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Intense pre-admission carriage and further acquisition of ESBL-producing Enterobacteriaceae among patients and their caregivers in a tertiary hospital in Rwanda

Mathis S. E. Kurz[1], Claude Bayingana[2], Jules M. Ndoli[2], Augustin Sendegeya[2], Anita Durst[1], Roland Pfüller[3], Jean Bosco Gahutu[2] and Frank P. Mockenhaupt[1]

1 *Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Berlin, Germany*
2 *University Teaching Hospital of Butare and School of Medicine and Pharmacy, University of Rwanda, Butare, Rwanda*
3 *Medizinisch-Diagnostische Institute, Berlin, Germany*

Abstract

OBJECTIVES To assess the presence and risk factors of intestinal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) among patients admitted to the University Teaching Hospital of Butare and among their attending caregivers, and to analyse the acquisition of ESBL-PE carriage during hospital stay and associated factors.

METHODS We screened 392 patients and their attending caregivers at admission and discharge for ESBL-PE carriage. Bacterial species were determined using the API-20E system, and antimicrobial susceptibility testing was performed by agar disc diffusion. Data on socio-economic status, diet, behaviour, household assets, livestock and hospital procedures were collected.

RESULTS At admission, 50% of the patients showed intestinal ESBL-PE carriage (*Escherichia coli*, 51%; *Klebsiella pneumoniae*, 39%; *Enterobacter cloacae*, 19%) as did 37% of their caregivers. Co-resistance was common but no carbapenem resistance was detected. At discharge, the proportion of ESBL-PE-colonised patients increased to 65% (caregivers, 47%) with almost complete carriage in paediatric patients (93%). The acquisition rate among initially non-colonised patients was 55% (or, 71/1000 patient days). Independent predictors of admission carriage included a colonised caregiver, prior antibiotic intake, egg consumption and neglecting to boil drinking water, whereas being a paediatric patient, undergoing surgery and male gender predicted acquisition during hospitalisation.

CONCLUSIONS Abundant admission carriage of ESBL-PE and a high acquisition rate in a Rwandan university hospital point to potential intrahospital transmission and community dissemination. Caregivers are an additional source of possible spread. Risk factors of colonisation such as diet and water source need to be tackled to prevent the further emergence and spread of ESBL-PE.

keywords ESBL, Rwanda, hospital, carriage, acquisition

Introduction

Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) have spread dramatically since the 1980s. ESBL-based resistance is easily transmitted on plasmids across species, promoting dissemination [1]. Scarce data from Asia, Latin America and Africa suggest exceptionally high ESBL-PE carriage [2], with pre-admission colonisation rates in African hospitals partially exceeding 50% [3–8]. Colonisation is a risk factor for ESBL-PE infections [9, 10], which are associated with increased morbidity and mortality [11]. ESBL-PE colonisation increases with antimicrobial treatment, hospitalisation, invasive procedures and surgery, and travel to high prevalence countries [2, 12–14]. However, compared to hospitals, transmission may be even more intense among household members of colonised individuals [15, 16].

As in many developing countries, most hospitalised patients in sub-Saharan Africa (SSA) are taken care of by family members who also stay on ward. Moreover, crowding, understaffing, sharing of cooking utensils and low compliance with hand hygiene are frequent features of African hospitals [17, 18]. Likewise, crowded households, close human–livestock contact, poor hygiene and lack of clean water, among others, favour the community spread of intestinal microbes [6, 19].

For East African Rwanda, hardly any data on ESBL-PE are available. In one hospital-based study from 2009, 6% and 38% of urinary tract infections among outpatients and inpatients, respectively, were due to ESBL-PE [20].

In this study, we aimed at assessing the presence and risk factors of intestinal carriage of ESBL-PE among patients admitted to the University Teaching Hospital of Butare (UTHB) and among their attending caregivers. In addition, we analysed the acquisition of ESBL-PE carriage during hospital stay and associated factors.

Materials and methods

Study population

This observational, prospective survey was conducted at UTHB from October to December 2014. Butare (population approx., 100 000) is located within the densely populated farmland hills of the country's central plateau. UTHB (500 beds) is one of three reference hospitals in the country. Rwanda has mandatory health insurance requiring adherence to a strict referral system. Consequently, the majority of patients admitted to UTHB had preceding health facility contact. For this study, patients were recruited within 48 h of admission to UTHB as well as one main caregiver *per* patient. Caregivers usually were relatives accompanying the patient at admission, staying in the patient's room and being involved in personal care of the patient and food preparation. Study purpose and procedures were explained, and informed written consent was obtained from participants (or parents in case of paediatric patients). The study protocol was approved by the Rwandan National Ethics Committee (297/RNEC/2014).

Socio-demographic and behavioural data, medical history

Trained staff interviewed patients (or parents in case of paediatric patients) at admission and at discharge. Admission questionnaire items included age, gender, education, occupation, residence, procedures and antibiotics during stay at a referring hospital and during the preceding 3 months, medical history, household information (number of people, assets, livestock ownership, water supply, toilet facilities), hygiene-related behaviour (hand washing, water treatment), travel and dietary pattern. Discharge questionnaire items included duration of stay, department, medication and medical procedures and were cross-checked with hospital files.

