proportion in the KPC-Kp group was assumed to be 0.0780. The test statistic used was the two-sided Z test with unpooled variance. The significance level of the test was 0.05.

The incidence rate of KPC, VIM and NDM rectal colonization was calculated as the counts of colonization cases per 10 000 patient-days. Actual confidence intervals (CIs) of the incidence rate estimate were calculated by means of the exact mid-p test [20]. Descriptive data were summarized as mean ± standard deviations or median (interquartile ranges) according to their distribution and percentages for continuous and categorical variables, respectively. Mann–Whitney and χ^2 tests (or Fisher's exact test, as appropriate) were performed to compare baseline characteristics among the groups.

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The primary outcome was analysed as a time-dependent incidence rate (event rate per 100 patient-months). Kaplan–Meier curves show a comparison of bacteraemia-free survival among the study groups. A multivariate logistic regression analysis was performed to identify factors independently associated with the risk of BSI. Variables included in the multivariate analysis were selected based on statistical significance in the univariate analysis ($p < 0.05$). Age and Charlson Co-morbidity Index were entered as continuous variables; all other variables were entered as categorical. KPC was defined as the reference for the exposure variable. The multivariate model was built using a forward stepwise procedure. The validity of the final model was assessed by estimating goodness of fit with the Hosmer–Lemeshow test. The discrimination ability of the model was assessed by estimating the area under the receiver operating characteristic curve. Results are expressed as OR and their 95% CIs. Values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed with the Statistical Package for the Social Sciences Windows, version 20.0 (IBM, Armonk, NY, USA).

Results

Study population

Among 677 patients with rectal colonization by CR-Kp, 382/677 (56.4%) were NDM, 247/677 (36.5%) were KPC, 39/677 (5.8%) were VIM and 9/677 (1.3%) were OXA-48 rectal carriers (Fig. 1). Prevalent cases (patients who tested positive at hospital admission) accounted for 33.2% of cases (225/677), incident cases accounted for 66.8% (452/677). Due to the small number of OXA-48 carriers, the nine OXA-48 rectal carriers (with no BSI) were excluded from further comparative analysis.

During the study period, there were 46 096 admissions for a total of 284 898 patient-days in Pisa Hospital [21]. The total incidence of CRE rectal colonization was 24.7 per 10 000 patient-days (95% CI 22.9–26.6). The number of patients screened was 12 426 with a prevalence of CRE rectal colonization of 5.7%. Baseline characteristics by study groups are reported in Table 1. There were no differences in age, gender and co-morbidities between KPC, NDM and VIM rectal carriers.

Among NDM-producing strains, we performed a sequence typing in 152 of 382 strains (all blood isolates were tested). All strains belonged to the same clonal lineage, namely sequence type (ST) 147 and carried the $bla_{\text{NDM-1}}$ gene, with the exception of one strain belonging to ST307 carrying the $bla_{\text{NDM-5}}$ gene. The clonal analysis revealed that all the ST147 isolates were closely related to each other, suggesting that a single NDM-1-producing clone had spread in our hospital [9,13]. The strains also produced the following extended-spectrum β-lactamases: CTX-M-15, OXA-1, OXA-9 and TEM-1. Among the 247 KPC-Kp strains we performed sequence typing on 41 isolates; all KPC-Kp tested belonged to the ST512 and carried the $bla_{\text{KPC-3}}$ gene [22]; *bla* genes encoding OXA-9 and SHV-11 were also detected. Among VIM-producing *K. pneumoniae*, we performed sequence typing on 9/39 isolates. The VIM-producing *K. pneumoniae* belonged to the ST-37 and carried the $bla_{\text{VIM-1}}$ gene.

Of 677 colonized patients, 80 (11.8%) developed BSI by the colonizing strain, 81 (12%) infections by the colonizing strain other than BSI (including 34 complicated urinary tract infections, 24 hospital-acquired/ventilator-associated pneumonias, 14 complicated intra-abdominal infections, 4 health-care-associated pneumonias, 5 skin and soft-tissue infections), while 516 (76.2%) had no subsequent infection.

