

## Colonization with third-generation cephalosporin-resistant Enterobacteriaceae on hospital admission: prevalence and risk factors

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Received 16 December 2015; returned 25 January 2016; revised 22 April 2016; accepted 9 May 2016

**Objectives:** The objectives of this study were to prospectively assess the rectal carriage rate of third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) in non-ICU patients on hospital admission and to investigate resistance mechanisms and risk factors for carriage.

**Methods:** Adult patients were screened for 3GCREB carriage at six German tertiary care hospitals in 2014 using rectal swabs or stool samples. 3GCREB isolates were characterized by phenotypic and molecular methods. Each patient answered a questionnaire about potential risk factors for colonization with MDR organisms (MDROs). Univariable and multivariable risk factor analyses were performed to identify factors associated with 3GCREB carriage.

**Results:** Of 4376 patients, 416 (9.5%) were 3GCREB carriers. *Escherichia coli* was the predominant species (79.1%). ESBLs of the CTX-M-1 group (67.3%) and the CTX-M-9 group (16.8%) were the most frequent  $\beta$ -lactamases. Five patients (0.11%) were colonized with carbapenemase-producing Enterobacteriaceae. The following risk factors were significantly associated with 3GCREB colonization in the multivariable analysis ( $P < 0.05$ ): centre; previous MDRO colonization (OR = 2.12); antibiotic use within the previous 6 months (OR = 2.09); travel outside Europe (OR = 2.24); stay in a long-term care facility (OR = 1.33); and treatment of gastroesophageal reflux disease (GERD) (OR = 1.22).

**Conclusions:** To our knowledge, this is the largest admission prevalence study of 3GCREB in Europe. The observed prevalence of 9.5% 3GCREB carriage was higher than previously reported and differed significantly among centres. In addition to previously identified risk factors, the treatment of GERD proved to be an independent risk factor for 3GCREB colonization.

### Introduction

Third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) have disseminated globally in recent years. Among 3GCREB, isolates producing ESBLs are involved in most cases of colonization and infection worldwide.<sup>1</sup> First reported in the 1980s in Europe as derivatives of narrow-spectrum  $\beta$ -lactamases (TEM/SHV type), ESBLs are now dominated by CTX-M types.<sup>2</sup> Apart

from ESBLs, resistance to third-generation cephalosporins can also be caused by production of the AmpC  $\beta$ -lactamase, especially in *Enterobacter*, *Citrobacter*, *Morganella* and *Serratia* spp.

Infection with 3GCREB frequently results in inappropriate antimicrobial therapy and may compromise treatment outcome. While the impact of inappropriate therapy on mortality remains a controversial issue, it is significantly associated with increased

length of stay and hospital costs.<sup>3–5</sup> 3GCREB colonization of the gut is the reservoir for infections with these organisms.<sup>2</sup> Colonization rates differ greatly between countries and continents, with a lower prevalence in Europe and much higher rates in Africa or South-East Asia, reaching up to 69.3% in healthy volunteers in Thailand.<sup>2,6–8</sup> Documented risk factors for colonization with 3GCREB are hospitalization within the previous 12 months, prior antimicrobial treatment, travel to regions with 3GCREB endemicity (e.g. India and South-East Asia) or eating pork.<sup>9–11</sup>

Currently, few data exist on the prevalence of and risk factors for 3GCREB carriage in Germany. As part of the multicentre Antibiotic Therapy Optimisation Study (ATHOS), admission screening for 3GCREB carriage was performed at six German tertiary care hospitals in 2014 and risk factors for colonization were investigated.

## Methods

### Participants and setting

This prospective prevalence survey was conducted at six large tertiary care hospitals, covering the north (Centre 6), west (Centre 2), east (Centre 1), south-west (Centres 4 and 5) and south-east (Centre 3) of Germany. Centres had between 1300 and 3200 inpatient beds. Patients aged  $\geq 18$  years from general wards that had been admitted between June and December 2014 were included in the study. Patients from ICUs, dermatology, obstetrics, ophthalmology, otorhinolaryngology and psychiatry were excluded.

