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The impact of antibiotic use on the incidence and resistance pattern of extended-spectrum beta-lactamase-producing bacteria in primary and secondary healthcare settings

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The emergence and spread of bacteria producing extended-spectrum beta-lactamases (ESBLs) has important therapeutic and epidemiologic implications.
- A key target for the establishment of hospital antibiotic stewardship is reducing the occurrence of additional antibiotic resistance.
- Further research is needed to accumulate supporting evidence that reducing antibiotic use will result in a parallel reduction in antibiotic resistance.

WHAT THIS STUDY ADDS

- Fluoroquinolone restriction reversed ciprofloxacin resistance in primary and secondary healthcare settings.
- Fluoroquinolone restriction reduced ESBL-producing bacteria incidence rates in both the primary and secondary healthcare settings.
- This study highlights the value of time-series analysis in designing efficient antibiotic stewardship.

AIMS

The objective of the present study was to study the relationship between hospital antibiotic use, community antibiotic use and the incidence of extended-spectrum beta-lactamase (ESBL)-producing bacteria in hospitals, while assessing the impact of a fluoroquinolone restriction policy on ESBL-producing bacteria incidence rates.

METHODS

The study was retrospective and ecological in design. A multivariate autoregressive integrated moving average (ARIMA) model was built to relate antibiotic use to ESB-producing bacteria incidence rates and resistance patterns over a 5 year period (January 2005–December 2009).

RESULTS

Analysis showed that the hospital incidence of ESBLs had a positive relationship with the use of fluoroquinolones in the hospital (coefficient = 0.174, $P = 0.02$), amoxicillin-clavulanic acid in the community (coefficient = 1.03, $P = 0.03$) and mean co-morbidity scores for hospitalized patients (coefficient = 2.15, $P = 0.03$) with various time lags. The fluoroquinolone restriction policy was implemented successfully with the mean use of fluoroquinolones (mainly ciprofloxacin) being reduced from 133 to 17 defined daily doses (DDDs)/1000 bed days ($P < 0.001$) and from 0.65 to 0.54 DDDs/1000 inhabitants/day ($P = 0.0007$), in both the hospital and its surrounding community, respectively. This was associated with an improved ciprofloxacin susceptibility in both settings [ciprofloxacin susceptibility being improved from 16% to 28% in the community ($P < 0.001$)] and with a statistically significant reduction in ESBL-producing bacteria incidence rates.

DISCUSSION

This study supports the value of restricting the use of certain antimicrobial classes to control ESBL, and demonstrates the feasibility of reversing resistance patterns post successful antibiotic restriction. The study also highlights the potential value of the time-series analysis in designing efficient antibiotic stewardship.

Introduction

Extended-spectrum beta-lactamases (ESBLs) are a heterogeneous group of bacterial enzymes that inactivate beta-lactam antibiotics by hydrolysis, resulting in the development of resistance to a variety of antibiotic agents [1]. The continued emergence of ESBL-producing pathogens poses significant therapeutic implications, i.e. complicated therapy and limited treatment options, predisposing infected patients to higher mortality and longer length of hospital stay, and causing serious consequences for infection control [2, 3]. In addition to being recognized as relevant to nosocomial infections, the production of ESBLs is also increasingly becoming an important public health issue with regard to community-acquired infections [4]. Established risk factors for infection or colonization by ESBL-producing organisms include greater severity of clinical status, intensive care unit stay and the insertion of various types of indwelling catheters [4, 5]. Antibiotic consumption (i.e. the use of third generation cephalosporins, other beta-lactams and fluoroquinolones) is also a well-established risk factor which has been shown to be associated with the acquisition of ESBL-producing organisms [2, 6, 7]. The widespread use of antibiotics in the community setting is also believed to be contributing to higher rates of resistance in hospitals [8–10]. However, few studies have investigated this hypothesis. A key reason for the establishment of hospital antibiotic stewardship is to attempt to reduce the occurrence of antibiotic resistance [11, 12], but uncertainty persists as to whether reducing antibiotic use will result in a parallel reduction in antibiotic resistance [13].

The objective of the present study was firstly to study relationships existing between hospital antibiotic use, community antibiotic use and the incidence of ESBL-producing pathogens in hospital. A secondary aim was to assess the impact of a fluoroquinolone restriction policy that was introduced in the study site hospital and in the primary care community following a major *Clostridium difficile* infection (CDI) outbreak [14], on hospital and community ciprofloxacin susceptibility patterns in ESBL-producing pathogens. The present study was conducted using time-series analysis, as applying simple regression analysis would be inappropriate to evaluate temporally sequenced observations on ESBL-producing organisms and antimicrobial drug use, which are not independent [10, 15–17].