Primary end point

The rate of BSI by the colonizing organism was significantly higher in the NDM group (59/382, 15.4%) than in the KPC (20/247, 8.1%) and VIM (1/39, 2.6%) groups (p 0.004 among groups, p 0.007 NDM versus KPC) (Table 1). Incidence rates of BSI per 100 patient-months were significantly higher in the NDM (22.33, 95% CI 17.26–28.88) than in the KPC group (9.56, 95% CI 6.17–14.82). Kaplan–Meier curves showed lower probability of event-free survival in the NDM group compared with the KPC and VIM groups (log rank p 0.002) (Fig. 2). Sources of BSI are reported in the Supplementary material (Table S2).

Comparison of patients who developed, or not, BSI is shown in Table 2. On multivariate analysis, multiple sites of extraintestinal colonization, solid organ transplantation, intravascular device, diabetes, previous use of cephalosporins and fluoroquinolones, invasive procedures and rectal colonization by NDM-Kp were

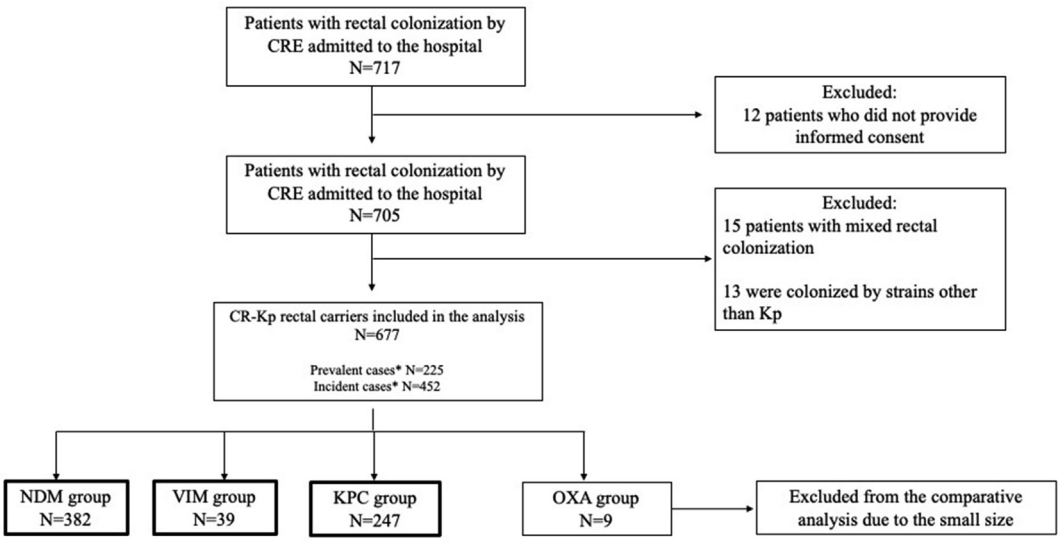

Fig. 1. Study flow chart. *Prevalent cases: patients who tested positive on hospital admission; incident cases: patients who became positive during the hospital course. CRE, carbapenem-resistant *Enterobacterales*; CR-Kp, carbapenem-resistant *Klebsiella pneumoniae*.

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Table 1
Baseline characteristics and BSI rates of the patients with rectal colonization by KPC versus NDM versus VIM

