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ORIGINAL PAPER

Findings from an outbreak of carbapenem-resistant *Klebsiella pneumoniae* emphasize the role of antibiotic treatment for cross transmission

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Abstract

Purpose In January 2015, we noticed by rectal swab analyses that seven of 23 patients at an early rehabilitation ward had been colonized with carbapenem-resistant *Klebsiella pneumoniae* (CKP). Here, we describe risk factors for CKP acquisition.

Methods In the present study, the outbreak is described and risk factors for CKP acquisition are examined, e.g., antibiotic treatment. Microbiological analyses including corresponding results were examined to study when colonization with CKP occurred and whether patients had suffered from diarrhea. To examine whether spread of bacteria was clonal, multi-locus sequence typing as well as XbaI macrorestriction and pulsed-field gel electrophoresis was performed. The presence of carbapenmase was examined by PCR analysis. Through univariate analysis of risk factors in the small study sample, the role of antibiotic consumption, isolation procedures, patient's age, gender, and Barthel index on colonization was elucidated.

Results Clonal spread of the novel sequence type (ST)2255 was identified. Additionally, one patient was colonized with *Escherichia coli* and *Serratia marcescens*, both resistant to carbapenems, while a further patient carried another carbapenem-resistant *E. coli* strain. In all isolates, carbapenemase gene $bla_{\text{OXA-48}}$ was found to be located on a conjugative plasmid (60 kb), suggesting in vivo transmission from CKP to *E. coli* and *S. marcescens*. Univariate tests indicated that antibiotic treatment was the only risk factor showing a significant association with being colonized by CKP. In addition, the likelihood of diarrhea appeared to be higher in this group. Antibiotic treatment was associated with CKP colonization, whereas patients´ age, gender, Barthel index at admission, and residence with a CKP-colonized roommate were not. Diarrhea also seemed to support to distribution of CKP.

Conclusions In this small outbreak, antibiotic treatment seemed to be the predominant risk factor for monoclonal transmission of $bla_{\text{OXA-48}}$ positive CKP.

Keywords OXA-48 · CTX-M-15 · Conjugative transfer · *Escherichia coli* · *Serratia marcescens*

Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CKP) initially were described in the 1990s in Japan and the USA [1–3]. Today, the bacteria are present all over the world, especially affecting hospitalized patients. Carbapenem resistance is predominately mediated by enzymes inactivating carbapenems and other β-lactam antibiotics. According to the Ambler classification, categorizing β-lactamases, CKP mainly produce class A, B, and D carbapenemases [4]. Among the class A carbapenemases, the worldwide emergence of KPC enzymes (mainly KPC-2 and KPC-3) in CKP is of concern [5]. Furthermore in CKP class B carbapenemases have been

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described, e.g., the VIM enzyme family. In 2008, a CKP, secreting a New Delhi metallo-β-lactamase (NDM), was isolated for the first time from a Swedish patient returning from India [6]. Meanwhile, NDM enzymes have become the most globally important class B carbapenemases [5]. Widespread in CKP is also the class D carbapenemase OXA-48, an enzyme which hydrolyses carbapenems but not third generation cephalosporins, e.g., cefotaxime and ceftazidime.

Since their first isolation about 25 years ago, CKP have emerged all over the world and their number is increasing steadily. Interestingly, the mechanism of carbapenem resistance varies geographically [5]. KPC-producing CKP are prevalent in various South American countries, the USA, Poland, Greece, Italy and China while the Indian subcontinent (India, Pakistan, and Bangladesh) bordering on China is the epicenter of NDM-producing CKP. OXA-48-producing CKP are endemic in India, Turkey, Egypt, Libya, and Morocco [5]. In Germany in 2008, many sporadic CKP findings were described [7–9] and several outbreaks were observed [10–12]. At the hospital in Ingolstadt, Germany, seven CKP-colonized patients were identified in January 2015. In the present study, the outbreak is described and patients' characteristics as well as risk factors for acquisition of these OXA-48-producing CKP were examined.

Methods

Setting and transmission description

The hospital in Ingolstadt is a tertiary care hospital in the center of Bavaria (South-East Germany). The hospital includes an early rehabilitation ward comprising 22 beds (Fig. S1). Each patient room contains a wet cell. Apart from the wet cells, there is a bathroom which is rarely used. Within the observation period, this bathroom had not been used. Patients at the ward commonly suffer either from neurological deficits (mostly strokes) or from trauma (≥ 2 injuries). Mobility of the patients is limited. Most patients are bedridden or able to move in a wheel chair. If patients are able to walk, the walk distance is 50 m at maximum. Although there is a dining room usually patients eat their meals in the patient rooms either at the table or in their bed at the nightstand. Depending on patients' conditions exercise training is individually performed in the patient rooms or in the corridor of the ward together with at least a physical therapist. Patients do not use a swimming pool or a gymnasium.

