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Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to *Escherichia coli* resistant to third-generation cephalosporins

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Objectives: This study determined excess mortality and length of hospital stay (LOS) attributable to bloodstream infection (BSI) caused by third-generation-cephalosporin-resistant *Escherichia coli* in Europe.

Methods: A prospective parallel matched cohort design was used. Cohort I consisted of patients with third-generation-cephalosporin-resistant *E. coli* BSI (REC) and cohort II consisted of patients with third-generation-cephalosporin-susceptible *E. coli* BSI (SEC). Patients in both cohorts were matched for LOS before infection with patients free of the respective BSI. Thirteen European tertiary care centres participated between July 2007 and June 2008.

Results: Cohort I consisted of 111 REC patients and 204 controls and cohort II consisted of 1110 SEC patients and 2084 controls. REC patients had a higher mortality at 30 days (adjusted odds ratio=4.6) and a higher hospital mortality (adjusted hazard ratio=5.7) than their controls. LOS was increased by 8 days. For SEC patients, these figures were adjusted odds ratio=1.9, adjusted hazard ratio=2.0 and excess LOS=3 days. A 2.5 times [95% confidence interval (95% CI) 0.9–6.8] increase in all-cause mortality at 30 days and a 2.9 times (95% CI 1.2–6.9) increase in mortality during entire hospital stay as well as an excess LOS of 5 days (95% CI 0.4–10.2) could be attributed to resistance to third-generation cephalosporins in *E. coli* BSI.

Conclusions: Morbidity and mortality attributable to third-generation-cephalosporin-resistant *E. coli* BSI is significant. If prevailing resistance trends continue, high societal and economic costs can be expected. Better management of infections caused by resistant *E. coli* is becoming essential.

Keywords: antibiotic resistance, Gram-negative bacteria, invasive infections, clinical impact

Introduction

Despite growing concerns about increasing antibiotic resistance there is a lack of information about the impact of antibiotic resistance on clinical outcomes in infected patients. Studies attempting to provide this information face considerable challenges. As most previous studies were carried out in single centres many have failed to enrol a sufficient number of patients, leading to imprecision and ambiguity about the true attributable mortality. To overcome this lack of power, results have been pooled in meta-analyses,[1,2] but this approach inherently suffers from heterogeneity as a result of data aggregation from studies with unequal endpoints, different designs and varying quality. Multicentre studies have the potential to improve precision and generalizability, but have only been reported incidentally.[3,4]

Another challenge in determining the impact of antibiotic resistance on clinical outcome is how to control for confounding. Patients infected with resistant pathogens are older, suffer from more chronic diseases, have more frequently been exposed to antibiotics and are generally more ill and consequently have longer hospital exposure than patients infected with susceptible bacteria.[5–7] Nevertheless, most studies compared these two patient groups directly, using multivariate models to adjust for differences, thereby ignoring the principle that control patients must be sampled from the population that produced the cases in order to permit causal inference.[8]

The present study was designed to improve precision, and internal and external validity, and provide representative estimates of the morbidity and mortality attributable to antibiotic-resistant bloodstream infection (BSI) for Europe as a whole. We chose to investigate *Escherichia coli* BSI for four reasons: (i) *E. coli* is the most frequent cause of BSI in European hospitals;[9–11] (ii) compared with other bacteria, antibiotic resistance in *E. coli* shows the most dramatic increase in Europe and beyond;[12–14] (iii) novel antibiotics that could fill the therapeutic void are unlikely to become available in the near future;[15,16] and (iv) little is known about the clinical impact attributable to antibiotic resistance caused by infections with this species.

This study is the first multicentre study to provide robust estimates of excess 30 day mortality, hospital mortality and hospital stay attributable to third-generation-cephalosporin-resistant *E. coli* (REC) BSI for tertiary care centres in Europe.

Methods

Setting

Thirteen tertiary care centres (WHO definition[17]) from as many European countries were selected from hospitals of the European Antimicrobial Resistance Surveillance System (EARSS).[14] They had representative levels of resistance for their countries. All were served by microbiological laboratories with good diagnostic practice, according to the results of the external quality assessment exercise carried out annually by EARSS.