Methods

Setting and study period

The Northern Health and Social Care Trust (NHSCT) consists of four acute hospitals: Antrim Area Hospital (AAH) (411 beds), Mid-Ulster Hospital (124 beds), Whiteabbey Hospital (130 beds) and Causeway Hospital (242 beds), serving a population of approximately 420 000 inhabitants. Antrim Area Hospital is a general teaching hospital that provides all acute, general medical and surgical services, supports a range of outpatient facilities and acts as a centre for the co-ordination of health service provision throughout a defined geographical area in Northern Ireland. All healthcare centres in primary care send their specimens to the AAH laboratory for assessment. The present investigation consisted of two components: (i) an ecological, retrospective investigation which involved collecting data on a monthly basis on the hospital use of antibiotics and the incidence of hospital ESBL-producing organisms over a 5 year period (January 2005–December 2009) and (ii) an ecological retrospective analysis which involved collecting monthly data on the usage of antibiotics in the community setting and the ciprofloxacin sensitivity patterns of community derived ESBL-producing pathogens over the same 5 year study period (January 2005–December 2009). The former component included only AAH. The latter included data on community antibiotic use across the Trust and ESBL-producing pathogens for three hospitals (for which electronic records were available within the AAH microbiology computer systems), i.e. AAH, Mid-Ulster Hospital and Whiteabbey Hospital.

Microbiology and pharmacy data

The monthly numbers of ESBL-producing organism cases identified in patient samples (hospital/community) were obtained from the clinical microbiology information system over the study period. Hospital ESBLs represent cases that were identified during a patient hospital stay. Community cases represent cases identified in community samples sent to the AAH laboratory for analysis. Duplication was removed from these data so that the first positive isolate only was considered. Within the hospital laboratory, samples were processed according to routine microbiology procedures. Mid-stream specimens of urine (MSSU) were cultured using a semi quantitative culture method of Leigh & Williams [18] and presumptive identification using the differential culture media. The MSSU was inoculated onto a Columbia blood agar plate containing 5% horse blood (Oxoid Limited, Basingstoke, UK) and a CPS ID 3 (CPS) agar plate (chromogenic medium, bioMerieux® sa., Marcy-Etoile, France) and incubated aerobically at 37°C for 18–24 h. The semi-quantitative count was recorded and colonies that appeared as either a pink or blue/green colour on CPS agar were recorded as coliforms. Antibiotic sensitivity testing employed the methodology of the Clinical and Laboratory Standards Institute (CLSI), using Mueller-Hinton agar and antibiotic sensitivity discs (BBL™ Sensi-Disc™, Becton, Dickinson and Company, Maryland, USA) with incubation performed at 35°C for 16–18 h. A cefpodoxime disc (CPD 10) was included to screen all coliform bacteria for the possibility of ESBL production. Any isolate displaying resistance to cefpodoxime was fully identified using the Vitek® (bioMerieux® sa., Marcy-Etoile,

France) Gram negative identification card (GN). Antibiotic sensitivity testing was performed using Vitek®, the AST-GN27 antibiotic sensitivity card and the Vitek® AST-N142 which incorporates cefotaxime and ceftazidime alone (at 0.5 μg ml^{-1}) and in combination with clavulanic acid (4 μg ml^{-1}) for the detection of ESBLs. ESBL ciprofloxacin susceptibility rates were calculated by dividing susceptible ESBL isolates over susceptible and non-susceptible ESBL isolates.

Co-morbidity scores (calculated using the Charlson Index) were obtained from the Hospital Episode Statistics (HES) in the AAH [19]. Bed occupancy data over the study period were obtained at monthly intervals to calculate the incidence of ESBL-producing pathogens per 1000 bed days. The monthly quantities of each antibiotic delivered for patient care to each ward of the hospital were obtained from the hospital pharmacy information system. These quantities were converted into defined daily doses (DDDs) following the recommendations of the World Health Organization (WHO) [20] The numbers of DDDs for individual antibiotics were then grouped into classes belonging to group J01 (antibacterials for systemic use) of the Anatomical Therapeutic Chemical (ATC) classification system from the WHO Collaborating Center for Drug Statistics Methodology. Hospital antibiotic use was expressed as DDDs/1000 bed days and community antibiotic use was expressed as DDDs/1000 inhabitants/day.

