Impact of a clonal outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in the development and evolution of bloodstream infections by *K. pneumoniae and Escherichia coli*: an 11 year experience in Oxfordshire, UK

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Objectives: The objectives of this study were: (i) to describe an outbreak of multidrug-resistant *Klebsiella pneumoniae* in our population; (ii) to identify the potential source of this outbreak by examining antibiotic resistance trends in urocultures; (iii) to evaluate the contribution of this outbreak to resistance patterns over time in the two commonest Gram-negative blood culture isolates, namely *K. pneumoniae* and *Escherichia coli*; and (iv) to assess risk factors for multidrug resistance and the impact of this resistance on mortality and length of stay.

Methods: We searched Microbiology and Patient Administration Service databases retrospectively and describe resistance trends in *E. coli* and *K. pneumoniae* bloodstream infections (BSIs) in Oxfordshire, UK, over an 11 year period.

Results: An outbreak of a multidrug-resistant, CTX-M-15 extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* clone was identified and shown by multilocus sequence typing to belong to a novel sequence type designated ST490. This was associated with a sporadic change in resistance rates in *K. pneumoniae* BSIs with rates of multidrug resistance (defined as resistance to three or more antibiotic classes) reaching 40%. A case–control study showed prior antibiotic exposure as a risk factor for infection with this organism. During the same time period, rates of ESBL-producing *Klebsiella* spp. isolated from urocultures increased from 0.5% to almost 6%. By contrast, the rate of multidrug resistance in *E. coli* rose more steadily from 0% in 2000 to 10% in 2010.

Conclusions: Changes in resistance rates may be associated with outbreaks of resistant clones in *K. pneumoniae*. Changing resistance patterns may affect important health economic issues such as length of stay.

Keywords: ESBLs, multidrug resistance, MDR

Introduction

Infections due to extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are increasingly seen worldwide. Widespread in South America, Eastern Europe and the Far East by the turn of the century, they have since become increasingly prevalent also in North America and Western Europe.[1] Though their prevalence in the UK is low compared with Asia or Latin America, national and European surveillance programmes have revealed dramatically increasing resistance to cephalosporins amongst *Escherichia coli* and *Klebsiella* spp., largely contingent on the spread of CTX-M ESBLs.[2] ESBL producers are often multidrug resistant, exhibiting co-resistance to other classes of antibiotics, including trimethoprim/sulfamethoxazole, aminoglycosides and the fluoroquinolones; this leaves few options for treatment.[3,4] The hospital epidemiology of these infections is often complex; multiple clonal strains causing focal outbreaks may co-exist with sporadic strains, with ESBL *E. coli* also having a reservoir in the community.[5]

Despite their prevalence, the clinical significance of these resistant organisms is not fully understood. Infections caused by ESBL-producing organisms have been associated with adverse clinical outcomes, including increased mortality, prolonged hospital stay and increased economic cost.[6] Individual

case–control studies have failed to demonstrate consistently a higher mortality for infections caused by these organisms;[1,7–9] however, a meta-analysis showed increased mortality associated with bloodstream infections (BSIs) caused by ESBL-producing organisms.[10] An excess mortality in severe infections or debilitated patients could be explained, in part, by inappropriate initial antimicrobial treatment, but again the data on this point are conflicting, with some studies suggesting that inadequate initial therapy for bacteraemia impacts on mortality whilst others found no such differences.[11–15]

A broad range of risk factors for infections with ESBL-producing Enterobacteriaceae have been identified in different studies, in part reflecting the heterogeneity of these studies. The prior use of antibiotics,[16] particularly cephalosporins,[9,17,18] is consistently associated with an increased risk of infection with ESBL-producing strains. Other risk factors identified by some studies include intensive care unit admission,[9] permanent urinary catheter,[16] anaemia,[16] duration of ventilation,[18] poor nutritional status or the need for artificial feeding,[19] recent surgery,[20] haemodialysis[21] and decubitus ulcers.[22]

Our aim was to describe patterns of antibiotic resistance over time in our population. Our routine surveillance identified a sporadic increase in rates of resistance in *Klebsiella pneumoniae* and we sought to further investigate this by molecularly characterizing representative strains during the assumed outbreak and by investigating risk factors for infection in a case–control study. We aimed to establish whether there was a potential source in the transmission dynamics of this outbreak by studying resistance patterns in urine cultures. Finally, to evaluate the contribution of this outbreak to resistance patterns over time, we compared trends in rates of resistance in our two commonest Gram-negative BSI isolates, namely *K. pneumoniae* and *E. coli*, and explored risk factors for multidrug resistance and the effect this trait had on mortality and length of stay.