### Ethics

The study was approved by the institutional ethics committees (approval number EA4/018/14). Patients were required to give informed consent prior to enrolment in the study.

### Collection of samples and patient data

Patients were screened for 3GCREB colonization within 3 days of admission to allow for inclusion of patients admitted on weekends. Each patient was asked to answer a short questionnaire (available as Supplementary data at JAC Online) on potential risk factors for colonization with MDR organisms (MDROs). Further potential risk factors included in the questionnaire were sex, age, current antibiotic treatment, animal contact and previous colonization with any MDRO (MRSA, 3GCREB, carbapenem-resistant Enterobacteriaceae and VRE). In addition, the following risk factors in the 6 months prior to admission were recorded: previous antibiotic therapy; travel abroad; stay at a rehabilitation centre; stay at a long-term care facility (LTCF); hospitalization; and medical management of gastroesophageal reflux disease (GERD).

### Phenotypic detection of 3GCREB carriers

Stool or rectal swabs (with Amies transport medium) were used for the screening of patients for 3GCREB. Samples were plated onto ChromID ESBL agar (bioMérieux, Nürtingen, Germany). Species identification of isolates growing on ESBL agar was performed using MALDI-TOF MS or the Vitek 2 GN ID card (bioMérieux). Susceptibility testing was carried out using Vitek 2 (bioMérieux). All isolates that were non-susceptible to cefotaxime, ceftriaxone or ceftazidime according to EUCAST breakpoints were included in the study and further characterized.

Phenotypic detection of ESBL production was performed with the combination disc test as recommended by EUCAST, using cefotaxime, ceftazidime and cefepime  $\pm$  clavulanate (Mast Diagnostica, Reinfeld, Germany).<sup>12</sup> All isolates were tested for AmpC production by the cefoxitin/cloxacillin disc test (Liofilchem, Roseto degli Abruzzi, Italy) as previously described.<sup>13</sup>

### Molecular characterization of isolates

All isolates were tested by PCR for *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> groups *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub> and *bla*<sub>CTX-M-9</sub> as previously reported.<sup>14</sup> Isolates that were negative for *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub> and *bla*<sub>CTX-M-9</sub> group but phenotypically ESBL positive were additionally tested for the presence of *bla*<sub>CTX-M-8/25</sub>, since CTX-M-8 has been described previously to be increasingly frequent in Germany.<sup>15</sup> *Escherichia coli* isolates that were identified phenotypically as AmpC positive were investigated for the presence of plasmid-mediated AmpCs as previously described.<sup>16</sup> All isolates with meropenem MICs  $>0.25$  mg/L were analysed for the presence of carbapenemase genes *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>OXA-48</sub> and *bla*<sub>NDM</sub> by multiplex PCR.<sup>17</sup>

### Statistical analysis

The prevalence rate of 3GCREB on admission was expressed as the number of patients positive for 3GCREB per 100 patients admitted. In the descriptive analysis, numbers and percentages were calculated; differences were tested using the  $\chi^2$  test. To evaluate independent risk factors for colonization on admission, univariable and multivariable regression analyses were performed. Since observations within a hospital (and/or within a region) are not statistically independent due to the patient population (especially the prevalence in the region), adjusted ORs with 95% CIs were calculated. They were based on generalized estimating equation models, which account for this clustering effect by using an exchangeable correlation structure. Parameters considered in the analysis were: centre (six different centres); age ( $\leq 45$ , 46–55, 56–65, 66–75 or  $>75$  years); sex (male/female); prior MDRO carriage; antibiotic use during the previous 6 months; travel abroad during the previous 6 months within or outside Europe; stay at a rehabilitation centre or LTCF in the previous 6 months; hospital stay in the previous 6 months in Germany, in a European country outside Germany or outside Europe; occupational or private animal contact; and treatment of GERD with antacids or proton-pump inhibitors during the last 6 months. Recorded parameters were categorized as no (reference), yes or unknown. In one hospital, hospital stay in the previous 6 months was answered incorrectly—only hospital stay within Europe but outside Germany and hospital stay outside Europe were answered; therefore, the information on hospital stay within Germany was set to unknown. In the multivariable analysis, the model building strategy was performed stepwise backward, significance level for excluding a parameter from the model was  $P=0.05$ . For epidemiological reasons, age and sex were included in all models.  $P$  values  $<0.05$  were considered significant. All analyses were performed using SPSS 22 (IBM SPSS Statistics, Somers, NY, USA) and SAS 9.3 (SAS Institute, Cary, NC, USA).