Study design

A prospective parallel matched cohort design was chosen. The first cohort consisted of patients with third-generation-cephalosporin-resistant *E. coli* BSI (REC cohort), and the second cohort consisted of patients with third-generation-cephalosporin-susceptible *E. coli* (SEC) BSI (SEC cohort). Any episode of REC or SEC BSI in an adult patient (≥18 years) was identified by daily laboratory liaison. Day of enrolment was defined as the date blood cultures were taken. To improve the power of this study each identified patient was not matched to one, but to two controls free of *E. coli* BSI, based on length of hospital stay (LOS) (±3 days) before enrolment. If more than two patients were eligible as controls, the patients closest to the exposed patient in the ward registry were selected.

Blood cultures were taken on clinical indication and all hospital patients with blood cultures positive for *E. coli*, irrespective of antibiotic resistance, were included as exposed patients. Third-generation cephalosporin resistance was determined using EARSS consensus protocols.[18]

Training of on-site investigators and data collection

For each hospital a dedicated on-site investigator was recruited. During two workshops, they were trained in standardized patient enrolment and data collection, using anonymized patient records provided by one of the co-authors (P. G. D.). Patients were enrolled for 12 months between July 2007 and June 2008. Anonymized data were recorded using a web-based and password-protected data submission tool hosted by the Netherlands National Institute of Public Health and the Environment. Built-in data checks secured data validity. To further ensure uniform application of criteria and definitions, one co-author (M. E. A. de K.) provided continuous helpdesk support. Data sources were patient records, the electronic hospital information system, the laboratory information system and nursing notes. Post-discharge surveillance to determine survival 30 days after enrolment was carried out by telephone contact with patients or their general practitioner.

Pre-enrolment data recorded consisted of patient referral history (long-term care facility, nursing home or other hospital admission), frequent hospital exposure, defined as two or more hospital admissions in the previous 12 months, current admission diagnosis, type of admission (emergency or elective), surgery and presence of co-morbidities from the Charlson co-morbidity index (CCI).[19] On enrolment, the presence of indwelling devices (tracheal tube, central venous catheter, arterial vascular access, peripheral vascular access, urinary catheter, tracheostomy, nasogastric tube, wound drainage tube) was recorded and the anatomical origin of the BSI (in the case of secondary BSI), the susceptibility profile of the causative pathogen and co-infections were ascertained. Main outcomes were mortality 30 days after enrolment, mortality during hospital stay and LOS after enrolment.

This study complied with the Dutch patient confidentiality regulations and ethical standards[20] and was approved by local institutional ethics committees.

Statistical analysis

Statistical analyses were performed using SAS 9.1 and R 2.8.1. Univariate comparisons of patients with *E. coli* BSI (either REC or SEC) and the unexposed controls were performed using Cochran's Q statistic for categorical variables and Friedman's ranks sum test for continuous variables.

Analyses of outcomes (30 day mortality, hospital mortality and LOS after enrolment) were performed separately for the REC and SEC cohorts. Variables were selected for multivariate analysis if they changed the effect estimate in bivariate regression by >5%. Collinearity was assessed by generating a correlation coefficient matrix. A robust sandwich covariance matrix estimator was used to account for the matched design. The effect attributable to third-generation cephalosporin resistance was determined by the ratio of the adjusted effect measures for REC BSI and SEC BSI from the parallel cohorts, and the 95% confidence intervals (95% CIs) were determined as described by Altman *et al.*[21]

Thirty day mortality and hospital mortality

The effect of *E. coli* BSI on mortality was determined by logistic regression for 30 day mortality (SAS command 'genmod') and by Fine and Gray's extended Cox's regression[22,23] for competing events for hospital mortality (SAS command 'bphreg'). Model fit for logistic regression was assessed by the Hosmer–Lemeshow test. Cumulative incidence graphs were created using a cause-specific hazard model where both discharge alive and hospital mortality were included as competing endpoints (R package 'cmprsk', command 'cuminc').[24,25]

LOS after enrolment

A generalized linear model (GLM) with gamma distribution and loglink function for positively skewed data was used to determine the impact of *E. coli* BSI on LOS after enrolment (SAS command 'genmod').[26,27] Excess LOS was calculated by comparing the mean outcomes predicted by the multivariate model for all patients in each group; 95% CIs for the difference in LOS in days were obtained by parametric bootstrapping.

Data heterogeneity

To test for group effects at hospital level, multilevel models for hierarchical data were used for logistic regression and the GLMs. Stratified analyses were used for the Fine and Gray model.

Results

During the study period, 1328 episodes of *E. coli* BSI were reported, of which 129 (10%) isolates showed resistance to third-generation cephalosporins (Table 1). For 107 (8%) patients with BSI no appropriate match with equal pre-enrolment time of admission ±3 days could be identified. For 160 (12%) exposed patients only a single control could be matched. Hence the analyses were based on 111 patients with REC BSI, 1110 patients with SEC BSI, matched with 204 and 2084 controls, respectively.