Antibiotic restriction policy

AAH: the use of fluoroquinolones was restricted (January 2008) by removal from the institutional guidelines for empirical antibiotic treatment, with the following exceptions: for the treatment of epididymo-orchitis, prostatitis, pelvic inflammatory disease, orbital cellulitis and class IV cellulitis in cases of penicillin allergy. Fluoroquinolones were removed from all wards and where treatment of a patient with a restricted antibiotic was required, a Trust exemption form had to be completed stating the diagnosis and this had to be approved by a consultant. All exemption forms were screened by the antimicrobial pharmacists and referred to the Consultant Medical Microbiologists if inappropriate use of restricted antibiotics was suspected.

NHSCT community: following the occurrence of the CDI outbreak in January 2008, a leaflet was sent to all general practitioners (GPs) in the area served by the Trust. The leaflet classified fluoroquinolones as high risk drugs. Non-prescribing of fluoroquinolones was continuously reinforced via prescribing meetings with GPs, regular feedback (quarterly) on GPs' prescribing patterns and training on appropriate antibiotic use.

Statistical analysis

Autoregressive integrated moving average (ARIMA) models, using the Box–Jenkins methodology [21], were used to evaluate whether relationships existed between antibiotic use and the incidence of ESBLs as described elsewhere [10, 16, 17]. To evaluate the effect of the fluoroquinolone restriction policy on ciprofloxacin sensitivity patterns, dummy variables were created, whereby 0 and 1 represented the pre and post intervention periods respectively. All variables were logarithmically transformed. A transfer function model, which consists of modelling a time series as a function of its past values and random errors, was built. For each individual series, an ARIMA model was identified and fitted according to the Box & Jenkins methodology [21]. The model was identified by determining the ARIMA model orders (p, d, q) using autocorrelation and partial autocorrelation. The model parameters were then estimated by the unconditional least squares method. Finally, the adequacy of the model was checked [16, 17] and statistical significance of the parameters determined. After identification of the multivariate transfer function models, the cross-correlation function was determined by estimating the correlations between the series describing antibiotic use at different time lags (up to 5 months) and the ESBL series. Significance tests for parameter estimates were used to eliminate the unnecessary terms in the model. A P value of 0.05 was considered to be statistically significant. The final model was derived by the econometric 'general-to-specific' approach. The most parsimonious model with the highest biological plausibility was presented in this research. All time series analyses were performed using EViews 6 software (QMS, Irvine, CA, USA).

Results

Over the 5 year study period (January 2005–December 2009), a total of 244 ESBL cases were identified in the AAH, and a total of 965 ESBL community cases were identified in the NHSCT. The average observed monthly ESBL incidence was 0.448/1000 bed days (range 0.102–1.26) and 0.001 per 1000 inhabitant days (range 0.0004–0.002) in the hospital and surrounding community, respectively. Trends in the use of each class of antibiotic, in the study site hospital and the NHSCT community setting, are presented in Table 1. The results showed that the use of some antibiotic classes (e.g. macrolides) increased over the study period, whereas other classes (e.g. second and third generation cephalosporins) showed a trend of decreased usage. The use of some other antibiotic classes (e.g. combinations of penicillins including beta-lactamase inhibitors) remained approximately constant (Table 1). The most widely used antibiotic class in the AAH was combinations of penicillins including beta-lactamase inhibitors (38.2%), followed by macrolides (18.7%), whereas in the community sample, penicillins with extended spectrum (28.0%), tetracyclines (20.7%) and combinations of penicillins including beta-lactamase inhibitors (11.5%) were the most widely used antibiotics (Table 1). The mean monthly co-morbidity

Table 1

Characteristics of the monthly antimicrobial usage in the Antrim Area Hospital (AAH) and the Northern Health and Social Care Trust surrounding community (January 2005–December 2009)