Methods

Data sources and collection

Our study took place at the Oxford Radcliffe Hospitals NHS Trust (Oxford, UK), a teaching hospital complex comprising the John Radcliffe Hospital, the Radcliffe Infirmary, the Churchill Hospital (all in Oxford) and the Horton Hospital, a district general hospital in Banbury, 48 km north of Oxford. These hospitals offer all of the specialist regional services, including cardiothoracic surgery, neurosurgery, nephrology and haematology/oncology. The Trust is the sole provider of acute clinical and microbiology services to approximately 600 000 people. Other hospitals in the region include a specialist orthopaedic hospital, psychiatric hospitals and several community hospitals; these were not included in our study.

Using the hospital microbiology information system, we generated a database of all blood cultures taken from patients admitted to the Oxford Radcliffe NHS Trust hospitals that cultured either *E. coli* or *K. pneumoniae* from 1 July 1999 to 30 June 2010. These were then transformed into 'cases of BSI' as defined below. Our analysis was confined to patients aged ≥16 years, as mortality was used as an outcome measure and preliminary analysis showed that mortality was too low in people under 16 to be a reliable marker of the effect of antibiotic resistance (data not shown). Susceptibilities for each isolate were recorded for the nine antibiotics consistently tested over the 11 year study period (amoxicillin, co-amoxiclav, ceftazidime, ciprofloxacin, ceftriaxone, gentamicin, meropenem, piperacillin/tazobactam and trimethoprim). Resistance rates to amoxicillin and trimethoprim were high and so these were disregarded;

Klebsiella spp. are anyway inherently resistant to amoxicillin. We separated antibiotics into five classes: (i) inhibitor combinations (co-amoxiclav and piperacillin/tazobactam); (ii) third-generation cephalosporins (ceftriaxone and ceftazidime); (iii) ciprofloxacin; (iv) gentamicin; and (v) meropenem. Multidrug-resistant isolates were defined as those resistant to three or more of these classes.

Further information regarding each case of BSI was extracted from the hospital Patient Administration System (PAS) (for the 10 years to 30 June 2009) and included dates of admission and discharge (and therefore length of stay for each admission with a BSI), age, gender, community or hospital acquisition, specialty, 7 and 30 day mortality and previous hospital exposure (for admission BSIs). If no admission was recorded in the PAS, the dates of admission and discharge were taken to be the date of the positive culture; this applied for numbers of renal, haematology and oncology patients, who are often managed as day cases.[23]

A simpler database of urinary isolates (from January 2005 to June 2010) was also constructed, detailing all urines that had yielded an *E. coli* or a 'coliform' and whether these were ESBL producers or not (see the Microbiological processing section).

Microbiological processing

BSI was detected using the Bactec 9240 system (Becton Dickinson, USA) with a standard incubation period of 5 days, extended to 10 days if endocarditis was suspected. Urinary infections were identified by growths of >10^4 cfu/mL on Cystine Lactulose Electrolyte Deficient agar or BD™ CHROMagar™ Orientation Medium (Becton Dickinson, New Jersey, USA). Identification of isolates was by standard methodology including API20E® (bioMérieux, Lyon, France). Susceptibility testing for BSI and urine culture isolates was performed using a modified Stokes disc diffusion method, controlled with *E. coli* NCTC 10418.[24] The method was subject to internal and external quality assurance (NEQAS) with satisfactory performance. From 2005 onwards, ESBL production was identified by synergy between clavulanate (as co-amoxiclav) and ceftriaxone and/or ceftazidime.[25] The method was subject to internal and external quality assurance (NEQAS) with satisfactory performance.

We grouped urine isolates into (i) *E. coli* and (ii) 'coliforms', meaning Gram-negative bacteria that were not *E. coli*, *Proteus* or *Pseudomonas* spp.), in accordance with the HPA National Standard Operating Procedure BSOP 41 Section 4.5.1.[26] *E. coli* and 'coliforms' exhibiting resistance to four or more of six 'first-line' antibiotics (amoxicillin, co-amoxiclav, cefradine, trimethoprim, nitrofurantoin and ciprofloxacin) were further characterized by testing for ESBL activity from January 2005. ESBL production was identified by synergy between clavulanate (as co-amoxiclav) and ceftriaxone and/or ceftazidime.[25] Isolates with ESBL activity were identified to species level by API20E® (bioMérieux, Lyon, France).

Case–control study

K. pneumoniae isolates from July 2007 to June 2009 that were resistant to ceftazidime and/or ceftriaxone were analysed further as potential ESBL-producing organisms. These isolates (5 of 53 BSIs from 2007, 4 of 45 BSIs from 2008 and 21 of 50 BSIs from 2009) were stored as frozen samples and the 28 that were recoverable (2 isolates from 2008 were omitted) were sent for further analysis to the Health Protection Agency's Centre for Infections (London, UK). MICs of antibiotics, with and without β-lactamase inhibitors, were determined using BSAC agar dilution methodology. PFGE of XbaI-digested genomic DNA was used to test for the clonal relatedness of isolates. Multilocus sequence typing (MLST) of a representative isolate of an 'outbreak strain' was undertaken in accordance with the 'Pasteur' scheme as described by Diancourt et al.[27] bla_{CTX-M} genes were identified by group-specific PCR as described by Poirel et al.[28] (prom+ 5′-TGC TCT GTG GAT AAC TTG C-′3 and preCTX-M 5′-CCG TTT CCG CTA TTA CAA AC-′3). As there were no apparent outbreaks

or surges in resistance rates in *E. coli*, we did not seek to characterize any of the isolates, though it is likely that many belong to the international ST131 clone, which has expanded in the UK, as elsewhere, since around 2002.[29]