Microbiology

Within 48 h of admission and at discharge, rectal swabs were collected from patients and caregivers (Amies swabs, Sarstedt, Germany). Specimens were plated onto chromogenic agar (CHROMagar-ESBL, Mast Diagnostica, Germany) and cultured for 18–24 h. For ESBL-PE-positive colonies, ESBL and/or AmpC production was verified (ESBL-AmpC-Detection Test, Mast Diagnostica), and isolates positive for AmpC only were excluded. Colonies suspicious of *Escherichia coli* were confirmed by indole reaction (DMACA Indole; Becton Dickinson, Germany). Further species identification used the API-20-E system (bioMérieux, Germany). Non-fermenting Gram-negative bacilli were determined by positive oxidase test (Mast Diagnostica) or after species identification and excluded. Antimicrobial susceptibility testing for imipenem, meropenem, ciprofloxacin, moxifloxacin, nalidixic acid, gentamicin, tobramycin, doxycycline, tigecycline, chloramphenicol, nitrofurantoin, cotrimoxazole and fosfomycin was performed using agar disc diffusion on Mueller-Hinton agar (Mast Diagnostica). Interpretation of results followed EUCAST guidelines [21], and CLSI guidelines for nalidixic acid, doxycycline and fosfomycin [22].

Statistical analysis

Variables were compared between groups by chi-squared test, Fisher's exact test, Student's t-test, Mann–Whitney *U*-test or Kruskal–Wallis test, as applicable. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were computed. Independent predictors of, for example, ESBL-PE carriage were identified by multivariate logistic regression with stepwise backward removal. In that, all factors found to be univariately associated at a level of $P < 0.1$ were entered into the model and removed if found to be not associated in multivariate analysis ($P \geq 0.05$). Acquisition of ESBL-PE carriage during hospitalisation was evaluated among patients who were ESBL-PE negative at admission. $P < 0.05$ was considered statistically significant.

Results

Patients' characteristics

Table 1 shows characteristics of 392 patients recruited within 48 h of admission. Their median age was 29 years (range, 0–94), two-thirds were female, and approximately half were referral cases. Among referred patients, 14.6% had had a urinary catheter; other procedures had been rare (Table 1). One-quarter of patients had taken antimicrobial drugs in the preceding 3 months. Among 361 recruited caregivers, 80.2% were women; the median age was 36 years (range, 10–76). Most patients originated from beyond the local Huye District. The educational status was generally low, and most patients were farmers.

Table 1 Selected clinical, socio-economic and behavioural characteristics of admitted patients

Parameter	Value
Demographic and clinical data	
No.	392
Female:male	252:140
Age (years); median, range	29 (0–94)
Referral (%, *n*)	53.1 (206/388)
No	46.9 (182/388)
From secondary hospital	51.0 (198/388)
From primary hospital	1.5 (6/388)
From any other	0.5 (2/388)
Characteristics during stay in referring hospital	
Duration of stay (days; median, range)	2 (0–204)
ICU stay (%, *n*)	7.7 (14/182)
Surgery (%, *n*)	4.1 (7/170)
Urinary catheter (%, *n*)	14.6 (30/206)
Intravenous catheter, peripheral (%, *n*)	46.1 (95/206)
Endotracheal tube (%, *n*)	5.9 (12/205)
Drainage (%, *n*)	9.2 (19/206)
Antibiotic intake (%, *n*)	34.5 (71/206)
Department (%, *n*)	
Surgery	33.2 (129/389)
Obstetrics/Gynaecology	32.6 (127/389)
Paediatrics	20.8 (81/389)
Internal Medicine	13.4 (52/389)
Antimicrobial intake in previous 3 months (%, *n*)	25.1 (98)
Urinary tract infection in previous 3 months (%, *n*)	4.9 (19/391)
Current comorbidity (%, *n*)	
Chronic disease	10.0 (39/389)
Urinary incontinence	0.5 (2/387)
Diarrhoea	2.5 (9/356)
Socio-economic and behavioural characteristics	
Residence in Huye District (%, *n*)	36.2 (142)
Time to next health centre (min.); median, range	40 (0–840)
Education (%, *n*)	
None	30.1 (117/389)
Primary	48.6 (189/389)
Secondary	16.2 (63/389)
Tertiary	5.1 (20/389)
Main occupation, farmer (%, *n*)	51.3 (191/372)
Possession of own farming land (%, *n*)	50.3 (192/382)
Utilisation of garbage as fertiliser (%, *n*)	77.2 (288/373)
No. of people in household (median, range)	5 (1–13) *n* = 389
No. of children under 16 ys in household (median, range)	2 (0–9) *n* = 369
No. of rooms in household (median, range)	4 (1–13) *n* = 387
People per room in household (median, range)	0.83 (0.2–3.8) *n* = 386
Taking care of a family member in last 3 months (%, *n*)	2.6 (10/386)
Household assets (%, *n*)	
Electricity	27.6 (108/382)
Bicycle	14.5 (57)
Radio	55.9 (219)
TV set	18.4 (72)
Mobile phone	57.9 (227)
Fridge	3.3 (13)
Livestock at household (%, *n*)	
Cows	26.7 (104/390)
Goats	19.7 (77/391)
Chicken	18.9 (74)
Pigs	16.3 (64)
Other	6.2 (24/390)
Main sources of drinking water (multiple answers) (%, *n*)	
Public tap/standpipe	56.3 (220/391)
Protected spring	25.8 (101/391)
Unprotected spring	19.9 (78/391)
Rainwater	5.4 (21/391)
Piped water into plot	3.3 (13/391)
Other	5.9 (23/391)
Boiling of drinking water (%, *n*)	52.2 (201/385)
Own toilet in household (%, *n*)	86.0 (333/387)
Cleaning of hands with soap after defecation (%, *n*)	53.5 (206/385)
Food mainly from own subsistence farming (%, *n*)	49.4 (134/271)
Mainly consumed food (three answers) (%, *n*)	
Beans	77.3 (303)
Vegetables	62.5 (245)
Sweet potatoes	58.4 (229)
Rice	58.1 (227/391)
Potatoes	52.0 (204)
Tomatoes	15.1 (59)
Fruits	8.9 (35)
Milk and dairy products	6.9 (27/391)
Noodles	6.6 (26/391)
Eggs	5.9 (23)
Other	13.3 (52/390)
Eating meat at least once per month (%, *n*)	60.5 (221/365)
Eating main meal outside at least once per month (%, *n*)	16.1 (61/379)