Antimicrobial class (ATC group)	Hospital use Average monthly use in DDD/1000 bed days (range)	% of J01 use	Trend 2005–2009 Coefficient	*P* value	Community use Average monthly use in DDD/1000 inhabitant-days (range)	% of J01 use	Trend 2005–2009 Coefficient	*P* value
Tetracyclines (J01A)	21.37 (0–93.75)	2.28	0.604223	<0.0001	4.60 (3.21–5.66)	20.68	0.025538	<0.0001
Penicillins with extended spectrum (J01CA)	45.35 (14.72–126.26)	4.83	0.923323	<0.0001	6.24 (3.58–13.03)	28.05	0.026611	0.0429
Beta-lactamase sensitive penicillins (J01CE)	22.42 (8.02–128.54)	2.39	0.112285	0.338	0.88 (0.61–1.17)	3.96	0.001638	0.0665
Beta-lactamase resistant penicillins (J01CF)	45.22 (17.70–85.05)	4.82	0.054682	0.6036	0.92 (0.69–1.23)	4.14	0.004907	<0.0001
Combinations of penicillins including beta-lactamase inhibitors (J01CR)	358.67 (232.9–583.4)	38.24	0.082277	0.9047	2.55 (1.92–3.26)	11.47	–0.002987	0.1948
First generation cephalosporins (J01DB)	3.59 (0.34–11.61)	0.38	–0.068703	<0.0001	0.70 (0.43–0.93)	3.15	–0.00677	<0.0001
Second generation cephalosporins (J01DC)	20.79 (0.53.28)	2.22	–0.847516	<0.0001	0.27 (0.12–0.47)	1.21	–0.004751	<0.0001
Third generation cephalosporins (J01DD)	6.00 (0.16–20.25)	0.64	–0.091052	0.001	0.004 (0–0.01)	0.02	–0.00002	0.0754
Carbapenems (J01DH)	13.63 (0–36.86)	1.45	0.342264	0.001	0.0001 (0–0.002)	0.0004	–0.000004	0.0475
Trimethoprim and derivatives (J01EA)	22.92 (10.39–40.28)	2.44	0.067854	0.169	1.33 (1.06–1.67)	5.98	0.008486	<0.0001
Combination of sulfonamides and trimethoprim (J01EE)	2.76 (0–9.92)	0.29	0.011876	0.4968	0.20 (0.12–0.29)	0.9	0.000174	0.5756
Macrolides (J01FA)	175.23 (90.78–359.23)	18.68	1.551382	<0.0001	3.07 (2.03–4.74)	13.8	0.015828	<0.0001
Lincosamides (J01FF)	6.45 (0.32–18.72)	0.69	–0.053604	0.0926	0.02 (0.01–0.03)	0.09	–0.000193	<0.0001
Aminoglycosides (J01GB)	11.92 (4.97–27.44)	1.27	0.104084	0.001	0.01 (0–0.03)	0.04	0.00004	0.2709
Fluoroquinolones (J01MA)	72.99 (4.56–156.01)	7.78	–2.042128	<0.0001	0.69 (0.51–0.82)	3.1	–0.001308	0.0086
Glycopeptide (J01XA)	26.14 (10.13–58.47)	2.79	0.500587	<0.0001	0.0007 (0–0.03)	0.003	0.00002	0.0012
Steroid antibacterials (J01XC)	13.47 (2.92–39.72)	1.44	0.023364	0.6571	0.02 (0–0.04)	0.09	–0.000189	0.0016
Imidazole derivatives (J01XD)	54.87 (26.13–89.15)	5.85	–0.382894	0.0002	0.23 (0.19–0.29)	1.03	0.000803	<0.0001
Nitrofuran derivatives (J01XE)	5.43 (0.46–16.04)	0.58	0.088432	0.0001	0.51 (0.26–0.85)	2.29	0.009523	<0.0001
Other antibacterials (J01XX)	4.24 (0–16)	0.45	0.01711	0.4658	0.01 (0–0.03)	0.04	0.00004	0.001
Antibacterials for systemic use, Total (J01)	938.03 (575.45–1201.34)	100	1.234052	0.2464	22.24 (15.77–33.01)	100	0.07749	0.0006

Table 2

Multivariate time series analysis model for monthly hospital ESBL incidence ($r^2 = 0.38$)

Term	Lag time†	Coefficient (SE)‡	T ratio	*P* value
Constant		–1.490571 (0.685698)	–2.174	0.0349
Fluoroquinolone use (hospital use)	1	0.175570 (0.072854)	2.410	0.0200
Amoxicillin-clavulanic acid use (community use)	4	1.026560 (0.464634)	2.209	0.0322
Mean co-morbidity index	2	2.148540 (0.974978)	2.204	0.0326
AR§	4	0.343144 (0.147462)	2.327	0.0244

†Represents the delay necessary to observe the effect (in months). ‡Indicates the size and the direction of the effect. §AR, autoregressive term representing past incidence density of ESBLs.

index was 0.627 (range 0.510–0.778) for hospitalized patients.