We performed a case–control study on the source patients for these isolates to identify potential risk factors for ESBL *K. pneumoniae* infection. The cases were patients with BSIs with *K. pneumoniae* that were resistant to ceftazidime and/or ceftriaxone (found to be ESBL producers) where medical notes were available (*n*=23). The controls were cases of BSI with *K. pneumoniae* resistant only to amoxicillin± trimethoprim (*n*=29). They were the closest susceptible isolate (resistant only to amoxicillin±trimethoprim) by date to a case, and were matched by gender and specialty. We also compared patients with the 'outbreak strain' (*n*=12; see the Results section) with all other isolates as controls (non-outbreak ESBL cases and control cases; *n*=40). The variables investigated for association with infections by ESBL producers and the outbreak clone included the following: hospital exposure; hospital acquisition; antibiotic exposure in the last 3 months; urinary catheter; vascular access device (peripheral cannula or central line); mechanical ventilation; renal impairment (creatinine >200 μmol/L); diabetes mellitus; immunosuppression (including corticosteroid treatment); malignancy; and residence in a nursing home.

Definitions

A case of BSI

A bacteraemic episode with isolation of *E. coli* or *K. pneumoniae* from blood culture when the same species had not been cultured from blood from the same patient within the previous 2 weeks. Cases were not counted if there was no corresponding entry on the PAS system (0.6% of positive cultures).

Multidrug resistant

An isolate of *K. pneumoniae* or *E. coli* resistant to three or more of the five antibiotic classes defined above.

Hospital-acquired BSI

A case of BSI arising >48 h after admission (as defined by the CDC, USA).[30]

Admission BSI

A case of BSI arising <48 h after admission. Renal and haematology/ oncology patients were excluded as they are often treated as outpatients or day cases.

Intensive care unit

A general adult intensive care unit, neurology intensive care unit or cardiothoracic critical care unit. Our hospitals did not have separate general medical and surgical intensive care units and all cultures taken on an intensive care unit were counted as 'intensive care' irrespective of other specialty.

Specialty of cases

Nationally recognized admission specialty codes were used to group cases. The PAS recorded each episode of admission to hospital, including admissions for haemodialysis and day care, as well as longer admissions. To simplify analysis, we grouped some specialties as follows: medicine (all medical specialties excluding renal medicine, haematology and

oncology); general surgery (including vascular and urological surgery); and specialist surgery (trauma, obstetrics and gynaecology, otorhino-laryngology, neurosurgery and cardiothoracic, eye and oral surgery). Haematology and oncology were grouped together, and renal medicine was counted separately. If only an Emergency Department attendance was recorded, then this was counted as the specialty.

Hospital exposure

Defined as having a record of previous hospital admission in the 365 days prior to the date of the case of BSI. The length of time (1–365 days) since discharge from this last admission to date of admission pertaining to the case blood culture was recorded. Renal and haematology/oncology patients were assumed to have had hospital exposure in the previous 365 days.

Seven and thirty day mortality

Death of a patient within 7 or 30 days of the date of BSI.

Statistical analysis

We analysed two sets of data: (i) the full BSI dataset; and (ii) a subgroup of admission BSIs. The 'admission BSI' subgroup excluded renal, haematology and oncology patients as these patients are regularly in hospital, often for short day-case procedures such as chemotherapy or dialysis, and so it is harder to be clear what is community acquired and what is hospital acquired.[23]

To examine secular trends in antibiotic resistance we plotted the proportion of blood-culture *E. coli* and *K. pneumoniae* that were resistant to each of the nine antibiotics examined in eleven 12 month blocks from 1 July 1999. We also plotted the proportions for each organism that were resistant to cephalosporins (ceftriaxone and/or ceftazidime) and also that were resistant to three or more of the antibiotic classes defined; 95% confidence intervals (CIs) for these proportions were calculated (modified Wald) and added as error bars to graphs. These data were examined by the χ^2 test for trends.

Trends in ESBL production rates among urine isolates were analysed by calculating the proportions of *E. coli* and proportions of 'coliforms' identified as *K. pneumoniae* that were identified as ESBL producers. These were plotted in eleven 6 month blocks from 1 January 2005; 95% CIs for proportions were calculated (modified Wald) and added as error bars to graphs.