	KPC ($n = 247$)	NDM ($n = 382$)	VIM ($n = 39$)	P Value
Age (years), median (IQR)	72 (62–81)	76 (64–83)	76 (58–81)	0.075
Male sex, *n* (%)	150 (60.7)	236 (61.8)	19 (48.7)	0.282
Co-morbidities, *n* (%)				
Diabetes mellitus	77 (31.2)	108 (28.3)	8 (20.5)	0.362
Cardiovascular disease	134 (54.3)	243 (63.6)	19 (48.7)	0.025
COPD	57 (23.1)	91 (23.8)	5 (12.8)	0.296
Chronic renal disease	50 (20.2)	92 (24.1)	4 (10.3)	0.102
Chronic liver disease	40 (16.2)	48 (12.6)	5 (12.8)	0.429
Solid organ transplant	15 (6.1)	10 (2.6)	1 (2.6)	0.083
Solid cancer	98 (39.7)	163 (42.7)	18 (46.2)	0.644
Haematologic malignancy	11 (4.5)	23 (6)	2 (5.1)	0.695
Ward of hospitalization, *n* (%)				
Medical wards	118 (47.8)	222 (58.1)	18 (46.2)	0.025
Surgery	81 (32.8)	90 (23.6)	14 (35.9)	0.020
ICU	48 (19.4)	70 (18.3)	7 (17.9)	0.934
Charlson Co-morbidity Index, median (IQR)	2 (0-5)	2 (0-5)	1 (0-5)	0.367
Antibiotic therapy in the last 30 days, *n* (%)	158 (64)	268 (70.2)	22 (56.4)	0.094
Type of antibiotic in the last 30 days, *n* (%)				
BLBLI[a]	66 (26.7)	106 (27.7)	5 (12.8)	0.131
Carbapenems	20 (8.1)	13 (3.4)	2 (5.1)	0.036
Cephalosporins	43 (17.4)	61 (16)	5 (12.8)	0.740
Fluoroquinolones	21 (8.5)	27 (7.1)	4 (10.3)	0.676
Other class	2 (0.8)	20 (5.2)	3 (7.7)	0.002
More than one class in the last 30 days	6 (2.4)	41 (10.7)	3 (7.7)	0.001
Radio/chemotherapy in the last 3 months, *n* (%)	18 (7.3)	35 (9.2)	5 (12.8)	0.459
Immunosuppressants in the last 30 days, *n* (%)	84 (34)	107 (28)	14 (35.9)	0.216
Intravascular device, *n* (%)	119 (48.2)	173 (45.3)	13 (33.3)	0.219
Invasive procedures during hospitalization, *n* (%)	51 (20.6)	87 (22.8)	6 (15.4)	0.513
Type of invasive procedure, *n* (%)				
Intra-abdominal	33 (13.4)	57 (14.9)	2 (5.1)	0.233
Bronchoscopy	6 (2.4)	10 (2.6)	0	0.595
Escharotomy	3 (1.2)	12 (3.1)	1 (2.6)	0.303
Angiography	9 (3.6)	8 (2.1)	2 (5.1)	0.353
Others	0	0	1 (2.6)	0.058
Abdominal surgery, *n* (%)	31 (12.6)	38 (9.9)	3 (7.7)	0.480
Number of additional colonized sites besides rectal swab >2, *n* (%)	3 (1.2)	14 (3.7)	0	0.095
Time from admission to positive rectal swab, median (IQRs)	4 (1–14)	5 (1–14)	5 (1–14)	0.482
Sequence typing ($N = 202$), *n* (%)				
ST512	41/41 (100)	—	—	
ST147	—	151/152 (99.3)[b]	—	
ST37	—	—	9/9 (100)	
Crude number of BSI	20 (8.1)	59 (15.4)	1 (2.6)	0.004

Abbreviations: BLBLI, β-lactam–β-lactamase inhibitor; BSI, bloodstream infections; COPD, chronic pulmonary obstructive disease; ICU, intensive care unit; IQR, interquartile range.
Data are median and IQR or frequency and proportion. Mann–Whitney test, χ^2 test or Fisher's Exact test were performed, as appropriate.
[a] BLBLI included amoxicillin/clavulanate, ampicillin/sulbactam and piperacillin/tazobactam. No patients received ceftolozane/tazobactam not ceftazidime/avibactam in the last 30 days.
[b] 1/152 NDM-Kp belonged to ST307.