Table 1. Activity data of participating hospitals from July 2007 to June 2008

Hospital number	Country	Beds	Admissions	Bed-days	BSI/ controls	National REC%[a]
H1	Austria	2137	99761	657268	84/171	7
H2	Belgium	856	26337	290790	64/128	4
H3	Croatia	1724	63804	479528	78/154	4
H4	England	1210	104680	292030	216/428	7
H5	Germany	1234	56193	391258	60/113	5
H6	Greece	949	44214	293632	84/112	10
H7	Ireland	819	22418	238166	124/244	6
H8	Italy	912	55600	292150	106/210	16
H9	Latvia	1029	46343	307006	17/34	9
H10	Malta	835	48504	252488	92/164	21
H11	Romania	1109	72739	427666	23/39	24
H12	Scotland	877	53276	255215	172/333	7
H13	Slovenia	2344	83161	614353	208/407	4
Overall	13	16035	777030	4791550	1328/2537	12

BSI, number of first episodes of *E. coli* bloodstream infection.
[a]Proportion of REC according to EARSS 2008.[14]

The 107 excluded exposed patients had a longer period between admission and enrolment than the included patients, other clinical characteristics were comparable. Hospital stay after enrolment was longer as well, while 30 day (χ^2 test, $P=0.86$) and hospital (χ^2, $P=0.28$) mortality were indistinguishable from the included exposed patients.

For the 3509 patients included in the analysis, the time from admission to enrolment was longer in the REC cohort than in the SEC cohort. Crucial differences existed between the patients in the two cohorts, which were independent of the exposure status of the patients. In the REC cohort, exposed patients as well as controls more often had an elective admission, more frequently received antibiotics before enrolment, had more surgery, suffered more frequently from cancer/leukaemia, more often had indwelling devices (such as central venous catheter, urinary catheter or wound drainage tubes) and their CCI was higher than for the patients in the SEC cohort. Similarly, some consistent differences between exposed and control patients could be identified in both cohorts. Patients with BSI in both cohorts more often had previous hospital admissions, genitourinary disorders or infections on admission, suffered more often from peripheral vascular diseases, diabetes with or without end-organ damage, cancer/leukaemia or dementia and more often had a central venous catheter or urinary catheter than the controls (Table 2).

Thirty day mortality

In the REC cohort, 34 of the 105 (32%) patients with a resistant *E. coli* BSI died within 30 days after enrolment, in contrast to only 11 of the 196 (6%) control patients. Almost all deaths occurred in hospital. Only one exposed patient died at home and only one control patient died in a nursing home. In the SEC cohort, 180 of the 1067 (17%) patients with a susceptible *E. coli* BSI died within 30 days of acquiring the infection, whereas mortality among controls was 137/1984 (7%). Again, most patients died in hospital, only 11 (6%) of the exposed and 24 (18%) of the controls died after discharge. Table 3 summarizes the results from the univariate and multivariate models. Multilevel analysis showed that group effects at hospital level did not change the coefficients for *E. coli* BSI exposure and therefore the presented results are based on regular logistic regression. After adjusting for potential confounders, REC BSI was associated with 30 day mortality with an adjusted odds ratio of 4.6 (95% CI 1.7–12.3) compared with controls without *E. coli* BSI. In the SEC cohort, the adjusted odds ratio was 1.9 (95% CI 1.4–2.5).

Comparison of excess 30 day mortality between the two cohorts showed that mortality associated with REC BSI was 2.5 (95% CI 0.9–6.8) times higher than for patients infected with susceptible strains.

Hospital mortality

Thirty-nine of 109 (36%) patients with REC BSI died while in hospital, whereas 11 of 204 (5%) control patients died in hospital. The median time from enrolment to death was 3 days for the patients exposed to REC BSI [interquartile range (IQR) 1–12], while median time to death was 9 days (IQR 4–17) among controls. In the SEC cohort, hospital mortality was 190/1101 (17%) among patients exposed to SEC BSI and 141/2070 (7%) among

Table 2. Characteristics of patients with *E. coli* BSI and matched controls, separately for the REC cohort and the SEC cohort