## Results

Overall, 4376 patients were enrolled. Of these, 416 patients were 3GCREB carriers, resulting in an admission prevalence of 9.5%. Of note, there was no difference in 3GCREB prevalence among patients screened within the first 2 days and those screened on day 3 of admission (data not shown).

Nineteen patients (0.4%) were colonized with two different 3GCREB strains, thus 435 isolates were phenotypically identified. The patients' median age was 62 years (IQR 49–73 years) and 49.2% were female (Table 1).

### Phenotypical and molecular epidemiology of 3GCREB isolates

*E. coli* was the most frequent 3GCREB species detected at all six centres, accounting for 344 of 435 isolates (79.1%), followed by *Klebsiella pneumoniae* (37 isolates, 8.5%), *Enterobacter* spp.

**Table 1.** Descriptive statistics of demographic patient data

Patient demographics	3GCREB negative, N=3960		3GCREB positive, N=416		3GCREB prevalence (%), overall=9.5%	P <sup>a</sup>
	n	%	n	%		
<b>Hospital</b>						
Centre 1	1770	44.7	224	53.8	11.23	<0.001
Centre 2	441	11.1	59	14.2	11.80	
Centre 3	469	11.8	31	7.5	6.20	
Centre 4	462	11.7	41	9.9	8.15	
Centre 5	369	9.3	37	8.9	9.11	
Centre 6	449	11.3	24	5.8	5.07	
<b>Age (years)</b>						
≤45	751	19.0	88	21.2	10.48	0.636
46–55	727	18.4	65	15.6	8.21	
56–65	801	20.2	83	20.0	9.39	
66–75	991	25.0	106	25.5	9.66	
>75	690	17.4	74	17.8	9.69	
<b>Gender</b>						
male	2001	50.5	222	53.4	9.98	0.273
female	1959	49.5	194	46.6	9.01	

<sup>a</sup>P value was obtained by using a  $\chi^2$  test and accounts for the whole group, e.g. Centre 1–6.

**Table 2.** Concomitant susceptibility/resistance to ciprofloxacin and meropenem among Enterobacteriaceae with resistance to third-generation cephalosporins

Species	3GCREB+CIP S+MEM	3GCREB+CIP I/R+MEM S,	MEM I/R,	Total, n (%)
	S, n (%)	n (%)	n (%)	
<i>E. coli</i>	191 (43.9)	153 (35.2)	0	344 (79.1)
<i>K. pneumoniae</i>	15 (3.4)	20 (4.6)	2 (0.5)	37 (8.5)
<i>Klebsiella oxytoca</i>	2 (0.5)	1 (0.2)	1 (0.2)	4 (0.9)
<i>Enterobacter</i> spp.	21 (4.8)	3 (0.7)	1 (0.2)	25 (5.7)
<i>Citrobacter</i> spp.	18 (4.1)	1 (0.2)	2 (0.5)	21 (4.8)
<i>Hafnia alvei</i>	2 (0.5)	1 (0.2)	0	3 (0.7)
<i>Proteus mirabilis</i>	1 (0.2)	0	0	1 (0.2)
	250 (57.5)	179 (41.1)	6 (1.4)	435 (100.0)

CIP, ciprofloxacin; MEM, meropenem; I, intermediate; R, resistant; S, susceptible.

(25, 5.7%) and *Citrobacter* spp. (21, 4.8%). These findings are summarized in Table 2. Of all 3GCREB isolates, 41.1% were resistant to fluoroquinolones.