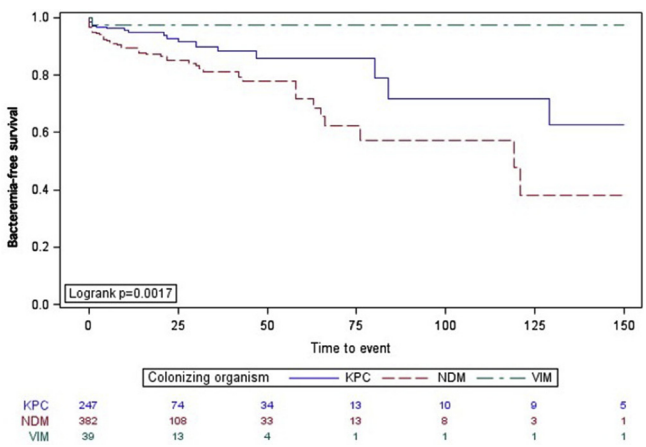

Fig. 2. Kaplan–Meier curves of event (bacteraemia)-free survival among the three study groups (KPC versus VIM versus NDMrectal carriers). Red line: NDM-group. Blue line: KPC-group. Green line: VIM-group.

factors independently associated with BSI by the colonizing organism (Table 3). The model displayed good predictive ability (C-statistic 0.872, 95% CI 0.833–0.910) and Hosmer–Lemeshow test was indicative of good calibration ($\chi^2 = 5.39$, p 0.715).

The rate of non-bacteraemic infections did not differ between KPC and NDM (13.4% versus 12.6%), no infections by VIM-producing strains were observed (see Supplementary material, Table S3).

Targeted antibiotic regimens of patients who developed BSI

The 60 patients with metallo-β-lactamase-Kp BSI received the following targeted antibiotic regimens: ceftazidime/avibactam plus aztreonam ($n = 42$, 71.2%), colistin-based regimens ($n = 7$, 11.8%), tigecycline +/− fosfomycin ($n = 4$, 6.8%), fosfomycin alone ($n = 1$, 1.7%). Six (10.2%) patients received no *in vitro* active therapy. Among 20 patients with KPC-Kp BSI treatments were ceftazidime-avibactam +/− aminoglycosides or fosfomycin ($n = 15$), colistin + gentamycin ($n = 2$), tigecycline + aminoglycosides ($n = 1$), trimethoprim-sulfamethoxazole ($n = 1$) and no *in vitro* active therapy ($n = 1$).

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Table 2
Comparison of rectal carriers who developed BSI by the colonizing organism and those who did not

	BSI ($n = 80$)	No BSI ($n = 597$)	p value
Age (years), median (IQR)	68 (54–77)	76 (64–83)	<0.001
Male sex, *n* (%)	55 (68.8)	354 (59.3)	0.104
Co-morbidities, *n* (%)			
Diabetes mellitus	30 (37.5)	165 (27.6)	0.067
Cardiovascular disease	31 (38.8)	369 (61.8)	<0.001
COPD	13 (16.2)	141 (23.6)	0.140
Chronic renal disease	14 (17.5)	132 (22.1)	0.346
Chronic liver disease	10 (12.5)	83 (13.9)	0.732
Solid organ transplantation	8 (10)	18 (3)	0.002
Solid cancer	26 (32.5)	255 (42.7)	0.082
Haematological malignancy	0	37 (6.2)	0.022
Ward of hospitalization, *n* (%)			
Medical wards	34 (42.5)	331 (55.4)	0.029
Surgery	11 (13.8)	176 (29.5)	0.003
ICU	35 (43.8)	90 (15.1)	<0.001
Charlson Co-morbidity Index, median (IQR)	1 (0–4)	3 (0–5)	0.005
Antibiotic therapy in the last 30 days, *n* (%)	63 (78.8)	389 (65.2)	0.015
Type of antibiotic in the last 30 days, *n* (%)			
Old BLBLI[a]	19 (23.8)	161 (27)	0.541
Carbapenems	4 (5)	32 (5.4)	0.893
Cephalosporins	27 (33.8)	82 (13.7)	<0.001
Fluoroquinolones	13 (16.2)	39 (6.5)	0.002
Other class[b]	0	25 (4.2)	0.061
More than one class	0	50 (8.4)	0.002
Radio/chemotherapy in the last 3 months, *n* (%)	5 (6.2)	53 (8.9)	0.430
Immunosuppressants in the last 30 days, *n* (%)	35 (43.8)	173 (29)	0.007
Intravascular device, *n* (%)	59 (73.8)	250 (41.9)	<0.001
Invasive procedures during hospitalization, *n* (%)	33 (41.2)	113 (18.9)	<0.001
Type of invasive procedure, *n* (%)			
Intra-abdominal	16 (20)	76 (12.7)	0.075
Bronchoscopy	3 (3.8)	13 (2.2)	0.385
Escharotomy	13 (16.2)	3 (0.5)	<0.001
Angiography	1 (1.2)	20 (3.4)	0.309
Others	0	1 (0.2)	0.714
Abdominal surgery, *n* (%)	6 (7.5)	68 (11.4)	0.295
Number of additional colonized sites besides rectal swab >2, *n* (%)	11 (13.8)	6 (1)	<0.001
Type of rectal carbapenemase, *n* (%)[c]			
NDM	59 (73.8)	323 (54.1)	0.001
KPC	20 (25)	227 (38)	0.023
VIM	1 (1.2)	38 (6.4)	0.065