	REC cohort					SEC cohort				
	BSI (%)	*n*	controls (%)	*n*	*P* value	BSI (%)	*n*	controls (%)	*n*	*P* value
Female gender	58 (52)	111	110 (54)	204	0.97	622 (56)	1110	1071 (51)	2084	0.05
Age[a] (years)	73 (60–83)	111	66 (50–79)	204	0.42	73 (60–81)	1110	68 (52–79)	2084	<0.01
Transfer from another institution	30 (31)	98	17 (9)	179	<0.01	137 (14)	960	158 (9)	1786	<0.01
More than two hospital stays in previous year	41 (42)	98	51 (28)	179	<0.01	232 (24)	960	359 (20)	1786	<0.01
Emergency admission	68 (62)	110	120 (59)	204	0.62	932 (84)	1110	1454 (70)	2082	<0.01
Antibiotic therapy before enrolment	54 (49)	111	103 (50)	204	0.23	396 (36)	1110	883 (42)	2084	<0.01
Surgery before enrolment	26 (23)	111	40 (20)	204	0.45	145 (13)	1110	282 (14)	2083	0.08
LOS before enrolment[a] (days)	5 (0–14)	111	4 (1–14)	204	0.43	0 (0–3)	1110	1 (0–3)	2084	<0.01
Admission diagnosis										
cardiovascular disease	7 (6)	111	25 (12)	204	0.25	76 (7)	1110	321 (15)	2084	<0.01
connective tissue disease	0	111	0	204	—	2 (0)	1110	20 (1)	2084	0.11
dermatological causes	2 (2)	111	4 (2)	204	1.00	6 (1)	1110	23 (1)	2084	0.25
endocrine/metabolic causes	1 (1)	111	9 (4)	204	<0.05	17 (2)	1110	88 (4)	2084	<0.01
gastrointestinal causes	16 (14)	111	36 (18)	204	0.64	231 (21)	1110	428 (21)	2084	0.77
genitourinary causes	23 (21)	111	14 (7)	204	<0.01	221 (20)	1110	201 (10)	2084	<0.01
gynaecological causes	0 (0)	111	2 (1)	204	0.14	14 (1)	1110	33 (2)	2084	0.36
haematological causes	9 (8)	111	19 (9)	204	0.90	27 (2)	1110	66 (3)	2084	<0.05
infectious disease	17 (15)	111	13 (6)	204	<0.01	287 (26)	1110	179 (9)	2084	<0.01
neurological causes	2 (2)	111	9 (4)	204	0.64	27 (2)	1110	95 (5)	2084	<0.01
oncological causes	15 (14)	111	33 (16)	204	0.77	82 (7)	1110	186 (9)	2084	0.09
orthopaedic causes	6 (5)	111	10 (5)	204	1.00	21 (2)	1110	45 (2)	2084	0.08
pulmonary causes	7 (6)	111	20 (10)	204	0.71	43 (4)	1110	226 (11)	2084	<0.01
trauma	2 (2)	111	2 (1)	204	1.00	20 (2)	1110	37 (2)	2084	0.54
undetermined	4 (4)	111	8 (4)	204	0.55	36 (3)	1110	136 (7)	2084	<0.01
CCI										
Charlson score[a]	3 (1–4)	111	2 (1–3)	204	<0.01	2 (1–3)	1110	2 (0–3)	2082	<0.01
myocardial infarct	9 (8)	111	11 (5)	204	0.70	101 (9)	1110	196 (9)	2083	0.88
congestive heart failure	18 (16)	111	25 (12)	204	0.36	150 (14)	1110	305 (15)	2082	0.45
cerebrovascular disease	12 (11)	111	24 (12)	204	0.15	119 (11)	1110	203 (10)	2083	0.12
chronic pulmonary disease	12 (11)	111	25 (12)	204	0.86	120 (11)	1110	310 (15)	2083	<0.05
mild liver disease	2 (2)	111	6 (3)	204	0.87	49 (4)	1110	53 (3)	2083	<0.05
severe liver disease	7 (6)	111	7 (3)	204	0.41	53 (5)	1110	72 (3)	2083	0.24
severe renal disease	17 (15)	111	22 (11)	204	0.53	183 (16)	1110	261 (13)	2083	<0.01
peripheral vascular disease	18 (16)	111	18 (9)	204	0.08	95 (9)	1110	160 (8)	2083	0.51
connective tissue disease	9 (8)	111	11 (5)	204	0.24	101 (9)	1110	196 (9)	2083	0.65
peptic ulcer	3 (3)	111	5 (2)	204	0.42	57 (5)	1110	101 (5)	2083	0.46
diabetes	30 (27)	111	28 (14)	204	<0.05	232 (21)	1110	365 (18)	2083	<0.05
diabetes with end-organ damage	15 (14)	111	4 (2)	204	<0.01	43 (4)	1110	69 (3)	2083	0.27
hemi/paraplegia	1 (1)	111	4 (2)	204	0.45	41 (4)	1110	58 (3)	2083	0.17
cancer/leukaemia	35 (32)	111	56 (27)	204	0.61	242 (22)	1110	425 (20)	2083	0.67
metastatic solid tumour	9 (8)	111	17 (8)	204	0.34	90 (8)	1110	124 (6)	2083	0.15
AIDS	2 (2)	111	0 (0)	204	—	1 (0)	1110	7 (0)	2083	0.61
dementia	10 (9)	111	9 (4)	204	0.35	73 (7)	1110	96 (5)	2083	0.09
Indwelling devices on enrolment										
tracheal tube	9 (8)	110	5 (2)	204	<0.05	56 (5)	1109	51 (2)	2084	<0.01
central venous catheter	36 (33)	110	36 (18)	203	<0.01	190 (17)	1109	219 (11)	2079	<0.01
arterial vascular access	9 (8)	110	9 (4)	204	0.41	82 (7)	1109	75 (4)	2082	<0.01
peripheral vascular access	83 (76)	109	134 (66)	204	<0.01	863 (78)	1108	1405 (68)	2070	<0.01
urinary catheter	52 (48)	111	48 (24)	204	<0.01	474 (43)	1109	408 (20)	2083	<0.01
tracheostomy	5 (5)	111	3 (1)	204	<0.05	14 (1)	1110	16 (1)	2083	0.70