Among the 435 phenotypically characterized isolates, 410 (94.3%) were available for molecular analysis of underlying resistance mechanisms. ESBL production was the predominant resistance mechanism and was detected in 370/410 isolates (90.2%), with some isolates carrying a combination of two

**Table 3.** Distribution of  $\beta$ -lactamases among 3GCREB isolates

	E. coli, N=335		K. pneumoniae, N=36		Enterobacter spp., N=19		Citrobacter spp., N=14		H. alvei, N=3		K. oxytoca, N=2		P. mirabilis, N=1		Total, N=410	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
CTX-M-1 group <sup>a</sup>	243	72.5	26	72.2	2	10.5	1	7.1	1	33.3	2	100	1	100	276	67.3
CTX-M-2 group <sup>b</sup>	1	0.3													1	0.2
CTX-M-8	3	0.9													3	0.7
CTX-M-9 group <sup>c</sup>	63	18.8	5	13.9	1	5.3									69	16.8
SHV ESBL	14	4.2	6	16.7	1	5.3					1	50			22	5.4
TEM ESBL	4	1.2													4	1.0
AmpC	7	2.1	1	2.8	18	94.7	11	78.6	3	100					39	9.5
VIM-1					1	5.3	1	7.1			1	50			3	0.7
NDM-1															1	0.2
IMP-8															1	0.2
Others <sup>d</sup>	2	0.6			1	5.3	1	7.1							3	0.7

<sup>a</sup>CTX-M-1 group: CTX-M-1, CTX-M-15, CTX-M-3, etc.

<sup>b</sup>CTX-M-2 group: CTX-M-2, CTX-M-4, CTX-M-6, etc.

<sup>c</sup>CTX-M-9 group: CTX-M-9, CTX-M-14, CTX-M-27, etc.

<sup>d</sup>Others: non-susceptibility to third-generation cephalosporins by other mechanisms (e.g. hyperproduction of narrow-spectrum  $\beta$ -lactamases).

or three  $\beta$ -lactamases (e.g. CTX-M-type ESBL + SHV ESBL or ESBL + carbapenemase). Among 3GCREB, the CTX-M-1 group was most frequently detected (276/410; 67.3%) followed by CTX-M-9 group (69/410; 16.8%), SHV ESBLs (22/410; 5.4%) and AmpC (39/410; 9.5%) (Table 3). Among seven *E. coli* isolates that were phenotypically AmpC positive, a plasmid-mediated AmpC was detected in six isolates (CMY-2 and CMY-42 in three isolates each). One *E. coli* isolate was negative for plasmid-mediated AmpC genes and AmpC production was likely a result of mutations in the promoter/attenuator region of the AmpC gene. A combination of ESBL and AmpC production was found in five isolates (1.2%).

Three isolates were phenotypically ESBL and AmpC negative and no ESBL gene was detected by PCR. All isolates possessed narrow-spectrum TEM  $\beta$ -lactamases; non-susceptibility to third-generation cephalosporins in these isolates was likely caused by other mechanisms such as a combination of hyperproduction of narrow-spectrum  $\beta$ -lactamases  $\pm$  porin loss.

Six isolates had elevated carbapenem MICs. Of these, five isolates (1.2% of all 3GCREB) produced a carbapenemase (VIM-1, three isolates, and NDM-1 and IMP-8, one isolate each; Table 3). In one ESBL-producing *K. pneumoniae* isolate with slightly elevated imipenem MIC, no carbapenemase was detected and carbapenem non-susceptibility was most likely caused by the combination of ESBL and porin loss.

There were marked differences observed between centres, with 3GCREB carriage rates ranging from 5.1% in Centre 6 to 11.8% in Centre 2 (Table 1). No statistically significant differences

were observed between centres for the various  $\beta$ -lactamase groups (data not shown).

### Risk factor analysis

Descriptive statistics revealed that patients with 3GCREB colonization significantly more often received antibiotic therapy (52.9% versus 33.7%,  $P < 0.001$ ), had prior MDRO carriage (9.4% versus 4.1%,  $P < 0.001$ ), were admitted to a German hospital (31.5% versus 25.1%,  $P < 0.001$ ), travelled outside Europe (13.5% versus 6.8%,  $P < 0.001$ ) and received treatment for GERD with antacids or proton-pump inhibitors within the previous 6 months (44.7% versus 36.7%,  $P = 0.003$ ). The complete descriptive statistics are summarized in Table S1.