Abbreviations: BLBLI, β-lactam–β-lactamase inhibitor; BSI, bloodstream infections; COPD, chronic pulmonary obstructive disease; ICU, intensive care unit; IQR, interquartile range.

[a] BLBLI included amoxicillin/clavulanate, ampicillin/sulbactam and piperacillin/tazobactam. No patients received ceftolozane/tazobactam nor ceftazidime/avibactam in the last 30 days.

[b] Other class included: 12 patients who received penicillin, nine received aminoglycosides, and four received trimethoprim-sulfamethoxazole.

[c] Nine patients with rectal colonization by OXA-48-like had no documented BSI.

Discussion

Our prospective cohort study reveals that among CR-Kp rectal carriers the risk of developing BSI by the same colonizing organism is higher in NDM-Kp than in KPC-Kp rectal carriers. Since the majority of NDM-Kp belonged to ST147, whereas all the tested KPC-Kp to ST512, carbapenemase types were strongly linked with a specific ST in our study. Our results suggest that the bacterial clone has a significant impact on the clinical course of patients with intestinal colonization.

Recent studies show that the dissemination of CRE is expanding across hospitals in Europe [2], with several countries reporting carbapenem-resistance percentages above 10% in *K. pneumoniae* [2]. A cross-border spread of uncommon resistance mechanisms such as $bla_{\text{NDM-1}}$- and $bla_{\text{OXA-48}}$-positive *K. pneumoniae* strains is also reported [10,23–25]. The rate of bacteraemia in KPC-Kp rectal carriers (8.1%) was consistent with previous data (7.8%) [6]. Instead, we detected an almost double rate of BSI among patients with NDM rectal colonization (15.4%). These results are of outstanding importance, because of the increasing detection of non KPC-producing organisms worldwide [10–13,26,27]. Our findings refer to a single clone of NDM (ST147). We found that ST147 NDM-Kp was associated with higher risk of BSI compared with ST512 KPC-3-Kp. The increased risk of BSI we observed in patients with rectal colonization by NDM-Kp may be related to the specific clone and strain type. This hypothesis is supported by both preclinical and clinical findings. NDM-1-Kp has been described as the most virulent isolate in a murine model of sepsis [27]. Moreover, ST147 is a high-risk *K. pneumoniae* clone with the potential to become a major threat to public health and to cause serious infections also by pan-resistant strains all over the world [28]. ST147 consists of multiple clades/clusters associated with various carbapenemases (i.e. KPCs, NDMs, OXA-48-like and VIMs) and has been responsible for several nosocomial outbreaks, including endoscopy-associated transmission and long-term care centre outbreaks and is endemic in India, Italy, Greece and certain North African countries [28]. Recently, a single ST147 clone carrying both $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$ in the same transferable IncR plasmid has emerged in the Spanish health-care system in Catalonia and is responsible for rapid dissemination and hospital outbreaks [29]. Studies addressing the prevalence of ST147 among *K. pneumoniae* isolates are relatively rare. Moreover, prevalence studies of human rectal colonization are