Continued

Table 2. *Continued*

	REC cohort					SEC cohort				
	BSI (%)	*n*	controls (%)	*n*	*P* value	BSI (%)	*n*	controls (%)	*n*	*P* value
nasogastric tube	16 (14)	111	15 (7)	204	0.09	86 (8)	1108	118 (6)	2083	0.14
wound drainage tube	11 (10)	111	19 (9)	204	0.43	54 (5)	1110	98 (5)	2082	0.99
Characteristics of the BSI										
polymicrobial BSI	9 (8)	111				103 (9)	1099			
hospital onset of BSI (>48 h)	63 (57)	111				308 (28)	1110			
Source										
bone/joint	0	111				5 (0)	1110			
CNS foci	0	111				0	1110			
intervention	1 (1)	111				22 (2)	1110			
ear–nose–throat	0	111				1 (0)	1110			
intra-abdominal	22 (20)	111				207 (19)	1110			
intravascular	2 (2)	111				22 (2)	1110			
lower respiratory tract	7 (6)	111				33 (3)	1110			
skin/soft-tissue	4 (4)	111				26 (2)	1110			
urinary–genital	49 (44)	111				544 (49)	1110			
unknown	26 (23)	111				250 (23)	1110			
Outcomes										
death in hospital	39 (36)	109	11 (5)	204	<0.01	190 (17)	1101	141 (7)	2070	<0.01
death within 30 days after enrolment	34 (32)	105	11 (6)	196	<0.01	180 (17)	1067	137 (7)	1984	<0.01
LOS after enrolment[a] (days)	12 (6–25)	109	6 (3–16)	204	<0.05	10 (6–17)	1101	7 (4–14)	2071	<0.01

P values correspond to Cochran's Q statistic and Friedman's ranks sum test, whenever appropriate.
[a]Median and interquartile range.

Table 3. Impact of REC or SEC BSI on 30 day mortality: univariate and multivariate logistic regression, and comparison of adjusted effect estimates from both cohorts

Type of analysis	*n*	OR for effect measure (95% CI)	Effect measure; potential confounders in model
REC versus controls			
univariate	294	7.2 (3.4–15.2)	REC BSI
multivariate	248	4.6 (1.7–12.3)	REC BSI; tracheal tube, central venous catheter, urinary catheter, transfer from another institution, CCI, number of indwelling devices
SEC versus controls			
univariate	2961	2.7 (2.2–3.4)	SEC BSI
multivariate	2494	1.9 (1.4–2.5)	SEC BSI; age, emergency admission, central venous catheter, urinary catheter, transfer from another institution, number of indwelling devices
REC cohort versus SEC cohort			
comparison of adjusted effect estimates		2.5 (0.9–6.8)	third-generation cephalosporin resistance of *E. coli* BSI

OR, odds ratio.

controls. SEC BSI patients died after a median of 7 days post-infection (IQR 1–17), while the control patients died after a median of 12 days after enrolment (IQR 6–28).