Risk factors for 3GCREB colonization in the univariable analysis that remained significantly associated in the final multivariable model included: admission at Centre 1 (OR = 2.17, 95% CI = 2.09–2.25), Centre 2 (OR = 2.25, 95% CI = 2.08–2.44), Centre 3 (OR = 1.10, 95% CI = 1.03–1.18), Centre 4 (OR = 1.44, 95% CI = 1.36–1.52) or Centre 5 (OR = 1.63, 95% CI = 1.45–1.85) compared with the centre with the lowest prevalence; prior MDRO colonization (OR = 2.12, 95% CI = 1.72–2.60); prior antimicrobial treatment (OR = 2.09, 95% CI = 1.84–2.39); travel outside Europe (OR = 2.24, 95% CI = 1.90–2.64); stay in an LTCF (OR = 1.33, 95% CI = 1.04–1.70); and medical treatment of GERD (OR = 1.22, 95% CI = 1.07–1.40) within the previous 6 months (Table 4). In addition, age between 46 and 55 years was significantly associated with a reduced risk of 3GCREB colonization (OR = 0.74, 95% CI = 0.58–0.94) compared with patients older than 75 years. The complete multivariable analysis is available in Table S2.

**Table 4.** Risk factors for 3GCREB carriage assessed by multivariable analysis; for calculation of the risk factor ORs the answer 'no' was set as 1 (reference)

Parameter	Category	OR	95% CI	P
Age (years)	$\leq 45$	0.96	0.71–1.31	0.806
	46–55	0.74	0.58–0.94	0.013
	56–65	0.87	0.66–1.13	0.294
	66–75	0.90	0.64–1.27	0.543
	>75	1		
Gender	male	1.17	1.00–1.38	0.056
	female	1		
Previous MDRO colonization/infection	yes	2.12	1.72–2.60	<0.001
Antibiotic use <sup>a</sup>	yes	2.09	1.84–2.39	<0.001
Travel outside Europe <sup>a</sup>	yes	2.24	1.90–2.64	<0.001
Stay in LTCF <sup>a</sup>	yes	1.33	1.04–1.70	0.024
Treatment of GERD <sup>a</sup>	yes	1.22	1.07–1.40	0.003
Hospital	Centre 1	2.17	2.09–2.25	<0.001
	Centre 2	2.25	2.08–2.44	<0.001
	Centre 3	1.10	1.03–1.18	0.007
	Centre 4	1.44	1.36–1.52	<0.001
	Centre 5	1.63	1.45–1.85	<0.001
	Centre 6	1		

<sup>a</sup>In the previous 6 months.

### Discussion

To the best of our knowledge, our study represents the largest study on the prevalence of 3GCREB carriage on hospital admission in Europe, including 4376 patients admitted to six tertiary care hospitals representing different regions of Germany. The admission prevalence of 3GCREB carriage was 9.5% in patients admitted to a general ward. Currently, few data are available on 3GCREB carriage on hospital admission and many studies have focused on *E. coli* or ESBL-producing Enterobacteriaceae only, not taking into account other resistance mechanisms.

Valenza et al.<sup>18</sup> reported a prevalence of 6.3% ESBL-producing *E. coli* among 3344 healthy volunteers in Germany who were in close contact with patients with diarrhoea and screened for faecal carriage of intestinal bacterial pathogens and ESBL-producing *E. coli*. Similarly, 329 of 4376 patients (7.5%) were colonized by ESBL-producing *E. coli* in our study.