Household assets were scarce; approximately a quarter of households had electricity. Livestock ownership was highest for cows (27%) followed by goats, chicken and pigs. A public tap was the main source of drinking water, which was boiled before use in roughly half of the

households. Food originated mainly from subsistence farming, beans, vegetables, (sweet) potatoes, and rice were the most commonly consumed items. Meat consumption and eating outside the home were uncommon (Table 1).

ESBL-PE carriage at admission and discharge

At admission, 49.7% (195/392) of patients and 37.4% (135/361) of caregivers carried at least one ESBL-PE species (mean, 1.3). After a median hospital stay of 6 days until discharge, these proportions increased to 64.6% (173/268, $P = 0.01$) and 46.5% (106/228, $P = 0.06$) among patients and caregivers, respectively. This increase was uneven across departments, reaching a discharge ESBL-PE positivity of 93% (52/56) in paediatrics (Figure 1).

Almost one-third of admitted patients (124/392) was not available for follow-up, mostly due to prematurely leaving the hospital. These patients did not significantly differ from those who were followed up in terms of sex, age, residence, referral, preceding intake of antibiotics and ESBL-PE admission carriage (data not shown). However, loss to follow-up was more frequent among those without formal education (41.0%, 48/117 *vs.* 27.2%, 74/272; $P = 0.007$) and among those who were admitted to an internal medicine ward (51.9%, 27/52 *vs.* 27.9%, 94/337; $P = 0.0005$).

Among ESBL-PE isolated from admitted patients, *E. coli* was the predominant species (51.3%, 100/195) followed by *Klebsiella pneumoniae* (38.5%, 75/195) and *Enterobacter cloacae* (18.5%, 33/195). Among caregivers, the proportion of *E. coli* was slightly increased (64.4%, 87/135; *K. pneumoniae*, 24.4%, 33/135; *E. cloacae*, 15.5%, 21/135; $P = 0.03$ for comparison between patients and caregivers). Further rare species included *Klebsiella oxytoca*, *Raoultella ornithinolytica*, *Raoultella terrigena*, *Citrobacter freundii*, *Cronobacter sakazakii*, *Morganella morganii*, *Enterobacter aerogenes*, *Enterobacter intermedius*, *Kluyvera* spp., *Pantoea* spp., *Salmonella enterica enterica gallinarum* and *Proteus mirabilis*.

Table 2 shows results of antimicrobial susceptibility testing. No carbapenem resistance was detected; all eighteen isolates with an intermediate carbapenem response were classified as sensitive upon verification with the Vitek®2 system (bioMérieux, France). High susceptibility was also observed for tigecycline, fosfomycin and nitrofurantoin, whereas resistance was common for cotrimoxazole, tobramycin and gentamicin. Fluoroquinolones, doxycycline and chloramphenicol also performed poorly, with susceptibility in approximately one in three isolates. Susceptibility differed between the main ESBL-PE species (Table 2), with particularly low susceptibility of *E. coli* against ciprofloxacin, doxycycline and cotrimoxazole, of *K. pneumoniae* against gentamicin, chloramphenicol and cotrimoxazole, and of *E. cloacae* against ciprofloxacin, gentamicin and chloramphenicol. Resistance patterns differed between patients and caregivers and between admission and discharge only for a few of the antibiotics tested.