In January 2015, an expert system had been put into operation at the laboratory of the hospital facilitating identification of carbapenem-resistant Gram-negative bacteria apparently exhibiting susceptibility to these antibiotics.

On 12th January 2015 (cutoff date), it was noticed that in total 3 patients (patients 1, 3, 6) had been tested positive for *K. pneumoniae* showing reduced susceptibility to ertapenem. On 13th January, rectal screening was initiated to analyze whether carbapenem-resistant enterobacteriaceae (CRE) unnoticeably had been spread at this ward. At that time, the ward had 19 residents. Screening results demonstrated that 6 of the 19 patients had been colonized with CKP. Patients not exhibiting CKP colonization were repeatedly tested until at least three negative swabs were obtained (patients 11–16, 18–23) or the patients were dismissed from hospital (patients 8, 9, 10, 17). Screening of CKP-colonized patients was also repeated until three swabs showed growth of CKP. If a patient exhibited CKP positive as well as CKP negative swabs, a multitude of swabs were taken to assess the CKP load of the patient. The rectal screening of colonized and non-colonized patients was repeated until dismissal from hospital.

After the outbreak had been detected, admitting new patients to the ward was stopped. Patients colonized by CKP underwent contact isolation in a single room or cohort isolation with another sex-matched CKP-colonized patient. All surfaces of the ward, including walls, floors, wheel chairs, nightstands, etc. were cleaned and disinfected. Each antibiotic treatment was discussed between the physicians of the ward and the head of the department of infectious diseases to restrict unnecessary prescriptions of (broad spectrum) antibiotics. E.g., antibiotics were administered only to those patients clearly suffering from infection and not to patients exclusively exhibiting increased concentration of C-reactive protein. Staff contacting CKP-colonized patients wore masks, gloves and coats. Depending on the clinical situation and room capacities, CKP-colonized patients were dismissed from hospital or moved to the infectious diseases ward. The rooms of that ward exhibit anterooms with storage space for coats, gloves, etc., rendering treatment of patients with multi-resistant bacteria easier to perform. Surfaces of the early rehabilitation ward were again cleaned and disinfected after the last CKP-colonized patient left the ward. After the second disinfection round new patients were admitted. From that point on, a weekly screening of patients for colonization with CRE and Gram-negative bacteria with extended spectrum beta-lactamase activity (ESBL) was implemented. After detection of the outbreak, there was no evidence of ongoing CKP transmission.

Apart from current patients on the ward, four patients with recent stays during the observation period were also screened for colonization. Of these a patient was readmitted to another ward (patient 7), while two patients were screened when visiting our out-patients departments (patients 10 and 17). After a short residence at the early rehabilitation ward, patient 23 was moved to the geriatric ward (demission 27th January). Of the additional patients, three had stayed in a room together with CKP-colonized patients: patient 7 together with patients 1 and 6 (room 13), patient 10 together with patient 4 (room 5) and patient 10 together with patient

2 (room 3). These patients were identified using an industry solutions healthcare module (SAP/IS-H; Siemens, Munich, Germany), adapted to the requirements of the hospital, that generated a list of patients who recently had stayed in a room together with a CKP-colonized patient. The list generated was composed of patients who remained in the room for the majority of the corresponding day. An alert system, implemented to the hospital IT system (Soarian, Siemens, Munich), allowed identification of these contact patients when using medical service of our facilities. While one of the additional patients had acquired CKP (patient 7), the three other patients had not (patients 10, 17, 23). As these four patients were included in our analysis, a total of 23 patients contributed to the study (seven CKP-colonized and 16 CKP non-colonized patients). Each patient that contributed at least a rectal swab was examined for prevalence of CKP.

Patient 7 was admitted on 17th October 2014 to the ward while the other CKP-colonized patients were admitted to the ward after patient 7. Using our SAP/IS-H system, a list was generated comprising all patients residing on the ward between 17th October 2014 and 12th January 2015 (observation period). In summary, of the 53 patients identified; 7 were CKP positive, 16 were CKP negative and 30 patients had an unknown colonization status. Using LabCentre l.i.c. laboratory software (i-SOLUTION Health GmbH, Mannheim, Germany), we examined if *K. pneumoniae* had been isolated from these patients within the observation period. Corresponding antibiotic resistance patterns were also regarded to assess if carbapenem resistance or extended spectrum beta-lactamase activity (ESBL) might have been prevalent in these bacteria (reduced susceptibility or resistance to carbapenems and/or cephalosporines of 3rd generation).

Antibiotic treatment regime and Barthel index (BI) of patients 1–23 were obtained from hospital IT system (Soarian, Siemens, Munich) adapted to the requirements of the hospital.