SPSS 17.0 for Windows was used for all analyses. We built multivariate logistic regression models describing 7 and 30 day mortality, multidrug resistance (resistance to three or more of the defined antibiotic classes) and length of stay, according to patient and BSI characteristics. The continuous variables 'age' and 'length of stay' were converted to dichotomous variables comparing patients aged <75 years versus those ≥75 years and those with a stay of <8 days versus ≥8 days. Univariate analysis was performed and then all significant variables were added to the multivariate models, with logistic regression performed using the forced entry method. The results are shown as odds ratios (ORs) with 95% CIs.

For the case–control study, OR and *P* values were calculated using logistic regression for categorical variables. Differences were analysed between cases and controls and then between 'outbreak strain' and 'non-outbreak strain' isolates.

Results

Resistance trends

Antibiotic resistance rates in *E. coli* rose steadily over time (χ^2=36, *P*<0.001 for multidrug resistance and χ^2=65, *P*<0.001 for co-amoxiclav) (Figure 1a), whereas resistance

rates in *K. pneumoniae* fluctuated, with two clear peaks in 2004 and 2009 (Figure 1b). In 2010 the ESBL rate in *K. pneumoniae* BSIs remained high but had reduced from the 2009 peak.

K. pneumoniae 'outbreak'

Antibiotic susceptibility testing of the 28 *K. pneumoniae* isolates sent for further analysis showed that 27 were ESBL producers with a CTX-M phenotype, whilst the remaining isolate had a ceftazidimase-type ESBL. PFGE showed that 15 of the 21 isolates from 2009 belonged to a single clone, designated JOHN23KL-8, a pattern not previously identified by the UK reference laboratory and not present among the seven isolates tested from 2007 and 2008. Group-specific PCR of bla_{CTX-M} genes identified the JOHN23KL-8 clone isolates to have CTX-M-15 enzyme, and MLST revealed them to belong to a novel sequence type, designated ST490.
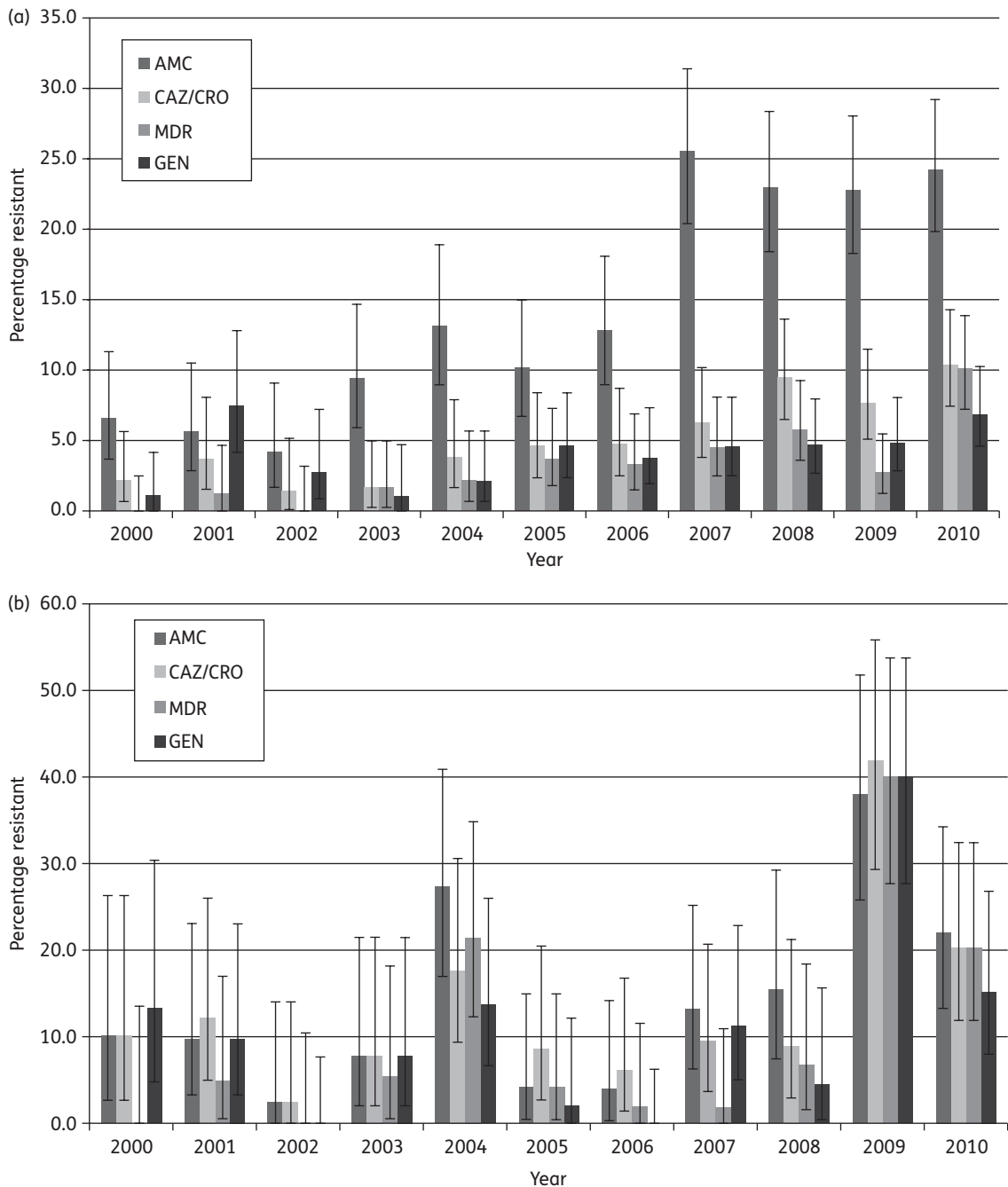