Factors associated with ESBL-PE carriage at admission

Patients accompanied by a caregiver colonised with ESBL-PE had three times increased odds of being carriers themselves (Table 3). This association was seen for non-referred patients (OR, 2.19 [95% CI, 1.06–4.55], $P = 0.02$), and it was pronounced for referred patients (OR, 3.45 [1.78–6.70], $P < 0.0001$). In 65.2% (58/89) of simultaneous colonisation of patient and caregiver, ESBL-

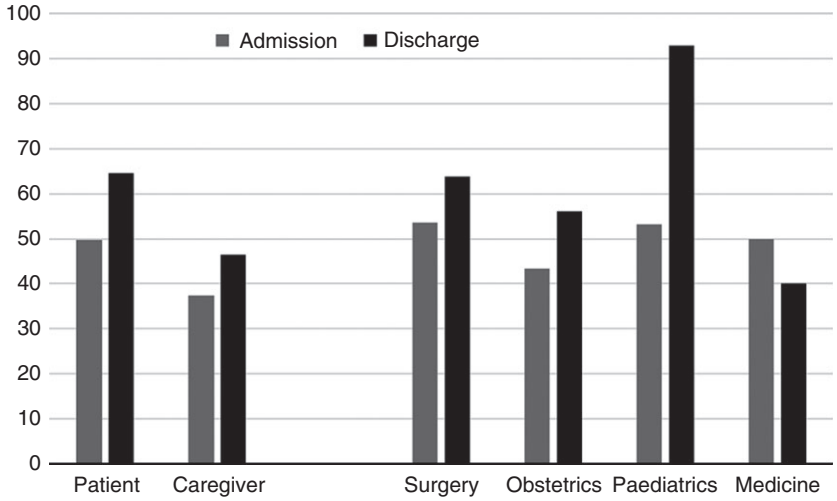

Figure 1 Prevalence (%) of carriage of ESBL-PE at admission and discharge among patients and caregivers.

Table 2 Susceptibility testing for ESBL-PE

	Proportion of sensitive isolates			
Species/antimicrobial	Patient admission	Patient discharge	Caregiver admission	Caregiver discharge
All ESBL-Species	*n* = 274	*n* = 255	*n* = 182	*n* = 142
Imipenem	100	100	100	100
Meropenem	100	100	100	100
Ciprofloxacin	26.3	25.1	29.7	20.4
Moxifloxacin	33.9	28.6	34.6	21.1
Nalidixic Acid*	31.4	25.6	25.3	22.5
Gentamicin	11.3	7.8	20.3§	12.0
Tobramycin	9.9	3.9‡	18.7§	8.5
Doxycycline*	30.3	22.7	28.6	23.2
Tigecycline†	(92.3)	(92.5)	(97.3)§	(91.5)
Chloramphenicol	36.5	40.8	39.0	49.3
Nitrofurantoin†	(74.8)	(73.3)	(79.7)	(78.9)
Cotrimoxazole	8.0	8.6	8.2	7.7
Fosfomycin*,†	(91.6)	(93.3)‡	(94.0)	(95.7)
ESBL-*E. coli*	*n* = 98	*n* = 98	*n* = 82	*n* = 65
Imipenem	100	100	100	100
Meropenem	100	100	100	100
Ciprofloxacin	12.2	14.3	15.9	7.7
Moxifloxacin	18.4	19.4	24.4	10.8
Nalidixic Acid*	8.2	8.2	11.0	9.2
Gentamicin	22.4	13.3	25.6	9.2
Tobramycin	12.2	5.1	20.7	3.1
Doxycycline*	7.1	10.2	8.5	9.2
Tigecycline†	99.0	99.0	100	100
Chloramphenicol	55.1	61.2	54.9	70.8
Nitrofurantoin†	87.8	95.9‡	98.8§	98.5
Cotrimoxazole	7.1	6.1	7.3	4.6
Fosfomycin*,†	95.9	99.0	100	98.5
ESBL-*K. pneumoniae*	*n* = 70	*n* = 65	*n* = 29	*n* = 28
Imipenem	100	100	100	100
Meropenem	100	100	100	100
Ciprofloxacin	55.7	43.1	51.7	46.4
Moxifloxacin	60.0	44.6	51.7	42.9
Nalidixic Acid*	62.9	50.8	55.2	46.4
Gentamicin	0	0	6.9	17.9§
Tobramycin	2.9	0	6.9	10.7
Doxycycline*	45.7	32.3	65.5	35.7
Tigecycline†	–	–	–	–
Chloramphenicol	30.0	40.0	34.5	53.6
Nitrofurantoin†	–	–	–	–
Cotrimoxazole	5.7	13.8	3.4	10.7
Fosfomycin*,†	–	–	–	–
ESBL-*E. cloacae*	*n* = 33	*n* = 30	*n* = 21	*n* = 17
Imipenem	100	100	100	100
Meropenem	100	100	100	100
Ciprofloxacin	9.1	13.3	42.9§	23.5
Moxifloxacin	24.2	23.3	42.9	29.4
Nalidixic Acid*	21.2	16.7	28.6	29.4
Gentamicin	3.0	13.3	4.8	5.9
Tobramycin	0	6.7	4.8	5.9
Doxycycline*	39.4	30.0	33.3	23.5
Tigecycline†	–	–	–	–
Chloramphenicol	12.1	13.3	19.0	17.6