Microbiological and molecular investigations

At the laboratory of the hospital carbapenem-resistant Gram-negative bacteria were identified during routine analyses, as recently described [13]. For susceptibility testing, CLSI breakpoints were used. In January 2015, additional measures were implemented, allowing identification of carbapenem resistance of Gram-negative bacteria obviously exhibiting susceptibility to ertapenem, meropenem, and/or imipenem. Species identification and antibiotic susceptibility testing were performed using VITEK 2 system (Becton-Dickinson, Heidelberg, Germany). The software of the VITEK 2 system was updated (version 06.01) to provide an alert when increased minimal inhibitory concentration (MIC) to carbapenem antibiotics occurred. In these cases, MIC of the corresponding antibiotics (imipenem, meropenem, ertapenem) was determined using MIC Test strips (Bestbiondx, Cologne, Germany). Probable presence of carbapenemase was phenotypically assessed using carbapenemase set D70C and temocillin cartridge disk TEM30C (Mast Diagnostica, Reinfeld, Germany). The presence of corresponding carbapenemase genes was examined by PCR using Xpert Carba-R panel (Cepheid Diagnostic, Frankfurt/Main, Germany).

Stool samples for *Clostridium difficile* diagnostic first were screened for the presence of *C. difficile* Antigen (Glutatmatdehydrogenase) using Serazym *Clostridium difficile* GDH (Seramun, Heidesee, Germany) test. Samples exhibiting reactivity in this screening assay were analyzed for the presence of *C. difficile* toxin A and B with the Serazym *Clostridium difficile* Toxin A + B (Seramun, Heidesee, Germany) test. Samples showing no reactivity in toxin A + B test were examined for toxin genes by PCR (Xpert *Clostridium difficile*, Cepheid, Frankfurt am Main, Germany). Patients were regarded to suffer from *C. difficile* infection (CDI) either when toxin A and B test or toxin gene PCR had been positive.

Nose and rectal swabs were routinely taken once per week from each patient to examine the presence of MRSA and VRE, respectively [13]. According to the current German guidelines, only patients colonized and/or infected with MRSA were isolated or underwent cohort isolation with a sex-matched roommate [14, 15].

In total, 28 contact plates (Oxoid [PO5172 C], Wesel, Germany) were taken from the near environment of patients 1, 4, 5, 6, and 7 (nightstands, wheelchairs, etc.) to examine contamination with CKP. Patient 18 had a leg splint which was difficult to disinfect. From this splint, eight contact samples were also taken. Furthermore, 48 contact slides were taken from the clothes of staff (nurses, physicians, physical therapists).

All enterobacteria isolates with increased MIC for ertapenem, imipenem or meropenem were sent to the Robert Koch Institute (RKI) for further analyses. At the RKI, antimicrobial susceptibilities were confirmed by VITEK 2 system (card AST-N248) using EUCAST breakpoints (version v 5.0). Since slightly increased MIC values for imipenem and meropenem (0.25–2 mg/L) were observed, Etests with ertapenem and a modified Hodge test were performed for confirmation of any carbapenemase production as recommended by CLSI (2013) [14].

The presence of carbapenemase genes ($bla_{\text{VIM-like}}$, $bla_{\text{IMP-like}}$, $bla_{\text{NDM-like}}$, $bla_{\text{KPC-like}}$, and $bla_{\text{OXA-48-like}}$ and further β-lactamase genes $bla_{\text{TEM-like}}$, $bla_{\text{SHV-like}}$, $bla_{\text{CTX-M-1-2-9group}}$, $bla_{\text{OXA1-2-9-10like}}$) was tested by PCR and sequencing using primers of previous studies [16–19]. Analysis of the upstream-located genetic environment of the $bla_{\text{OXA-48}}$ genes and additional identification of

plasmid-located genes contributing to fluoroquinolone resistance (*qnrA/B/S-like*, *aac(6')1b-cr*) was performed, as previously described [18–22].

Transfer of resistance was tested by broth mating assays using a sodium azide-resistant *Escherichia coli* J53 recipient [23] and selective Luria-Bertani agar plates containing 100 mg/L ampicillin and 200 mg/L sodium azide. Plasmid DNA of clinical isolates and transconjugants was isolated using S1 nuclease restriction followed by pulsed-field gel electrophoresis (PFGE) [24]. In addition, genetic relationship of all isolates was analyzed by PFGE using XbaI-restricted whole genomic DNA with interpretation of the results according to Tenover et al. [25]. PCR-based assay for determination of the phylogenetic group was performed on *E. coli* isolates [26], and for selected *K. pneumoniae* isolates multi-locus sequence typing (MLST) and PCR-based determination of the capsular type was done, respectively [27, 28].