Figure 1. (a) Percentages of *E. coli* BSIs from 1 July 1999 to 30 June 2010 resistant to different antibiotics. (b) Percentages of *K. pneumoniae* BSIs from 1 July 1999 to 30 June 2010 resistant to different antibiotics. Error bars are 95% CIs (modified Wald). AMC, co-amoxiclav; CAZ/CRO, ceftazidime and/or ceftriaxone; MDR, multidrug resistant; GEN, gentamicin.
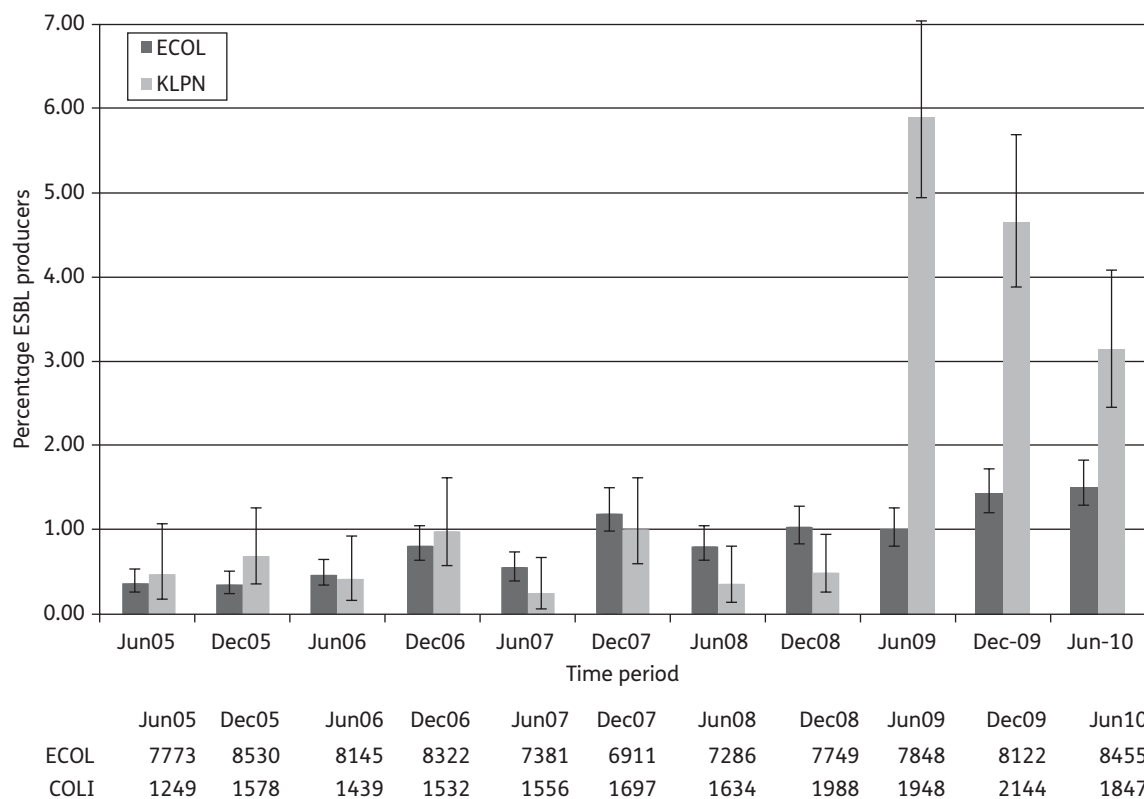
Figure 2. Percentages of urinary isolates testing positive for ESBL enzyme identified as *E. coli* or *K. pneumoniae* from 1 July 2005 to 30 June 2010. Error bars are 95% CIs (modified Wald). The table shows the total number of *E. coli* and coliforms cultured for each time period. ECOL, *E. coli*; KLPN, *K. pneumoniae*; COLI, coliforms (excluding *E. coli* and *Proteus* spp.).