The dynamics of hospital mortality and discharge for both cohorts is illustrated in Figure 1. In both panels (Figure 1a and b) the upper two curves show the cumulative discharge rate for the controls (top curve) and the exposed patients, the lower two curves show the cumulative incidence of hospital mortality, with the lowest curve representing mortality of control patients. Figure 1(a) shows that most patients with REC BSI die relatively soon after ascertainment of the infection, while the discharge rate for exposed patients was lower and much more dispersed

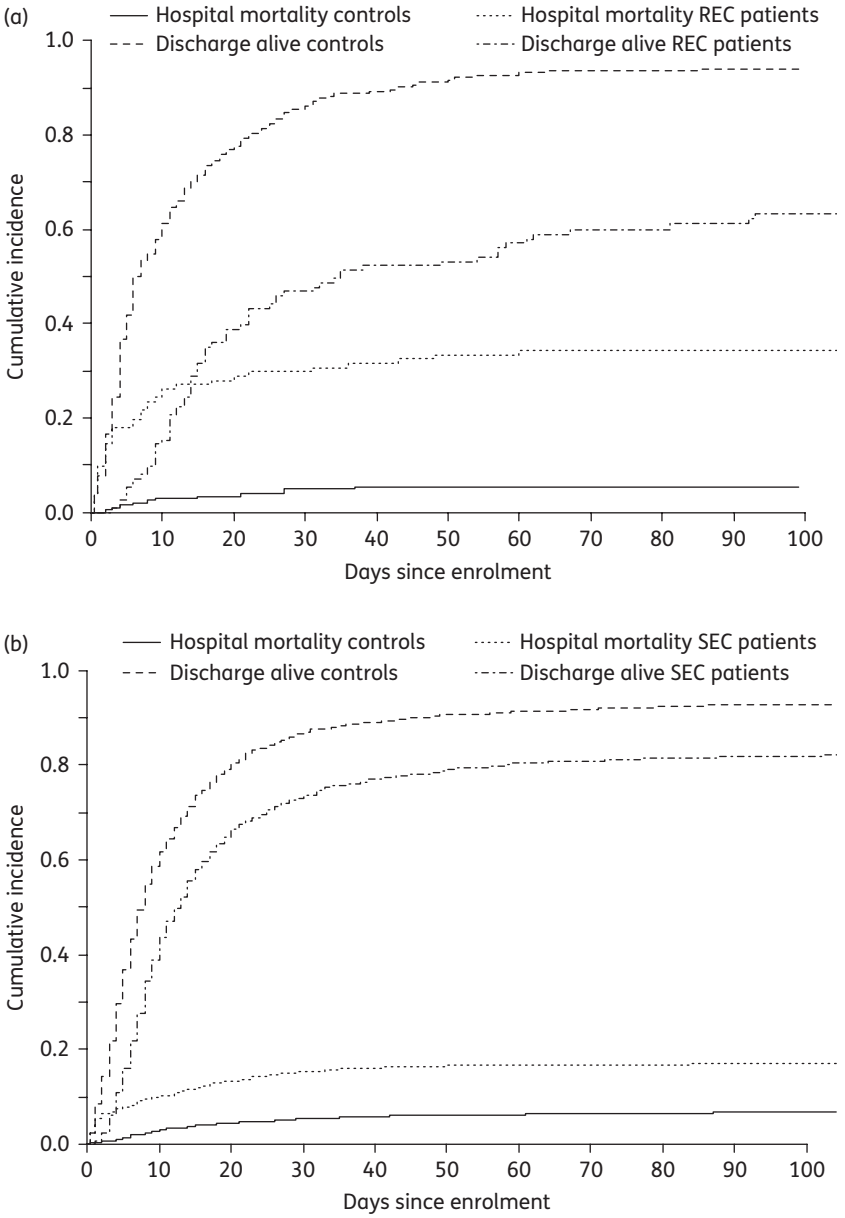

Figure 1. Unadjusted cumulative incidence functions for the competing risks discharge alive and hospital mortality, for patients with and without *E. coli* BSI. (a) Patients with REC BSI and their matched controls. (b) Patients with SEC BSI and their matched controls.

than that for controls. Conversely, Figure 1(b) reveals that patients with SEC BSI were discharged more quickly after ascertainment of the infection and almost at the same rate as the controls.