Statistical analyses

STATA IC 11.1 (Statacorp, College Station TX, USA) was used for statistic calculations. p values < 0.05 were considered to be significant. Due to small sample sizes, Fisher's exact test (STATA command "tabulate" with the option "exact") was performed to compare dichotomous variables. The alternative hypotheses are one-sided, as we are interested in the evidence as to whether an exposition may be a risk factor for CKP colonization, but not whether it could be a protective factor. For categorical data with more than two values, the p value for Kendall's tau-b correlation coefficient (STATA command "ktau") was employed as a non-parametric test with appropriate correction for ties. This correction was inevitable as the variables "age" and "BI" contain several ties.

Results

Within the observation period between 17th October 2014 and 12th January 2015, a total of 53 patients were treated at the ward. The median age of the patients was 75 years, 34 patients (64.1%) were male and 19 patients (35.9%) were female. Systematic screening for CKP carriage was initiated on 12th January 2015 and patients 1–7 were colonized with the bacteria. After the cutoff date, no transmission of CKP was noticed. Neither of the colonized patients developed a CKP infection or died within the observation period. Most likely, the bacteria were isolated at earlier points in time from patients 1, 3, and 6. These patients exhibited *K. pneumoniae*, showing similar antibiotic resistance patterns to CKP, including intermediate susceptibility to ertapenem but were apparently susceptible to meropenem and imipenem. However, at that time, the bacteria were not identified as CKP, since a corresponding expert system had not been implied and tests for carbapenemase detection (Hodge test, PCR) were not performed.

Apart from patients 1–7, *K. pneumoniae* were isolated from patients 11, 36–40, and 43 but none of those isolates showed reduced susceptibility to carbapenems and/or third generation cephalosporines. Aside from patient 13, who was colonized with *Escherichia coli* ESBL, no further Gram-negative ESBL secreting bacteria were detected, while patients 17, 24, 40, 45, and 46 were colonized with methicillin-resistant *Staphylococcus aureus* (MRSA).

From 23 patients residing on the ward within the observation period, at least one rectal swab had been examined for the presence of CKP. Consequently, further analysis of risks factors for CKP colonization (residence with a CKP-colonized roommate, antibiotic treatment, age, gender, BI) was restricted to these patients. Patients 1–7 were CKP positive, patients 8–23 were CKP negative. All CKP-colonized patients received antibiotics (100%). Antibiotics and duration of individual antibiotic treatment are shown in Table S1 and Fig. S2. Five CKP-colonized patients (71.4%) resided in a room with at least a further CKP-colonized patient; BI at admission was 15. From the 16 non-colonized patients, seven received antibiotics (43.8%) and 12 stayed in a room together with at least a CKP-colonized patient (75.0%), and the BI was 20. The proportion of non-colonized patients receiving antibiotics (7 out of 16) was significantly lower than that of CKP-colonized patients (7 out of 7) in one-sided Fisher's exact test ($p < 0.02$), while age, sex, and BI did not show any significant association with CKP colonization in univariate tests. Interestingly, BI of patients 1–14 was significantly lower (BI = 15) than BI of patients 15–23 (BI = 40) (Kendall's tau-b correlation coefficient—0.40; $p = 0.03$). In contrast to patients 15–23, patients 1–14 had been treated with antibiotics suggesting that low BI may indicate the need for antibiotic treatment during hospital stay.

Individual characteristics of patients 1–23 are summarized in Table 1. The proportion of screening swaps exhibiting growth of CKP varied between CKP-colonized patients. This variation might be indicative of a different bacterial load among the patients. E.g., 10 of 11 rectal swabs from patients 1 showed growth of CKP and CKP was isolated from the throat of this patient, suggesting a systemic colonization with relative high load of CKP. By contrast, only 2 of 8 swabs taken from patients 2 and 4 resulted in growth of CKP, while no growth was obtained in microbiological samples from other body sites, indicating a lower CKP load of these patients than patient 1.

Figures 1 and 2S show the occupancy of patient rooms, antibiotic treatment of patients 1–23 and the temporal points when stool samples were submitted to the laboratory for *C. difficile* analysis, indicative of a diarrheal episode.