Table 1. Case–control study

Factor	Outbreak strain (n=12), n (%)	Non-outbreak strain *Klebsiella* (n=40), n (%)	OR (95% CI)	P value
Age (years)				
mean	71.8	67.5		
SD	19.2	16.3		
range	26–92	37–96		
30 day mortality	3 (25)	9 (23)	1.61 (0.47–5.53)	0.45
Male:female	7:5	29:11		
Hospital exposure	10 (83)	23 (58)	3.70 (0.72–19.1)	0.12
Hospital acquired	7 (58)	19 (48)	1.55 (0.42–5.70)	0.512
Antibiotic exposure	12 (100)	25 (63)	a	≤0.05
Catheter	8 (67)	21 (53)	0.55 (0.14–2.13)	0.39
Peripheral cannula	9 (75)	22 (55)	2.46 (0.58–10.44)	0.22
Central line	7 (58)	14 (35)	2.60 (0.70–9.7)	0.16
Ventilated	5 (42)	10 (25)	2.14 (0.55–8.29)	0.27
Renal impairment	5 (42)	8 (20)	2.86 (0.72–11.41)	0.14
ITU	6 (50)	11 (28)	2.64 (0.70–9.94)	0.15
Diabetes	3 (25)	10 (25)	1.00 (0.23–4.44)	1.0
Immunosuppressed	2 (17)	10 (25)	0.60 (0.11–3.21)	0.55
Malignancy	6 (50)	14 (35)	1.86 (0.50–6.85)	0.35
NH resident	0 (0)	4 (10)	a	0.56

ITU, intensive care unit; NH, nursing home.
[a]Unable to calculate OR using logistic regression as all of the cases have or none of the cases has the predictor variable.

Urine cultures

Alongside the upsurge in multidrug-resistant *K. pneumoniae* BSI isolates in 2009, there was a corresponding rise in ESBL-producing *Klebsiella* spp. amongst coliforms in urine cultures (Figure 2). In the subsequent 12 months, the proportion of urinary isolates with this resistance decreased but did not return to pre-2009 levels.

Case–control study

There were no obvious epidemiological links between the cases infected with ESBL *K. pneumoniae* in general, nor among those infected with the JOHN23KL-8 clone. The JOHN23KL-8 isolates were isolated from both admission- and hospital-acquired blood cultures and were scattered across several different units with no identifiable links between patients.

The only risk factor identified for ESBL status was exposure to antibiotics in the previous 3 months (24/27 versus 17/29, $P=0.015$). This was also the only risk factor for having an isolate being of the outbreak strain (Table 1).

Outcomes and risk factors

Mortality

Crude 7 and 30 day mortality for all *E. coli* and *K. pneumoniae* BSIs over the 10 year period (to 30 June 2009) are shown in Table 2. Irrespective of resistance, isolation of *K. pneumoniae* (OR 1.29, 95% CI 1.01–1.65) was a predictor of 30 day mortality, as were age ($\geq$75 years), male gender, hospital-acquired BSI and blood cultures being taken in an intensive care unit or from a haematology/oncology patient (Table 3). Isolation of a multidrug-resistant isolate did not predict either 7 or 30 day mortality.

Table 2. *E. coli* and *K. pneumoniae* BSI demographics over 10 years[a]

	All BSIs			Admission BSIs		
	all ($n=2516$)	*E. coli* ($n=2070$)	*K. pneumoniae* ($n=446$)	all ($n=1386$)	*E. coli* ($n=1220$)	*K. pneumoniae* ($n=166$)
Sex						
male	1255	990	265	767	698	69
female	1261	1080	181	619	522	97
Age (years)						
mean	68.5	69	65	71.6	71.5	72.8
SD	17.9	17.9	17.5	18.1	18.4	15.2
median	72.9	68.2	73.7	76.5	76.7	75.1
range	16–102	16–102	17–96	16–102	16–102	20–96
Acquired						
community	1760	1505	255	NA	NA	NA
hospital	756	565	191	NA	NA	NA
Specialty						
general medicine	1305	1142	163	1028	917	111
general surgery	365	288	77	250	204	46
specialist surgery	139	114	25	58	53	5
intensive care	179	120	59	42	38	4
renal[b]	203	164	39	NA	NA	NA
haematology/oncology[b]	317	234	83	NA	NA	NA
emergency	8	8	0	8	8	0
Hospital exposed[b]						
none	NA	NA	NA	725	667	58
in previous 365 days	NA	NA	NA	661	553	108
Resistance						
not MDR	2358	1970	388	1318	1169	149
MDR	107	59	48	39	26	13
Mortality						
7 day	284 (11.3%)	222 (10.7%)	62 (13.9%)	146 (10.5%)	117 (9.6%)	29 (17.5%)
30 day	505 (20.1%)	399 (19.3%)	106 (23.8%)	235 (17%)	190 (15.6%)	45 (27.1%)

MDR, multidrug resistant; NA, not applicable.
[a]Demographic data available for first 10 years of BSI data.
[b]Renal and haematology/oncology patients assumed to have been in hospital in previous 365 days.