Table 2 (*Continued*)

Species/antimicrobial	Proportion of sensitive isolates			
	Patient admission	Patient discharge	Caregiver admission	Caregiver discharge
Nitrofurantoin†	–	–	–	–
Cotrimoxazole	6.1	10.0	9.5	11.8
Fosfomycin*,†	–	–	–	–

*CLSI breakpoints.
†Breakpoint only applicable for *E. coli*; proportions are therefore in parentheses.
‡Comparison of admission and discharge isolates, $P < 0.05$ by McNemar test.
§Comparison of patient and caregiver isolates, at admission and at discharge, $P < 0.05$.

PE species were at least partially concordant. Further factors associated with patients' ESBL-PE carriage in univariate analysis (Table 3) included male gender, referral case, preceding antibiotic treatment, few household rooms, chicken husbandry, an unprotected spring as main source of drinking water, preferential consumption of tomatoes and eggs as well as a distant health centre. Factors reducing the odds involved nearby residence, formal education, electricity or a TV set at home, hand cleaning with soap after defecation and boiling drinking water. In multivariate analysis, independent predictors of ESBL-PE carriage were a colonised caregiver, intake of antibiotics within the preceding 3 months and having eggs among the mostly consumed food items, whereas boiling drinking water reduced the odds (Table 3).

Separation by species revealed increased odds of ESBL-PE carriage due to caregiver colonisation with the respectively matching ESBL-PE species. Also, preceding antibiotic treatment was associated with colonisation for all three species in addition to species-specific factors (Table S1). In multivariate analysis, caregiver positivity was confirmed to predict the colonisation of patients with the respective ESBL-PE species (Table S2). Further factors associated with ESBL-*E. coli* carriage in multivariate analysis were preceding antibiotic intake, chicken husbandry, piped water and the omission of boiling drinking water. For ESBL-*K. pneumoniae*, independent predictors included being a referral or a paediatric patient, own or parents' occupation being farmer, omission of cleaning hands after defecation, tomatoes as favourite food item and a distant health centre. For ESBL-*E. cloacae*, preceding antibiotic intake and chicken husbandry increased the odds, whereas favourite consumption of sweet potatoes and beans was protective in multivariate analysis (Table S2).

Referral from another health facility almost doubled the odds of carrying ESBL-PE (Table 3). As to preceding procedures in the referring hospital, ESBL-PE admission positivity was increased in case of endotracheal intubation (OR, 9.03 [1.26–393.0], $P = 0.01$), urinary catheter (OR, 3.57 [1.33–11.15], $P = 0.006$), antibiotic treatment (OR, 2.42 [1.26–4.67], $P = 0.004$) and i.v. catheter (OR, 1.76 [0.97–3.21], $P = 0.047$), whereas the duration of hospitalisation had an only borderline influence (≥3 days, OR, 1.73 [0.88–3.41], $P = 0.09$). For *K. pneumoniae*, one specific risk factor was an intensive care unit stay at the referring hospital (OR, 3.42 [0.95–12.13]; $P = 0.046$).

Hospital acquisition of ESBL-PE

At discharge, samples and data were collected from 68.4% (268/392) of patients and 63.2% (228/361) of caregivers. Among patients non-colonised at admission, 55.0% (72/131) acquired ESBL-PE carriage during hospitalisation corresponding to an incidence of 71.5/1000 hospital days. For caregivers, these figures were 39.4% (56/142) and 52.9/1000. In patients with newly acquired ESBL-PE carriage, ESBL-*K. pneumoniae* (50%, 36/72) predominated over ESBL-*E. coli* (47.2%, 34/72) and ESBL-*E. cloacae* (18.1%, 13/72). Among newly colonised caregivers, ESBL-*E. coli* (66.1%, 37/56) was the leading species (ESBL-*K. pneumoniae*, 30.4%, 17/56; ESBL-*E. cloacae*, 14.3%, 8/56). In 63.2% (12/19) of cases in which both patient and caregiver acquired carriage in hospital, the species of ESBL-PE were at least partially concordant.