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Table 3
Multivariate analysis of factors independently associated with bloodstream infections by colonizing CR-Kp in the overall population

Covariate	OR (95%CI)	p value
Sites of colonization beyond stool >2	9.48 (2.62–34.02)	0.001
Solid organ transplantation	4.66 (1.66–13.05)	0.003
Invasive procedures [a]	1.91 (1.05–3.49)	0.035
Intravascular device	3.72 (2.03–6.83)	<0.001
Type of rectal colonization		
KPC rectal colonization (reference variable)	1.0	—
VIM rectal colonization	0.4 (0.47–3.39)	0.401
NDM rectal colonization	3.27 (1.73–6.18)	<0.001
Antibiotic therapy last 30 days		
No antibiotic therapy (reference variable)	1.0	—
BLBLI	0.81 (0.36–1.83)	0.619
Cephalosporin	5.04 (2.27–11.16)	<0.001
Carbapenems	1.48 (0.41–5.39)	0.552
Fluoroquinolones	7.13 (2.72–18.7)	<0.001
Other class	—	0.998
More than one class	—	0.997
ICU	2.22 (1.14–4.29)	0.018
Cardiovascular disease	0.48 (0.25–0.93)	0.029

Abbreviations: BLBLI, β-lactam–β-lactamase inhibitors; CR-Kp, carbapenem-resistant *Klebsiella pneumoniae*; ICU, intensive care unit.
The multivariate model was performed using a forward stepwise procedure. Variables entered to the regression but not retained were: age, haematological malignancy, Charlson Co-morbidity index, immunosuppressants in last 30 days (see variables with statistical significance at the comparison reported in Table 2).
[a] Invasive procedures occurring after rectal colonization.

currently lacking, and it is unknown whether the prevalence or duration of intestinal colonization in humans is different for ST147 than for other *K. pneumoniae* clones or isolates [28]. Strain typing is not routinely performed; it might be important to identify strain types in clinical practice, especially in regions at high endemicity of multidrug-resistant organisms, when outbreaks occur or in hospitals with high mortality rates due to infections by multidrug-resistant organisms.

Our study has some limitations. First, because of variability in the epidemiology of CRE throughout the world, the generalizability of our results should be validated in different settings. Second, the presence of a clonal spread of ST147 NDM-1-Kp may have influenced the results, and the imbalance in the rate of progression from colonization to BSI between NDM- and KPC-producers may reflect differences in the virulence of the predominant NDM clone. Third, one-third of BSI were central-line-associated BSI, which may potentially be indicative of a high risk of procedures-related BSI during the hospital outbreak. However, central-line-associated BSI are not always related to central line care, but may result from translocation of gut microorganisms related to mucosal barrier injury, especially if enteric bacteria (e.g. *K. pneumoniae*) are involved [30]. Finally, we did not perform a long-term follow up to evaluate the duration of colonization and delayed infections after 30-day follow up.

Our study highlights the importance of systematic screening for rectal colonization by specific carbapenemase type. In our cohort, patients colonized by NDM-Kp are at higher risk of bacteraemia compared with those colonized by KPC-Kp and the carbapenemase type is strongly related to the specific ST. Hence, our findings may reflect an increased virulence of the ST147 clone of *K. pneumoniae*. The knowledge of mechanisms of resistance together with the study of virulence and presence of specific risk factors may help to stratify the risk of infection in hospitalized patients with CRE rectal colonization. Strategies to achieve intestinal decolonization of CRE are urgently needed.