Table 1 Characteristics of patients analyzed in the present study

Patient no.	Gender	Age (years)	Rectal screening (*N*)	CRE (*N*)	Species	Date of first isolation	Other sites (*N*)	Diagnosis	BI
1	M	77	11	10	*K. pneumoniae*	2014-12-21	Throat (1)	Various injuries including multiple leg fractures after being hidden by a truck	40
				4	*E. coli*	2015-01-13	–		
				1	*S. marcescens*	2015-05-27*	–		
2	M	75	8	2	*K. pneumoniae*	2015-01-13	–	Stroke	0
3	F	85	5	5	*K. pneumoniae*	2014-12-23	Urine (3)	Fracture of femur	30
4	F	70	8	2	*K. pneumoniae*	2015-01-13	–	Guillain–Barré syndrome	15
5	M	86	6	4	*K. pneumoniae*	2015-01-12	Throat (1)	Bleeding of basal ganglia during rivaroxaban treatment	15
				1	*E. coli*	2015-01-22	Sputum (1)		
6	M	62	5	5	*K. pneumoniae*	2015-01-06	–	Spondylodiscitis	15
7	M	79	3	2	*K. pneumoniae*	2015-01-23	–	Cardiac and renal insufficiency, pneumonia	35
8	F	77	1	0	–	–	–	Stroke	5
9	M	71	1	0	–	–	–	Stroke after previous strokes	15
10	F	76	1	0	–	–	–	Stroke	5
11	F	74	3	0	–	–	–	Decubitus ulceration. Stroke previously	20
12	M	78	4	0	–	–	–	Stroke	25
13	M	90	6	0	–	–	–	Urethral bleeding, bladder cancer. Stroke previously	15
14	F	77	3	0	–	–	–	Stroke, pneumonia	10
15	M	68	3	0	–	–	–	Recurrent bleeding of basal ganglia, stroke	10
16	M	53	3	0	–	–	–	Various fractures of pelvis and rips after fall	40
17	M	56	2	0	–	–	–	Various fractures of pelvis after fall	35
18	M	41	6	0	–	–	–	Fractures of pelvis, femur, tibia and fibula	40
19	M	75	5	0	–	–	–	Fractures of pelvis and olecranon after fall	40
20	M	27	5	0	–	–	–	Fractures of patella and forearm bones after motorbike accident	40
21	F	83	7	0	–	–	–	Ischemia of basal ganglia, pneumonia	10
22	F	57	4	0	–	–	–	Ischemia of basal ganglia	20
23	F	89	5	0	–	–	–	Global heart failure and atrial fibrillation	55

BI Barthel index at admission, *Rectal screening* number of rectal screening swabs taken, *CRE* (*N*) number of rectal swabs showing growth of CRE, *Other Sites* body sites other than rectum exhibiting CKP, *Species* prevalence of CRE species, *CRE* Carbapenem-resistant enterobacteriaceae, *F* female, *M* male

*Until finding of CKP (patients 1–6) or discharge (patients 7–23). Carbapenem-resistant *S. marcescens* was isolated from patient 1 four months after the outbreak when the patient subsequently visited the hospital

Furthermore, the dates are given when CKP (arrow heads) and VRE (*X*) had been isolated at first. It seems probable that room 13 was the hot spot of CKP transmission, since five of seven CKP-colonized patients had stayed in this room. On the other hand, residence together with a CKP-colonized patient in the same room was not sufficient to cause CKP colonization because patients 9, 12, 13, 15, and 16 also stayed in room 13 but did not acquire CKP. Although it remains unknown who the index patient was, it seems likely that patients 1, 6, or 7 initiated the outbreak. There were two findings apparently linked to the spread of CKP between these patients: Between 22nd November and 28th November, each of them suffered from diarrhea. Moreover, patients 1, 6, 5, and 7 received antibiotics within this period or somewhat later. During this time, patient 4 also suffered from diarrhea and received antibiotics thereafter, possibly making her susceptible to CKP acquisition. Interestingly, at that time, patient 4 stayed in room 5 but not in room 13. In contrast to patient 4, patient 2 resided in room 13 for some days. As he was treated with two antibiotics while staying in this room neighboring room 13 during the "hot spot period", it seems more