The prevalence of 3GCREB recorded in the present study among patients from regular wards is similar to that reported among ICU patients in Germany. A case-control study including 1706 patients from 15 ICUs in a German university hospital reported a prevalence of 3GCREB carriage of 9.6%.<sup>19</sup> In a Swiss study investigating 235 patients transferred from a hospital outside Switzerland or in a high-prevalence region of Switzerland, a prevalence of 17.9% was reported for Gram-negative MDRO carriage.<sup>20</sup> A recent study including 1351 patients in the Netherlands reported an ESBL prevalence of 8.2% on admission.<sup>7</sup> In contrast, a higher prevalence has been observed in studies from Singapore or Israel (12.4% and 10.7%, respectively).<sup>21,22</sup>

The predominant species found in our study was *E. coli*, followed by *K. pneumoniae*, *Enterobacter* spp. and *Citrobacter* spp. Confirming previous studies, ESBL-producing *E. coli* were detected much more frequently on admission than ESBL-producing *K. pneumoniae*.<sup>7,22</sup>

Resistance to third-generation cephalosporins was most commonly caused by ESBLs of the CTX-M-1 group in the present study (67.3% of all 3GCREB), followed by ESBLs of the CTX-M-9 group (16.8%). When comparing our data with other studies from Germany investigating only *E. coli*, the prevalence of the CTX-M-1 group among *E. coli* ESBLs was similar (72.8% in the present study, compared with 75.5% in the study by Valenza *et al.*<sup>18</sup> or 75.3% in the study by Leistner *et al.*<sup>10</sup>). This underscores the importance of the CTX-M-1 group ESBLs, outnumbering all other  $\beta$ -lactamases in Germany and in many other countries.<sup>7,23</sup>

The admission prevalence of carbapenem-resistant Enterobacteriaceae was low at 0.11%. Interestingly, VIM-1 was the most frequently detected carbapenemase in our study. This contrasts with most other studies, which report OXA-48 to be the most frequent carbapenemase among Enterobacteriaceae in Germany and the neighbouring countries Belgium, France and the Netherlands.<sup>24,25</sup> This difference might be explained by a different study design, as most of the currently available data stem from pre-selected carbapenem-resistant clinical isolates, mainly of nosocomial origin sent to national reference laboratories. Therefore, these data might not be representative for the general population.

There were significant regional differences in 3GCREB prevalence. Patients admitted to hospitals in the east or west of Germany (Centres 1 and 2) had a significantly higher rate of 3GCREB colonization as compared with patients admitted to a hospital in the north, south-east or south-west. A possible explanation could be differences in food intake. Leistner *et al.*<sup>10</sup> identified the frequent consumption of pork as an independent risk factor for colonization with ESBL *E. coli*. Evidence of dissemination of MDR Enterobacteriaceae via the food chain has been shown previously.<sup>8,11,26</sup> Another reason for the higher prevalence in east and west German centres could be the population structure in these areas. The centres are located in areas that have a higher proportion of inhabitants originating from east and south European countries and from Asian countries, where the ESBL prevalence is higher.<sup>2,27</sup> Further evidence for higher ESBL carriage rates in selected populations comes from another study, where ESBL *E. coli* was more commonly found in patients with an Asian mother tongue.<sup>10</sup>

In the present study, we did not obtain information on the patients' dietary habits and their nationality or ethnicity. Thus, the reasons for the observed regional differences could not be clearly elucidated and require further study.

Previous antibiotic therapy was an independent risk factor for 3GCREB colonization in our study. This finding is in concordance with the results of several studies in which the development of 3GCREB colonization after antibiotic treatment especially with third-generation cephalosporins was demonstrated.<sup>19,22,28,29</sup> With our questionnaire, we did not obtain information on any specific antimicrobial class or compound that the patients had taken within the previous 6 months, assuming that most of them would not be able to recall the antimicrobial class. Therefore, we could not analyse the association of 3GCREB prevalence with prior antimicrobial treatment with different drug classes.

In the present study, travel outside Europe within the previous 6 months was also an independent risk factor for colonization with 3GCREB. This concurs with investigations demonstrating international travel as a possible means of spreading ESBL-producing Enterobacteriaceae, mainly ESBL *E. coli*, from high- to low-prevalence countries through healthy travellers.<sup>30,31</sup> Kuenzli *et al.*<sup>9</sup> recently demonstrated that colonization rates with 3GCREB were especially high in travellers returning from South Asia, ranging from 34.7% in those visiting Sri Lanka to 86.8% in those returning from India. As a clinical consequence, recent travel of patients presenting with an infection should always be considered when choosing empirical antibiotic treatment.