Author contributions

MF and FM designed the study; GT created the case report form, developed the database, and analysed and interpreted data; GT coordinated the data collection; VG, GT and EM recruited patients and collected clinical data; MF, FM and GT wrote the study; CG, AL and SB performed microbiological analyses; MF, PDS, GB, UB and SB revised and contributed to the critical revision of the final manuscript.

Transparency declaration

MF received grants and speaker honoraria from MSD, Angelini and Shionogi. FM has participated in advisory boards and/or received speaker honoraria from Angelini, Correvio, MSD, Pfizer, Astellas, Gilead, BMS, Jansenn, ViiV, BioMérieux, Biotest, Becton-Dickinson, Nordic Pharma, Pfizer and Shionogi. All conflicts of interest are outside the submitted work. The remaining authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.06.031.

References

[1] Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin Infect Dis 2019;69:S521–8.
[2] Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, et al. Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill 2019;24:1900123.
[3] Falcone M, Tiseo G, Dentali F, La Regina M, Foglia E, Gambacorta M, et al. Predicting resistant etiology in hospitalized patients with blood cultures positive for Gram-negative bacilli. Eur J Intern Med 2018;53:21–8.
[4] Falcone M, Russo A, Iacovelli A, Restuccia G, Ceccarelli G, Giordano A, et al. Predictors of outcome in ICU patients with septic shock caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. Clin Microbiol Infect 2016;22:444–50.
[5] Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, et al. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. Clin Infect Dis 2017;65:208–15.
[6] Giannella M, Trecarichi EM, De Rosa FG, Del Bono V, Bassetti M, Lewis RE, et al. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection among rectal carriers: a prospective observational multicentre study. Clin Microbiol Infect 2014;20:1357–62.
[7] Cano A, Gutiérrez-Gutiérrez B, Machuca I, Gracia-Ahufinger I, Pérez-Nadales E, Causse M, et al. Risks of infection and mortality among patients colonized with *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: validation of scores and proposal for management. Clin Infect Dis 2018;66:1204–10.
[8] Falcone M, Bassetti M, Tiseo G, Giordano C, Nencini E, Russo A, et al. Time to appropriate antibiotic therapy is a predictor of outcome in patients with bloodstream infection caused by KPC-producing *Klebsiella pneumoniae*. Crit Care 2020;24:29.
[9] Falcone M, Tiseo G, Antonelli A, Giordano C, Di Pilato V, Bertolucci P, et al. Clinical features and outcomes of bloodstream infections caused by New Delhi