Table 3. Univariate analysis of predictors of 30 day mortality for all BSIs

Factor	OR	95% CI	P value
Sex			
male	1.45	1.19–1.77	<0.0005
female	1.0		
Age			
<75 years	1.0		
≥75 years	2.42	1.98–2.56	<0.0005
Organism			
E. coli	1.0		
K. pneumoniae	1.29	1.01–1.65	0.039
Acquired			
community	1.0		
hospital	1.67	1.36–2.05	<0.0005
Specialty			
medicine	1.0		
general surgery	0.60	0.43–0.83	0.002
specialist surgery	0.60	0.36–0.99	0.045
intensive care	1.48	1.04–2.10	0.03
renal	0.53	0.34–0.82	0.005
haematology/oncology	1.32	0.99–1.75	0.056
Resistance			
not MDR	1.0		
MDR	1.37	0.87–2.14	0.172

MDR, multidrug resistant.

Table 4. Multivariate analysis of predictors of length of stay ≥8 days

Factor	OR	95% CI	P value
Age			
<75 years	1.0		
>75 years	1.65	1.30–2.11	<0.0005
Organism			
E. coli	1.0		
K. pneumoniae	1.51	1.02–2.22	0.038
Hospital exposure			
none	1.0		
last 365 days	1.21	0.95–1.53	0.121
Specialty			
medicine	1.0		
general surgery	0.91	0.67–1.23	0.520
specialist surgery	0.61	0.35–1.09	0.094
intensive care	12.01	2.78–51.91	0.001
Resistance			
not MDR	1.0		
MDR	2.91	1.27–6.63	0.011

MDR, multidrug resistant.

Subgroup analysis of admission BSIs showed the same predictors for both 7 and 30 day mortality, with the additional predictor of hospital exposure in the previous 365 days (for 30 day mortality: OR 1.78, 95% CI 1.34–2.37, $P<0.0005$). This remained significant on multivariate analysis. Again, multidrug resistance did not predict mortality.

Length of stay

For admission BSIs, multidrug resistance (OR 2.77, 95% CI 1.23–6.22, $P=0.014$), previous hospital admission (OR 1.27, 95% CI 1.01–1.60, $P=0.041$) and isolation of *K. pneumoniae* (OR 1.53, 95% CI 1.05–2.22, $P=0.026$) predicted a longer hospital stay (i.e. equal to or greater than the median length of stay of 8 days), as did age ≥75 years (OR 1.58, 95% CI 1.26–1.99, $P<0.0005$) on univariate analysis. On multivariate analysis, multidrug resistance remained a strong independent predictor of increased length of stay (Table 4). Resistance to third-generation cephalosporins (taken as a surrogate for ESBL production though probably also including a minority of isolates with AmpC-type enzymes) also predicted an extended length of stay.

Risk factors for multidrug-resistant BSIs (resistant to three or more antibiotic classes)

The factors predicting BSI with a multidrug-resistant isolate were blood cultures taken in hospital versus the community (OR 1.86, 95% CI 1.34–2.58) and isolation of *K. pneumoniae* versus *E. coli* (OR 2.89, 95% CI 2.05–4.08). These remained independent predictors on multivariate analysis (Table 5). For admission BSIs, isolation of *K. pneumoniae* versus *E. coli* (OR 2.62, 95% CI 1.47–4.65) and hospital exposure in the last 365 days (OR 2.85, 95% CI 1.67–4.85) predicted multidrug resistance; however, only isolation of *K. pneumoniae* remained an independent predictor on multivariate analysis (Table 5). Our preliminary analysis showed that there was no difference in the effect of 'previous admission within 90 days' and 'previous admission within 91 to 365 days' (data not shown) and hence the use of 365 days as the cut-off.

Discussion

We have identified, and describe here, an interesting pattern of sporadic increases in multidrug resistance, including ESBL production, in *K. pneumoniae* in Oxfordshire, in contrast to a steady increase over time in resistance in *E. coli*. This pattern in *E. coli is* in keeping with other published data for the UK and elsewhere.[2] Surges in rates of resistance in *K. pneumoniae* in 2004 and 2009 suggest outbreaks of multidrug-resistant organisms. In 2009, this was found to be due to an outbreak of a single, novel CTX-M-15 ESBL-producing *K. pneumoniae* clone within our population. Our search for the source of this outbreak revealed a corresponding increase in the isolation of ESBL-producing *Klebsiella* spp. from community and hospital urine cultures. We have described this outbreak in the context of an 11 year experience of *E. coli* and *K. pneumoniae* BSIs in which multidrug resistance is associated with an extended length of stay but not mortality.