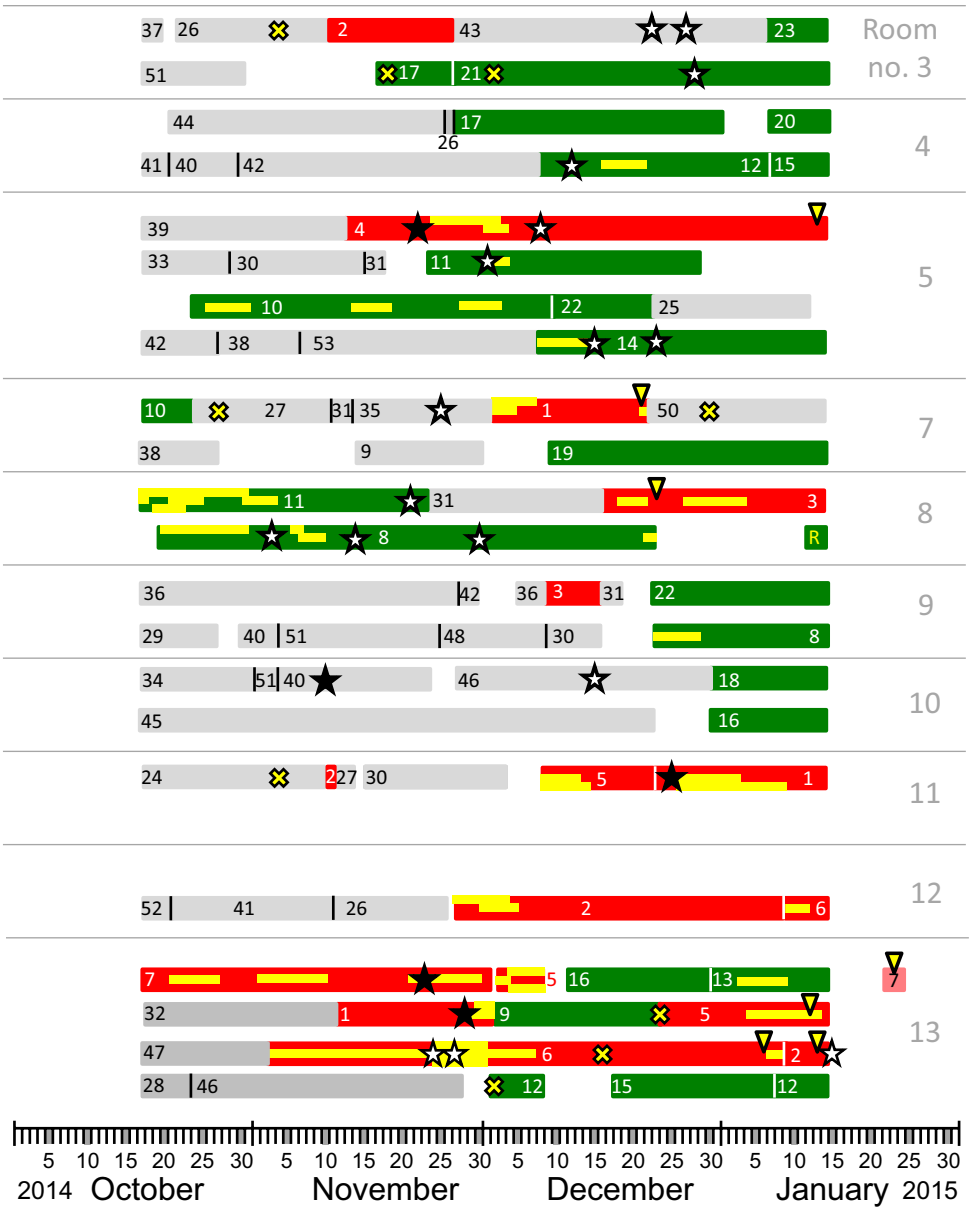

Fig. 1 Occupancy of patient rooms, antibiotic treatment and microbiological findings of patients at an early rehabilitation ward. Observation period was between 17th October 2014 and 12th January 2015. Red bars: Patients colonized with CKP (patients 1–7). Green bars: Patients not colonized with CKP (patients 8–23). Gray bars: Unknown CKP colonization status (patients 24–50). Small yellow bars: Antibiotic treatment. Each yellow bar represents an antibiotic. Arrow heads: First isolation of CKP. CKP of patient 7 were discovered after the patient had been readmitted to the hospital. Stars: Patients examined for *C. difficile* infection. Solid black stars: *C. difficile* toxin positive, stars with white filling: *C. difficile* toxin negative. *X* = First isolation of VRE. *R* = Daughter of patient 2 performing rooming-in

probable that CKP was acquired within this time frame, rather than during his residence in room 13.

Only one contact plate exhibited growth of CKP. That plate had been taken from the tray of a wheel chair in front of patient 6. The other 73 contact plates exhibited no growth of CKP.

Two CKP-colonized (patients 5, 6) and three non-colonized patients (patients 12, 17, 21) were additionally colonized with VRE. Furthermore, VRE were isolated from patients 24, 26, 27, and 50.

All CKP were resistant to piperacillin, piperacillin/tazobactam, cefotaxime, ceftazidime, ciprofloxacin, moxifloxacin, and ertapenem, while MIC for imipenem and meropenem (0.25–2 mg/L) was slightly increased, but still in the range of susceptible according to the current EUCAST breakpoint tables. The modified Hodge test confirmed carbapenemase production. All CKP remained susceptible to tigecycline, colistin, fosfomycin and sulfamethoxazole/trimethoprim (Table S2).

XbaI-macrorestriction patterns of the CKP isolates were identical (Table S2, Fig. S3), confirming the presence of the same clone in all colonized patients. By MLST analysis, this clone could be assigned to the new sequence type (ST)2255 which differs from the known ST35 only by a single nucleotide polymorphism (SNP) within the gapA allele. Accordingly, capsular type *wzi*-37 K22.37 matched to that of *K. pneumoniae*-ST35 in the MLST database (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html).

Follow-up analyses revealed that patient 3 additionally carried a carbapenem-resistant *Serratia marcescens* strain

and a carbapenem-resistant *E. coli* strain while patient 6 was also colonized with another carbapenem-resistant *E. coli* strain (Table 1, Table S2, Fig. S3). Antibiotic susceptibilities of *E. coli* from patient 3 matched that of CKP while *E. coli* from patient 6 was susceptible to quinolones. Antibiotic resistance of *S. marcescens* was restricted to piperacillin, piperacillin/tazobactam, and ertapenem (Table S2).