The same dynamics are captured by the hazard ratios in Table 4. Since stratified analysis showed that group effects at hospital level did not change the coefficients for *E. coli* BSI exposure, the presented results are based on the non-stratified Fine and Gray model. Compared with controls, the adjusted hazard ratio for hospital mortality among patients exposed to REC BSI was 5.7 (95% CI 2.5–13.0), and for patients with SEC BSI 2.0 (95% CI 1.5–2.5). Comparison of excess hospital mortality in the two cohorts showed that the adjusted hazard ratio for patients infected with REC strains was 2.9 (95% CI 1.2–6.9) times higher than the adjusted hazard ratio for patients who had SEC BSI.

LOS

On average, patients with an *E. coli* BSI stayed in hospital longer than control patients. This effect was more pronounced for patients with REC BSI than for patients with SEC BSI. Patients with REC BSI stayed in hospital for a median of 12 days (IQR 6–25) after ascertainment of the infection compared with 6 days (IQR 3–16) for the controls. The patients exposed to

Table 4. Impact of REC or SEC BSI on hospital mortality: univariate and multivariate proportional subdistribution hazards regression model of Fine and Gray, and comparison of adjusted effect estimates from both cohorts

Type of analysis	*n*	HR for effect measure (95% CI)	Effect measure; potential confounders in model
REC versus controls			
univariate	315	7.9 (4.0–15.3)	REC BSI
multivariate	268	5.7 (2.5–13.0)	REC BSI; tracheal tube, central venous catheter, urinary catheter, transfer from another institution, number of indwelling devices
SEC versus controls			
univariate	3193	2.7 (2.2–3.3)	SEC BSI
multivariate	2699	2.0 (1.5–2.5)	SEC BSI; age, emergency admission, tracheal tube, central venous catheter, arterial vascular access, urinary catheter, transfer from another institution, CCI, number of indwelling devices
REC cohort versus SEC cohort comparison of adjusted effect estimates		2.9 (1.2–6.9)	third-generation cephalosporin resistance of *E. coli* BSI

HR, hazard ratio.

Table 5. Impact of a REC or SEC BSI on length of hospital stay after infection: univariate and multivariate analysis (generalized linear model with gamma distribution and loglink function), and comparison of multivariate effect estimates from both cohorts

Type of analysis	*n*	Ratio of mean LOS for effect measure (95% CI)	Extra LOS in days for effect measure (95% CI)	Effect measure; potential confounders in model
REC versus controls				
univariate	311	1.7 (1.3–2.1)	8.4 (4.3–13.0)	REC BSI
multivariate	264	1.5 (1.2–2.0)	7.9 (3.5–13.0)	REC BSI; antibiotic therapy before enrolment, central venous catheter, urinary catheter, nasogastric tube, more than two hospital stays in previous year, transfer from another institution, diabetes with end-organ damage, CCI, number of indwelling devices
SEC versus controls				
univariate	3154	1.2 (1.1–1.3)	2.8 (1.6–4.0)	SEC BSI
multivariate	3116	1.2 (1.1–1.3)	2.9 (1.7–4.0)	SEC BSI; antibiotic therapy before enrolment, central venous catheter, urinary catheter, number of indwelling devices
REC cohort versus SEC cohort comparison of adjusted effect estimates		1.3 (1.0–1.7)	5.0 (0.4–10.2)	third-generation cephalosporin resistance of *E. coli* BSI

SEC BSI stayed for a median of 10 days (IQR 6–17) as compared with 7 days (IQR 4–14) for control patients.

Multilevel analysis showed that group effects at hospital level did not change the coefficients for *E. coli* BSI exposure and therefore the presented results are based on regular GLMs. After taking into account underlying differences between exposed and control patients, patients with REC BSI still had a longer hospital stay; they stayed 1.5 (95% CI 1.2–2.0) times longer than controls, resulting in an excess LOS of 8 days (95% CI 4–13). Patients with SEC BSI also stayed longer in hospital, although the adjusted ratio was 1.2 (95% CI 1.1–1.3), resulting in 3 excess days. Comparing the effects of the REC and SEC cohorts, the excess LOS attributable to third-generation cephalosporin resistance in *E. coli* BSI was estimated to be 5 days (95% CI 0.4–10.2) (Table 5).