Our data show that 71% of the multidrug-resistant *K. pneumoniae* isolates (15 of 21 examined) from the 2009 'surge' belonged to the same PFGE clone, indicating the dissemination

Table 5. Multivariate analysis of predictors of multidrug-resistant BSIs (all BSIs and admission BSIs)

Factor	All BSIs			Admission BSIs		
	OR	95% CI	P value	OR	95% CI	P value
Organism						
E. coli	1.0			1.0		
K. pneumoniae	3.53	2.35–5.31	<0.0005	3.29	1.63–6.67	0.001
Acquired						
community	1.0			NA	NA	NA
hospital	1.86	1.21–2.85	0.005	NA	NA	NA
Hospital exposure						
none	NA	NA	NA	1.0		
previous 365 days	NA	NA	NA	1.93	0.97–3.83	0.061
Specialty						
medicine	1.0			1.0		
general surgery	1.61	0.93–2.77	0.087	1.65	0.79–3.45	0.181
specialist surgery	0.81	0.31–2.14	0.668	2.40	0.69–8.30	0.167
renal	1.70	0.89–3.25	0.109	NA	NA	NA
haematology/oncology	1.43	0.70–2.92	0.331	NA	NA	NA
intensive care	0.92	0.47–1.80	0.813	[a]	[a]	[a]

NA, not applicable.

[a]No admission cultures from ITU had multidrug-resistant organisms; therefore there was no logistic regression.

of a single resistant clone among our population. This contrasts with data in recent publications from Jamaica and Mexico, which show the endemic persistence of different clones of ESBL-producing *K. pneumoniae*.[9,31] However, the ESBL *K. pneumoniae* isolates and 'outbreak clone' occurred across a variety of clinical areas with no evidence of patient-to-patient transmission or epidemiological link. In view of the relatively low numbers of ESBL *K. pneumoniae* BSI (∼50 per year) it is possible that this pattern of sporadic resistance peaks could occur by chance, but the concurrent increase in rate of isolation of ESBL-producing *K. pneumoniae* in urine samples in 2009 suggested this was a real phenomenon. This also highlights the possible role of urosepsis in the dynamics of this outbreak. The ESBL *Klebsiella* rate in urine cultures decreased after the 2009 upsurge but remained significantly higher than baseline 12 months later, suggesting persistence of this resistant clone in our population.

Rates of resistance to co-amoxiclav, a commonly used first-line antibiotic, rose steadily in *E. coli* before increasing to >20% in the last 4 years studied. This rise coincided temporally with its introduction in our hospitals as the first-line antibiotic for community-acquired sepsis (as a replacement for cefuroxime). By contrast, resistance rates to gentamicin in non-ESBL isolates remained low. A striking feature of the rates of resistance is the collinearity of the rates of third-generation cephalosporin and multidrug resistance for both *E. coli* and *K. pneumoniae*. This is in keeping with observations that ESBL producers are often multidrug resistant.[3,4]

Isolation of a multidrug-resistant organism did not predict mortality in this study. Crude 30 day mortality rates for *E. coli* and *K. pneumoniae* BSIs were high at 19.3% and 23.8%, respectively, with the excess for *K. pneumoniae* not significant once their greater likelihood of being hospital-acquired was factored in.

A systematic review of 16 studies of BSIs caused by ESBL producers concluded that resistance (in the form of ESBL production) was associated with increased mortality, but only one of the studies included had corrected for potential confounders.[10] Confounding remains a limitation of the present study, exactly as for many of those on the mortality risk of BSIs with methicillin-susceptible and -resistant *Staphylococcus aureus*. Another potential limitation is that our data only include information on *E. coli* and *K. pneumoniae* isolates and it is possible that a small number of these patients were contemporaneously bacteraemic with other organisms, i.e. that they had polymicrobial sepsis or that others had infections due to ESBL-producing strains of other species.

The identification of risk factors for multidrug-resistant BSIs may help clinicians in their choices of empirical antibiotic therapy for individual patients, potentially resulting in better outcomes. In this study, as expected, hospital-acquired BSIs were more likely to be associated with multidrug-resistant isolates than community-acquired BSIs. In the subanalysis of admission BSIs, previous hospital exposure (a hospital admission within the last 365 days) was an additional risk factor, though this only tended towards significance (P=0.061) on multivariate analysis. Broader-spectrum antibiotics could therefore be targeted to these patients, and possibly the more elderly, who we have shown have poorer outcomes in terms of both mortality and length of stay.

In our case–control study, the only risk factor identified for ESBL *K. pneumoniae* BSIs and for the specific 'outbreak' clone was exposure to antibiotics in the preceeding 3 months. Antibiotic exposure exerts strong selective pressure and is an important modifiable risk factor for antibiotic resistance. Failure to identify other risk factors may have reflected the relatively

small size of the study. Alternatively, it may be indicative of the heterogeneous nature of the population with no apparent epidemiological links.

In summary, we have shown a steady increase in antibiotic resistance including ESBL activity in *E. coli* and sporadic increases in *K. pneumoniae* BSIs associated with the recent dissemination of a clone of ESBL-producing *K. pneumoniae*. We have demonstrated a significant association of multidrug resistance with increased length of stay but not mortality. Prior hospital admission may be a risk factor for antibiotic-resistant *E. coli* and *K. pneumoniae* BSIs.

Transparency declarations
None to declare.

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