PCR screening for resistance genes revealed the presence of carbapenemase gene $bla_{\text{OXA-48}}$ in all CKP as well as in both *E. coli* and the *S. marcescens* isolates. The CKP-ST2255 clone and the two *E. coli* clones with phylogenetic groups A and D and non-related XbaI-macrorestriction patterns harbored additionally ESBL gene $bla_{\text{CTX-M-15}}$ and further β-lactamase genes ($bla_{\text{SHV-33}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-9}}$), as well as genes contributing to fluoroquinolone resistance (*aac(6')Ib-cr*; *qnrB1*) and aminoglycoside resistance (*aacA4*) (Table S2). However, both *E. coli* lacked the $bla_{\text{SHV-33}}$ gene which might be chromosomally encoded in *K. pneumoniae*. Furthermore, in one *E. coli* the genes $bla_{\text{OXA-1}}$, *qnrB1* and *aac(6')Ib-cr* were not detected (Table S2).

Conjugation assays revealed randomly selected transconjugants carrying either single plasmids of variable size (95–110 kb) with ESBL gene $bla_{\text{CTX-M-15}}$ and further resistance genes or a plasmid (ca. 60 kb) harboring only the $bla_{\text{OXA-48}}$ gene (Table S3, Fig. S4). Transconjugants producing only OXA-48 were resistant to piperacillin and ertapenem. In contrast, transconjugants producing CTX-M-15 but not OXA-48 were carbapenem-susceptible but resistant to cefotaxime, ceftazidime, gentamicin, amikacin and ciprofloxacin.

Discussion

At a first glance, the results of our single center study suggest that antimicrobial treatment may be the predominant risk factor for acquisition of CKP. Besides antimicrobial treatment diarrhea appeared more often in patients with CKP, but this may be just a confounder especially in diarrhea from *Clostridium difficile*. We were surprised that the variable of staying in the same room did not show stronger impact on our analysis. This is not in line with current expert opinion exclusively focusing on infection control measures to prevent the spread of multidrug-resistant pathogens in hospitals [29].

Results of outbreak analysis suggest that room 13 had been a hot spot area for transmission of CKP. The finding that patients 9, 12, 13, 15, and 16 did not acquire CKP despite staying in this room with CKP-colonized patients 2, 5, and/or 6 indicates that residence in the same room is not sufficient for CKP acquisition. In total, six non-colonized patients (patient 9, 10, 11, 14, 15, 16) stayed for more than 20 days in a room with a CKP-colonized patient, suggesting that even long-term exposition is not alone sufficient to become colonized.

The correlation between antibiotic consumption and CKP acquisition was examined in previous studies, demonstrating that all or at least most CKP-colonized or infected patients were treated with antibiotics before CKP detection [11, 30–33]. However, in those studies the impact of antibiotic treatment was not compared to close spatial contacts. In some studies, consumption of carbapenems was a major risk factor for CKP acquisition [29, 33], whereas carbapenem consumption was not increased in another study [11]. Due to the small number of patients, we cannot assess whether carbapenem use results in an over proportional risk for CKP acquisition.

It seems probable that one of patients 1, 6, or 7 had been the index patients transmitting CKP to the others. Within a 7-day period, each of these patients suffered from diarrhea. During residence of non-colonized patients 9, 12, 13, 15, and 16 in room 13 no patient suffered from diarrhea, possibly contributing to the absence of CKP acquisition. However, from our data it is not clear whether diarrhea of a CKP-colonized patient is more supportive for CKP transmission or diarrhea of the possible recipient. As diarrhea is often induced by antibiotic treatment avoidance of unnecessary antibiosis might additionally restrict this risk factor for CKP acquisition.

Besides the five male patients, two female patients (patients 3, 4) were colonized with CKP. As room 13 was occupied only by male patients during the outbreak period, colonization of females obviously occurred outside of room 13. As described in the results, it is also likely that patient 2 acquired CKP outside hot spot room 13, indicating that residence in the same room is not essential for CKP acquisition.

In contrast to other studies [12, 34], only one sample taken from patients´ environment revealed growth of CKP, suggesting that contamination of surfaces inferiorly contributed to CKP transmission. We assume that transmission of CKP to patients 2, 3, and 4 was mediated by the hands of the staff. In comparison to other wards, there is more contact between patients and the staff at an early rehabilitation ward, facilitating above average bacterial spread when violating basic measures of hand hygiene. Therefore, the finding that no further transmissions occurred after the outbreak seems most likely to be due to consequent compliance of basic hygiene measures.