Discussion

In Europe, the most rapid increase in antibiotic resistance in *E. coli* has been seen in the past decade.[14] This has been due mainly to the expansion of extended-spectrum β-lactamase (ESBL) genes of the CTX type that confer resistance to most third-

generation cephalosporins. In order to assess the impact of this disquieting trend on the lives of patients and the burden on the health service, we conducted a prospective, multicentre study to determine mortality and prolongation of hospital stay attributable to REC in Europe. Patients with REC BSI were 2.5 (95% CI 0.9–6.8) times more likely to die within the first 30 days after infection and had a 2.9 times (95% CI 1.2–6.9) higher instantaneous probability of dying during their hospital stay compared with patients infected with a susceptible strain. At the same time, third-generation cephalosporin resistance prolonged the duration of hospitalization by 5 days (95% CI 0.4–10.2).

We studied three different outcome measures, which allowed our assessment of the clinical impact of antimicrobial-resistant *E. coli* to be more comprehensive than previously reported. Whilst 30 day mortality is a static measure indicating excess mortality within the first month, hospital mortality, if analysed by appropriate time-to-event methods, provides insight into the temporal dynamics of mortality during the entire hospital stay. Prolongation of hospital stay served as a surrogate marker for morbidity as well as an indicator of costs borne by the healthcare system and society.[28]

The main strength of our study is that through the parallel cohort design and matching on LOS before infection we were able to comprehensively adjust for confounders, including unmeasured residual confounders. This increased the probability that the observed effects are attributable to the resistance phenotype *per se*. The CCI was used to further reduce confounding by differences in health status. As has been shown by others, the CCI is an adequate predictor of mortality in patients with BSI.[29] More importantly, CCI is independent of acute severity of disease and thus is not part of the chain of events between infection and outcome.[30]

A further strength of our study is that we focused on *E. coli*, avoiding non-specific effect estimates arising from studying a combination of pathogens.[31] In this respect, our results can be compared with two single-centre studies. In one large study no impact of resistance in *E. coli* on 30 day mortality was found.[32] However, by including variables that are on the causative pathway such as 'shock' and 'inappropriate therapy', the true effect of resistance was likely underestimated.[30] Results from the second, smaller study were consistent with our estimate for 30 day mortality.[33]

Adjusting for confounding is important to improve the validity of the estimate of interest, but it compromises the precision of the estimate. After controlling for confounding variables, the estimated effect on 30 day mortality [adjusted odds ratio=2.5 (95% CI 0.9–6.8, P=0.08)] was not precise enough to exclude random error at the conventional threshold for an alpha error of $\leq$0.05. However, the increases in hospital mortality and LOS associated with third-generation cephalosporin resistance were significant, indicating that chance is not a very likely explanation. Apart from this limitation there are two possible sources of bias that need to be considered. First, blood culture frequency varied between the centres. This could have caused ascertainment bias, whereby exposed patients were erroneously included as controls, potentially underestimating the impact of *E. coli* BSI on clinical outcome. However, the incidence of BSI is low and therefore the impact of this bias is likely to be low. Furthermore, sensitivity analysis excluding centres with low blood culturing frequency did not change overall effect estimates (data not shown). Second, heterogeneity between participation study centres regarding clinical management of patients or virulence among locally prevalent *E. coli* clones could influence the association between antibiotic resistance and mortality. If this is ignored during the analysis, it could conceal the true relationship between exposure and outcome. However, we showed by multilevel analyses that differences between participating centres did not modify the results. Moreover, other studies have shown that variation in the phylogenetic background of *E. coli* strains[34] was not related to mortality in patients with BSI. Nevertheless, a small effect due to differences in healthcare systems can never be ruled out.

Our results are increasingly relevant, because multiresistant *E. coli* BSIs impose a growing burden on populations in Europe and worldwide.[12,14,35] Moreover, faecal carriage of antibiotic-resistant, frequently ESBL-producing, *E. coli* is increasing in the community[36,37] and is associated with a greatly increased risk of subsequent infection.[37] Reservoirs for multiresistant *E coli* that can spill into the community include nursing homes[38] and food production animals.[39] Finally, few options exist to treat REC infections and new compounds will not become available in the near future.[15] Carbapenems are suggested for empirical treatment of serious infections involving REC; however, over the last few years *E. coli* resistant to third-generation cephalosporins as well as carbapenems have been increasingly reported.[40]

To conclude, we showed that REC BSIs have a considerable impact on mortality and morbidity in Europe. In conjunction with the rapid progression of REC in hospitals and the emergence of these pathogens in the community, high societal and economic costs can be expected. Control strategies aimed at decreasing the overall biomass of REC, and for that matter all other third-generation-cephalosporin-resistant Enterobacteriaceae, as well as additional investments in novel therapeutic approaches, are urgently needed.

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