Another independent risk factor for 3GCREB carriage in our study was prior stay in an LTCF within 6 months before admission. Various studies on the prevalence of MRSA in LTCFs in Germany and other European countries have been published, but few data exist on the current prevalence of 3GCREB in LTCFs in Germany. In a point prevalence study to assess carriage rates of ESBL-producing Enterobacteriaceae in LTCF residents in the German Rhine-Main region, 455 residents were screened for rectal carriage and MDROs were detected in 17.8% of patients.<sup>32</sup>

The medical management of GERD with proton-pump inhibitors or antacids was also an independent risk factor for 3GCREB carriage in our study. These drugs can promote enterobacterial overgrowth in the upper gastrointestinal tract by raising the gastric pH.<sup>33</sup> Interestingly, it has been demonstrated previously that treatment with H<sub>2</sub> blockers in neonates was significantly associated with carriage of ESBL-producing Enterobacteriaceae.<sup>34</sup>

Even though five risk factors could be identified in the present study, further studies are needed to establish if a risk factor-based screening approach is reasonable for the detection of MDR Gram-negative organisms. In the present study, 79.3% of all patients colonized with 3GCREB were positive for at least one of the five significant risk factors, but also 61.7% of all patients not colonized with 3GCREB.

Our study has several limitations. It was performed in large tertiary care facilities and thus generalizability of our results to the general hospital patient population may be limited. The study design did not allow follow-up evaluation or discharge screening of enrolled patients. Another limitation may be the limited sensitivity of screening for 3GCREB using rectal swabs as compared with stool samples. However, rectal swabs have been used for practical reasons in most epidemiological studies, as they are easier to obtain. Furthermore, no pre-enrichment of samples was done, which has recently been shown to improve the sensitivity of screening for 3GCREB.<sup>35</sup> Another limitation of our study might be the screening agar used (ChromID ESBL). This agar has good sensitivity for the detection of ESBLs and has also been demonstrated to have higher sensitivity for the detection of carbapenemase producers than most carbapenem-containing agars.<sup>36</sup> However, it does not allow the detection of OXA-48 strains without ESBL expression. Most OXA-48-positive isolates coproduce an ESBL and will therefore be detected; however, we cannot exclude that occasional OXA-48 isolates without an ESBL were missed. Furthermore, some AmpC-positive isolates are also suppressed by this medium.<sup>37</sup>

In conclusion, a prevalence of 3GCREB carriage of 9.5% among patients on hospital admission was observed in this large multi-centre study, which was higher than previously reported in Germany. Colonization with 3GCREB was common among patients not considered 'high risk' and who therefore are usually

not screened on admission. Currently, there is no universal recommendation in Germany for screening patients for MDR Gram-negative bacteria and most hospitals screen only patients who report previous hospitalization abroad. In the multivariable analysis, hospital stay outside Europe was not significantly associated with an increased risk of 3GCREB colonization, so it might not be a good marker to initiate patient screening.

Given the diversity of risk factors and the high prevalence of 3GCREB colonization on admission, the results of the present study suggest that vertical prevention strategies, as currently employed in many institutions for MRSA, are unlikely to be effective for the prevention of 3GCREB transmission.<sup>38</sup> In contrast, since 'high-risk' patients cannot be identified easily, it might be more reasonable to reinforce horizontal prevention strategies such as hand hygiene and antimicrobial stewardship programmes.

## Acknowledgements

We would like to thank Ahmad Saleh and Olivia Käsgen for excellent technical assistance, Hanna Birkholz, Anne C. Boldt, Minh Trang Bui, Vera Ihle, Marina Kipnis, Nayana Martin and Andrea Pelzer for obtaining screening samples and Solvy Wolke for study assistance.

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## Funding

The study was supported by the German Center for Infection Research (DZIF).

## Transparency declarations

None to declare.

## Supplementary data

Table S1, Table S2 and the patient questionnaire are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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