Table 4 shows factors associated with the acquisition of ESBL-PE among patients during hospitalisation. The caregiver's ESBL-PE admission carriage was not linked to the patient's acquisition: 49.5% (46/93) and 62.5% (20/32) of patients accompanied by ESBL-PE-negative and ESBL-PE-positive caregivers, respectively, acquired ESBL-PE during hospitalisation ($P = 0.20$). Only 10.6% (7/66) of acquired isolates matched (at least partially) in species with the caregiver's admission ESBL-PE. Neither did an intensive care unit stay ($n = 6$), urinary ($n = 47$) or i.v. catheter ($n = 98$), or drainage ($n = 11$) associate with ESBL-PE acquisition (data not shown). Hospitalisation of ≥6 days (median) tended to increase ESBL-PE carriage (64.8%, 35/54 *vs.* 48.0%, 36/75; $P = 0.06$). The same

Table 3 Univariate and multivariate analysis of factors associated with ESBL-PE carriage among patients at admission

Parameter	Value	No.	ESBL-PE positive (%)	Univariate analysis				Multivariate analysis†			
				OR	95% CI		*P*	aOR	95% CI		*P*
Caregiver admission ESBL status	Negative	225	38.7	1				1			
	Positive	135	65.9	3.07	1.92	4.72	**<0.0001**	2.88	1.80	4.61	**<0.0001**
Gender	Female	252	44.8	1							
	Male	140	58.6	1.74	1.12	2.70	**0.009**				
Residence in Huye District	No	250	53.6	1							
	Yes	142	43.0	0.65	0.42	1.01	**0.04**				
Hospital department	Surgery	129	53.5	1							
	Paediatrics	81	53.1	0.98	0.54	1.78	0.95				
	Gynaecology	127	43.3	0.66	0.39	1.12	0.10				
	Medicine	52	50.0	0.87	0.43	1.74	0.67				
Referral	No	182	41.2	1							
	Yes	206	56.8	1.88	1.23	2.87	**0.002**				
Admittance to any other healthcare facility within last 3 months	No	323	47.7	1							
	Yes	69	59.4	1.61	0.92	2.82	0.08				
Intake of antibiotics within the last three months	No	293	44.0	1				1			
	Yes	98	67.3	2.62	1.58	4.37	**<0.0001**	2.70	1.59	4.58	**0.0002**
Any level of education	No	117	58.1	1							
	Yes	272	46.0	0.61	0.39	0.97	**0.03**				
Ratio of people per room	>1	204	53.4	1							
	≤1	179	44.7	0.70	0.46	1.08	0.09				
No. of rooms in household	> 3	219	44.7	1							
	≤ 3	168	55.4	1.53	1.0	2.34	**0.04**				
Presence of electricity in household	No	284	54.6	1							
	Yes	108	37.0	0.49	0.30	0.79	**0.002**				
Presence of TV set at home	No	320	52.5	1							
	Yes	72	37.5	0.54	0.31	0.95	**0.02**				
Presence of chicken in household	No	318	46.9	1							
	Yes	74	62.2	1.86	1.08	3.24	**0.02**				
Use of garbage as fertiliser	No	85	41.2	1							
	Yes	288	52.4	1.57	0.94	2.65	0.07				
Cleaning hands with soap after toilet use	**No**	**179**	**58.7**	**1**							
	Yes	206	41.3	0.50	0.32	0.76	**0.0007**				
Piped water into plot as main source of drinking water	No	378	48.7	1							
	Yes	13	76.9	3.51	0.88	20.1	0.05				
Public tap/standpipe as main source of drinking water	No	171	55.0	1							
	Yes	220	45.5	0.68	0.45	1.04	0.06				
Unprotected spring as main source of drinking water	No	313	45.4	1							
	Yes	78	66.7	2.41	1.39	4.19	**0.0008**				
Boiling of water prior to drinking	No	184	59.8	1				1			
	Yes	201	40.3	0.45	0.30	0.70	**0.0001**	0.59	0.37	0.92	**0.02**
Sweet potatoes as favourite food item*	No	163	44.8	1							
	Yes	229	53.3	1.41	0.92	2.15	0.10				
Tomatoes as favourite food item*	No	333	46.5	1							
	Yes	59	67.8	2.42	1.30	4.54	**0.003**				
Eggs as favourite food item*	No	369	47.7	1				1			
	Yes	23	82.6	5.21	1.68	21.38	**0.001**	6.52	1.75	24.31	**0.005**
Vegetables as favourite food item*	No	147	43.5	1							
	Yes	245	53.5	1.49	0.97	2.30	0.06				
Fruits as favourite food item*	No	357	48.5	1							
	Yes	35	62.9	1.80	0.84	3.91	0.10				

Table 3 (*Continued*)

Parameter	Value	No.	ESBL-PE positive (%)	Univariate analysis OR	95% CI		*P*	Multivariate analysis† aOR	95% CI	*P*
>40 min. (median) needed to reach next health centre	No	201	42.8	1						
	Yes	186	57.5	1.81	1.19	2.77	**0.004**			

Table shows factors associated with ESBL-PE at a level of $P < 0.10$. OR, odds ratio; aOR, adjusted odds ratio; 95% CI, 95% confidence interval.
*One out of three food items mostly consumed.
†$n = 354$; $R^2 = 0.11$.

was true for surgery during hospitalisation (68.6%, 24/35 *vs.* 50.5%, 46/91; $P = 0.07$) and for cephalosporin treatment (69.2%, 18/26 *vs.* 51.5%, 53/103; $P = 0.10$). The odds of ESBL-PE acquisition were significantly increased for males, young age, paediatric department, absence of formal education as well as ventilation or cloxacillin treatment during hospitalisation (Table 4). In multivariate analysis, being a paediatric patient (aOR, 11.28 [2.36–53.83], $P = 0.002$), surgery (aOR, 3.19 [1.32–7.70], $P = 0.01$) and male gender (aOR, 2.93 [1.07–7.99], $P = 0.04$) independently predicted the acquisition of ESBL-PE.