Beside antibiotic treatment, bacterial load also seems to have some influence on CKP transmission. Only two out of eight rectal swabs of patient 4 revealed proof of CKP, suggesting relative low bacterial load. Accordingly, patient 4 did not pass the bacteria to her roommates, although three of them received antibiotics and two had diarrheal episodes.

Apart from CKP, various patients were colonized with VRE. However, there was no common pattern of bacterial

transmission routes, suggesting that acquisition of VRE might be associated with risk factors other than acquisition of CKP.

In the present study, outbreak was caused by the novel strain *K. pneumoniae*-ST2255. ST2255 is related to ST35 which has been reported mainly as an ESBL-producing colonizer or agent of infections of patients in Tunisia, Spain, Denmark and France [35]. However, according to the MLST database, isolates belonging to this ST were also found in milk (USA) or slurry (China), indicating a widespread of this clonal lineage, and that the acquisition of resistance genes by transfer of conjugative plasmids might have facilitated their spread. In our study, we could confirm this kind of resistance transfer. One plasmid (ca. 60 kb) carrying carbapenemase gene $bla_{\text{OXA-48}}$ was found in the *K. pneumoniae*-ST2255 clone as well as in two *E. coli* and one *S. marcescens* strains that occurred as co-colonizers in two patients. The in vivo exchange of this carbapenemase gene carrying plasmid between the three different bacterial species is most likely. Another plasmid carrying ESBL and other resistance genes (e.g., $bla_{\text{CTX-M-15}}$ and *qnrB1*) was identical in size (ca. 110 kb) in all ST2255 isolates and one *E. coli* strain whereas the other *E. coli* strain carried a smaller plasmid (ca. 95 kb) with $bla_{\text{CTX-M-15}}$ but without additional fluoroquinolone resistance genes (Table S3; Fig. S2). In the latter case, sequence analyses of the whole plasmids are needed to elucidate their similarity.

Our study has several limitations. This is a single center study with a small cohort and the results have to be interpreted with caution. As CKP colonization of most patients was discovered after initiating rectal screening, it is not clear when colonization happened in real and, therefore, true transmissions routes remain speculative. Furthermore, there are various factors possibly disguising patient to patient transmissions. (1) Many patients had missing colonization status. (2) Many room changes happened within the observation period. (3) For analysis of residence together with a CKP-colonized patient, only the rooms in which the patients had stayed the major part of the corresponding day were recorded. Therefore, short contacts to CKP-colonized patients might have been neglected.

Furthermore, it is not clear who the index patient was. Initially, CKP was isolated from patient 1 but it is doubtful that this patient was the index patient. Patient 1 was admitted to our clinic because of a traffic accident. In the years before admission, he lived independently in his own apartment and rarely stayed in hospitals. By contrast, patient 6 lived in a nursing home for many years because of cognitive deficits. Therefore, it would have been interesting whether his roommate had also been colonized by CKP. However, local health authorities denied examination for CKP carriage of the roommate. In addition, our findings may not be generalized without further studies on further multidrug-resistant pathogens in other hospital settings. The database is small and, therefore, we did not more in-depth investigate the influence of duration of therapy, type of antibiotic substance and other factors. We could also not use multivariate tests to control for confounders.

Altogether, small figures, univariate tests, and typical biases of an observational study that cannot be controlled inevitably leave considerable uncertainty. In addition, it gives the puzzling impression that isolation procedures might have a lesser impact than antibiotic treatment on cross transmission. This raises questions about treatment procedures, as isolation aims to be a perfect cut in transmission. But it is commonly believed in practice that this assumption does not hold true. The fact that the outbreak was soon limited after noticing its presence indicates that basic hygiene measures were consistently maintained by the staff after its perusal. Therefore, gloves, gowns, mouth masks and single rooms cannot compensate totally for any remaining deficits in basic hygienic procedures, such as hand hygiene. Despite the limitations of our small observational study, it deserves reporting and discussion.

Despite the fact that our evidence for an association between CKP colonization and antibiotic treatment is weak (small figures, univariate test), it is worth reporting. Fortunately, most outbreaks of newly introduced multiresistant bacteria can be controlled early on and, therefore, occasions to study this association in real outbreaks with large figures are rare. Although the evidence may be weak, what has been uncovered through research may be a call to reconsider our priorities in outbreak control of nosocomial infections and study the impact of antibiotics more in depth.

In conclusion, we recommend paying more attention to the association of multi-resistant pathogens and antimicrobial use in future outbreaks. If our assertion holds true, antimicrobial stewardship should be emphasised even more in infection control.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical standards The study does not contain clinical studies or patient data allowing conclusion to patients' identity or individual clinical course. According to the ethics committee of the Bavarian medical association (Bayerische Landesärztekammer, http://www.blaek.de), having jurisdiction over medical matters in our state (Federal State Bavaria), ethics approval is not necessary.

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