metallo-β-lactamase-producing *Enterobacterales* during a regional outbreak. Open Forum Infect Dis 2020;7:ofaa011.
[10] Tavoschi L, Forni S, Porretta A, Righi L, Pieralli F, Menichetti F, et al. Prolonged outbreak of New Delhi metallo-beta-lactamase-producing carbapenem-resistant *Enterobacterales* (NDM-CRE), Tuscany, Italy, 2018 to 2019. Euro Surveill 2020;25:2000085.
[11] Falcone M, Mezzatesta ML, Perilli M, Forcella C, Giordano A, Cafiso V, et al. Infections with VIM-1 metallo-beta-lactamase-producing *Enterobacter cloacae* and their correlation with clinical outcome. J Clin Microbiol 2009;47:3514–9.
[12] Livermore DM, Nicolau DP, Hopkins KL, Meunier D. 'CRE, CRO, CPE and CPO': terminology past its 'sell-by-date' in an era of new antibiotics and regional carbapenemase epidemiology. Clin Infect Dis 2020. ciaa122.
[13] Falcone M, Daikos GL, Tiseo G, Bassoulis D, Giordano C, Galfo V, et al. Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by MBL-producing *Enterobacterales*. Clin Infect Dis 2020. ciaa586.
[14] Falcone M, Paterson D. Spotlight on ceftazidime/avibactam: a new option for MDR Gram-negative infections. J Antimicrob Chemother 2016;71:2713–22.
[15] CDC/NHSN bloodstream infection event (central line-associated bloodstream infection and non-central line associated bloodstream infection). Available at: https://www.cdc.gov/nhsn/pdfs/pscmanual/4psc_clabscurrent.pdf. [Accessed 1 April 2021].
[16] CDC/NHSN surveillance definitions for specific types of infections. Available at: https://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf. [Accessed 1 April 2021].
[17] Liu JL, Xu F, Zhou H, Wu XJ, Shi LX, Lu RQ, et al. Expanded CURB-65: a new score system predicts severity of community-acquired pneumonia with superior efficiency. Sci Rep 2016;6:22911.
[18] Lautenbach E, Fishman NO, Bilker WB, Castiglioni A, Metlay JP, Edelstein PH, et al. Risk factors for fluoroquinolone resistance in nosocomial *Escherichia coli* and *Klebsiella pneumoniae* infections. Arch Intern Med 2002;162:2469–77.
[19] European Committee on Antimicrobial Susceptibility Testing (EUCAST). The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2019. Available at:, Version 9.0. http://www.eucast.org. [Accessed 15 September 2020].
[20] Rothman KJ, Boice Jr JD. Epidemiologic analysis with a programmable calculator. Bethesda: MD: NIH Publication; 1979.
[21] Relazione sanitaria aziendale azienda ospedaliera universitaria pisana. 2019. Available at: https://www.ao-pisa.toscana.it/index.php?option=com_attachments&task=download&id=8091. [Accessed 21 January 2021].
[22] Tiseo G, Falcone M, Leonildi A, Giordano C, Barnini S, Arcari G, et al. Meropenem-vaborbactam as salvage therapy for ceftazidime-avibactam-, cefiderocol-resistant ST-512 *Klebsiella pneumoniae* producing KPC-31, a D179Y variant of KPC-3. Open Forum Infect Dis 2021;8:ofab141.
[23] Ludden C, Lötsch F, Alm E, Kumar N, Johansson K, Albiger B, et al. Cross-border spread of blaNDM-1- and blaOXA-48-positive *Klebsiella pneumoniae*: a European collaborative analysis of whole genome sequencing and epidemiological data, 2014 to 2019. Euro Surveill 2020;25:2000627.
[24] Falcone M, Giordano C, Barnini S, Tiseo G, Leonildi A, Malacarne P, et al. Extremely drug-resistant NDM-9-producing ST147 *Klebsiella pneumoniae* causing infections in Italy, May 2020. Euro Surveill 2020;25:2001779.
[25] van Duin D, Arias CA, Komarow L, Chen L, Hanson BM, Weston G, et al. Molecular and clinical epidemiology of carbapenem-resistant *Enterobacterales* in the USA (CRACKLE-2): a prospective cohort study. Lancet Infect Dis 2020;20:731–41.
[26] Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, et al. Phenotypic and genotypic characterization of carbapenem-resistant *Enterobacteriaceae*: data from a longitudinal large-scale CRE study in China (2012-2016). Clin Infect Dis 2018;67(Suppl. 2):S196–205.
[27] Fuursted K, Schøler L, Hansen F, Dam K, Bojer MS, Hammerum AM, et al. Virulence of a *Klebsiella pneumoniae* strain carrying the New Delhi metallo-beta-lactamase-1 (NDM-1). Microbe. Infect 2012;14:155–8.
[28] Peirano G, Chen L, Kreiswirth BN, Pitout JDD. Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. Antimicrob Agents Chemother 2020;64. e01148-20.
[29] Marí-Almirall M, Cosgaya C, Pitart C, Viñes J, Muñoz L, Campo I, et al. Dissemination of NDM-producing *Klebsiella pneumoniae* and *Escherichia coli* high-risk clones in Catalan healthcare institutions. J Antimicrob Chemother 2021;76:345–54.
[30] See I, Iwamoto M, Allen-Bridson K, Horan T, Magill SS, Thompson ND. Mucosal barrier injury laboratory-confirmed bloodstream infection: results from a field test of a new National Healthcare Safety Network definition. Infect Control Hosp Epidemiol 2013;34:769–76.

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