Approximately one-quarter of initially colonised patients (26.3%, 36/137) lost ESBL-PE carriage during hospital stay. Of note, while surgery rate and gender were similar to patients acquiring ESBL-PE carriage, only 2.8% (1/36) were paediatric patients.

Discussion

Colonisation with ESBL-PE is a risk factor for ESBL-PE infections in industrialised countries [9, 10], which are associated with delays in effective treatment, prolonged hospitalisation, increased costs and increased morbidity and mortality [11]. We found that 50% of patients admitted to a university hospital in Rwanda carry ESBL-PE and that 55% of non-colonised patients acquire carriage during hospitalisation. Colonised caregivers, prior antibiotic intake, egg consumption and neglect to boil drinking water independently predicted carriage among admitted patients, whereas being a paediatric patient, undergoing surgery and male sex predicted acquisition.

The fragmentary data from SSA indicate that ESBL-PE are common in hospital and community settings, partially reaching >50% prevalence [2, 3]. Community ESBL-PE data are particularly rare but admission colonisation rates ranging from 10–21% in Madagascar [4, 5], 33% in Guinea-Bissau [6] and 34% in Gabon [7] to 55% in Cameroon [8] have been reported. This contrasts with an ESBL-PE carriage rate of 6% in Germany [23] and even less in the USA [2]. In Rwanda in 2009, 6% of outpatient urinary tract infections contained ESBL-PE [20], and in 2014, 4.9% of >1000 schoolchildren in the local district were carriers (FPM, unpublished observations). Compared to the latter figure, ESBL-PE carriage was eight times more common among our non-referred patients, and it was increased almost 11-fold among referred patients. Prior antibiotic treatment, a well-known risk factor [12, 13, 24], contributed to ESBL-PE carriage also in our study. Increased carriage due to specific foods and unsafe drinking water conceivably is present in other SSA settings as well and confirms the role of the environment including animals, animal products and water in the transmission of antimicrobial resistance [19]. Nevertheless, even if excluding all independent predictors of ESBL-PE carriage, one-quarter of patients still carried these bacteria. So far, we are unable to provide conclusive arguments to explain the remaining difference in prevalence as compared to the above-mentioned community figures. Water source and chicken husbandry were specific risk factors for ESBL-*E. coli*, and being a referral or paediatric patient for ESBL-*K. pneumoniae*, which accords with common transmission concepts [2, 19]. A peculiarity in many areas of limited resources is that usually family members take care of hospitalised patients including personal hygiene and food preparation. At admission, these caregivers showed a high proportion of colonisation themselves (37%), which was strongly associated with ESBL-PE carriage of the admitted patients. The difference in admission carriage rate of some 13% between patient and caregiver agrees with 10% in a Cameroonian study [25]. Although we are unable to comment on the causality of this association, let alone, directionality, our data highlight the caregiver as a reservoir of ESBL-PE and, likely, source of ESBL-PE transmission into, within and out of the hospital. Intrahousehold transmission of ESBL-PE preceding hospitalisation likely contributed to this finding [15, 16]. In addition, shared prior exposure to health facilities might have exerted parallel

Table 4 Factors associated with the acquisition of ESBL-PE during hospital stay among 131 patients

Parameter	Value	No.	% ESBL-PE acquisition	OR (95% CI)	*P*
Gender	Female	94	45.7	1	
	Male	37	78.4	4.30 (1.16–11.47)	0.0007
Age (years)	n.a.	131	n.a.	0.98 (0.96–1.00)	0.049
Department	Surgery	38	60.5	1	
	Paediatrics	25	88.0	4.78 (1.10–28.63)	0.02
	Gynaecology	59	42.4	0.48 (0.19–1.19)	0.08
	Medicine	9	22.2	0.19 (0.02–1.20)	0.06
Any level of formal education	No	27	77.8	1	
	Yes	103	49.5	0.28 (0.09–0.80)	0.009
Ventilation during hospital stay	No	116	52.6	1	
	Yes	12	83.3	4.51 (0.89–43.66)	0.04
Cloxacillin treatment during hospital stay	No	117	52.1	1	
	Yes	12	83.3	4.59 (0.91–44.43)	0.04

OR, odds ratio; 95% CI, 95% confidence interval; n.a., not applicable.

acquisition of ESBL-PE among patients and caregivers, which is partially supported by the stronger association among referred patients. Unfortunately, we have no information whether the caregiver attended the patient also in the referring hospital, even though this is likely. On the other hand, ESBL-PE species only incompletely overlapped between patients and caregivers, suggesting the presence also of non-uniform sources of colonisation.

Rates of acquiring ESBL-PE during hospitalisation were remarkably high for both patients (55%) and caregivers (39%). These figures greatly exceed the 4.4% observed in Switzerland [24], are in the range of the 48% reported in a paediatric hospital in Madagascar [4] but are below an extraordinary 94% seen in a paediatric re-nutrition centre in Niger [26]. Nevertheless, in the present study, the acquisition rate among paediatric patients was 88%, and 93% of discharged paediatric patients were ESBL-PE carriers. Crowding in paediatrics may have contributed to this excessive acquisition rate [6]. Overall, the absence of formal education, ventilation and cloxacillin treatment were risk factors of acquisition in univariate analysis. While education may correlate with personal hygiene and ventilation with disease severity and duration of stay, treatment with the closely related dicloxacillin promoted ESBL-*E. coli* colonisation in a murine model [27]. The reason for male sex predicting acquisition remains obscure; for ESBL-PE infections, associations with both genders have been reported [28, 29]. Only in a minority of cases, the caregivers' admission isolates matched with the ESBL-PE species acquired by the patient during hospitalisation. This indicates that the caretaker's admission carriage status does not substantially contribute to the acquisition by the individual patient but rather is outweighed by the abundance of other sources of ESBL-PE such as other patients and environmental contamination.

Our study has limitations. It was meant to describe ESBL-PE prevalence among patients and caregivers, but in subgroups, for example by hospital department, it might be underpowered. One-third of participants were not available for discharge sampling, mainly because they left the hospital prematurely. Patients available and non-available for follow-up were similar in virtually all baseline characteristics. The absence of formal education and admission to an internal medicine ward were frequent among patients not followed up. While poor education increased the odds of ESBL-PE acquisition, the opposite was seen for admission to internal medicine. Together, this indicates no major impact of loss to follow-up on the estimated rate of ESBL-PE acquisition. We used single rectal swabs and chromogenic culture plates for ESBL-PE detection. As the sensitivity of this approach is imperfect [30], the actual prevalence and acquisition of ESBL-PE may be even higher. Imperfect sensitivity might also have contributed to the observation of a considerable proportion of initially positive patients found negative for ESBL-PE carriage at discharge. In addition, strains with OXA-48-type carbapenemase without any other resistance mechanism might have been missed [31]. Responses to the questionnaire-based interviews could not be verified in all cases, for example by home visits, and details of preceding medical procedures and treatment might have been subject to variable recollection leaving the possibility of response and recall bias. Multiple testing was performed in this study without subsequent Bonferroni correction or similar adjustments. Thus, some of the reported significant associations may be spurious, and this should be kept in mind when interpreting the results. On the other hand, Bonferroni or similar corrections inflate type two errors, which are no less false. We therefore decided to describe

the findings as they are, and have discussed the main findings cautiously. Moreover, biological plausibility should not be disregarded when interpreting observed associations [32]. Not lastly, due to limited resources and funds, we were unable to genotype the isolated ESBL-PE, which would have allowed for analysing transmission pathways. Refusal of hospital staff made it impossible to screen this group for ESBL-PE carriage.

Resistance to more than two antibiotic classes was frequent, including three in four isolates with resistance to ciprofloxacin and almost 90% gentamicin resistance. Doxycycline, cotrimoxazole and chloramphenicol showed high resistance rates, mirroring their broad use. Carbapenem resistance was absent, and sensitivity to other second-line antibiotics, that is tigecycline, nitrofurantoin and fosfomycin, was good to complete. However, the high costs of carbapenems and tigecycline limit their availability at limited resources. Promising are the high rates of nitrofurantoin and fosfomycin susceptibility in ESBL-*E. coli*, allowing their usage in empiric treatment of urinary tract infections.

In this study, the extent of ESBL-PE carriage and acquisition exceeded our expectations. Beyond the individual risk of the colonised patient [9–11], transmission to other patients and dissemination into the community are a substantial concern. Improving diagnostic capacities, routine admission screening and monitoring community spread are desirable. Reducing crowding in the hospital and increasing the number of staff are likely effective countermeasures of transmission. Beyond a critical re-evaluation of antibiotic prescription, hand hygiene measures also for caregivers should be enforced. Our data show that community water sources and animal husbandry contribute to ESBL-PE emergence, which needs attention in further studies. The high proportion of patients and caregivers colonised at discharge warrants investigation into their role in spreading multiresistant micro-organism into the community.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Factors associated with carriage of ESBL-*E. coli*, ESBL-*K. pneumoniae*, and ESBL-*E. cloacae* among admitted patients.

Table S2. Independent predictors of admission carriage of ESBL-PE according to species and by multivariate analysis.

Corresponding Author Frank P. Mockenhaupt, Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany. Tel.: +49 30 450 565 721; Fax: +49 30 450 565 989; E-mail: frank.mockenhaupt@charite.de