Time series analysis showed that the incidence of ESBL-producing pathogens in the AAH had a positive relationship with the use of fluoroquinolones (mainly ciprofloxacin) in hospital, amoxicillin-clavulanic acid in the community and mean co-morbidity scores for hospitalized patients (AAH), with various time lags (Table 2). For example, temporal variations in the incidence of ESBL-producing pathogens followed temporal variations in fluoroquinolones use with an average delay of 1 month. This means that, on average, an increase (or decrease) in fluoroquinolone use by 1% resulted 1 month later in an increase (or decrease) of the incidence of ESBLs by 0.18%. Effects of different sizes with different delays were observed for community amoxicillin-clavulanic acid use (average delay = 4 months, coefficient = 1.027, $P = 0.032$), and mean co-morbidity index (average delay = 2 months, coefficient = 2.149, $P = 0.033$). One stochastic term was introduced into the model, i.e. an autoregressive term (AR) with a lag time of 4 months (Table 2), which reflected auto-correlation in the incidence of ESBLs in AAH, i.e. this incidence was related to the incidence observed in the previous months. The determination coefficient (r^2) of the final model was 0.38, i.e. 38% of the variation in the incidence of ESBLs in AAH over the study period was explained by the factors included in the model. Graphical representations of the relationships between the monthly use of fluoroquinolones, amoxicillin-clavulanic acid, mean co-morbidity index *vs.* the monthly incidence of ESABLs in AAH are presented in Figure 1.

Analysis showed that the restriction policy relating to fluoroquinolones led to a statistically significant reduction in their use (mainly ciprofloxacin) (coefficient = –96, $P < 0.001$, $r^2 = 0.88$; immediate effect), with the mean use being reduced from 113 DDDs/1000 bed days to 17 DDDs/1000 bed days. Interestingly, this was associated with an improved susceptibility of ESBL-producing pathogens to ciprofloxacin in hospital (Figure 2) and with a reduction in the ESBL-producing pathogen incidence rates (average delay = 2 months, coefficient = –0.44, $P = 0.017$, $r^2 = 0.24$). Similarly, analysis showed that the restriction policy in the NHSCT community had a positive impact on reducing fluoroquinolone use (mainly ciprofloxacin) (average delay = 2 months; coefficient = –0.11; $P = 0.0007$; $r^2 = 0.66$), with the mean use being reduced from 0.65 DDDs/1000 inhabitants/day to 0.54 DDDs/1000 inhabitants/day. The latter was also associated with an improved susceptibility of ESBL-producing pathogens to ciprofloxacin (Figure 3), with susceptibility being improved from 16% to 28% (coefficient = 12, $P < 0.001$, $r^2 = 0.27$, immediate effect), and with a reduction in the ESBL-producing pathogen incidence rates (average delay = 2 months, coefficient = –0.22, $P = 0.016$, $r^2 = 0.10$).

Discussion

The increasing prevalence of infections caused by antibiotic-resistant pathogens, for which antibiotic consumption has been recognized as the main driver, remains a challenging issue worldwide. The objective of the present study was to study relationships between hospital antibiotic use, community antibiotic use, and the incidence of ESBL-producing pathogens and to assess the impact of a fluoroquinolone restriction policy on the susceptibility of these pathogens to ciprofloxacin. The study demonstrated statistically significant temporal relationships between the use of certain antibiotic classes and the incidence of ESBL-producing pathogens identified in hospital inpatients. This latter incidence was also linked to mean co-morbidity scores for hospitalized patients.

The hospital use of fluoroquinolones was positively correlated with an increased incidence of ESBL-producing pathogens in the study site hospital. The latter findings were consistent with those reported by others in relation to the contribution of this antibiotic class to high incidence rates of ESBL-producing pathogens in health care settings [7, 9, 10], thus following the lines of evidence for a cause–effect relationship between antibiotic use and resistance proposed by McGowan [22]. Additionally, the results of this research demonstrated an association between antibiotic use in the community (i.e. amoxicillin-clavulanic acid) and the incidence of ESBLs in hospitals, highlighting the importance of the interaction between antibiotic use in the community and the development of antibiotic

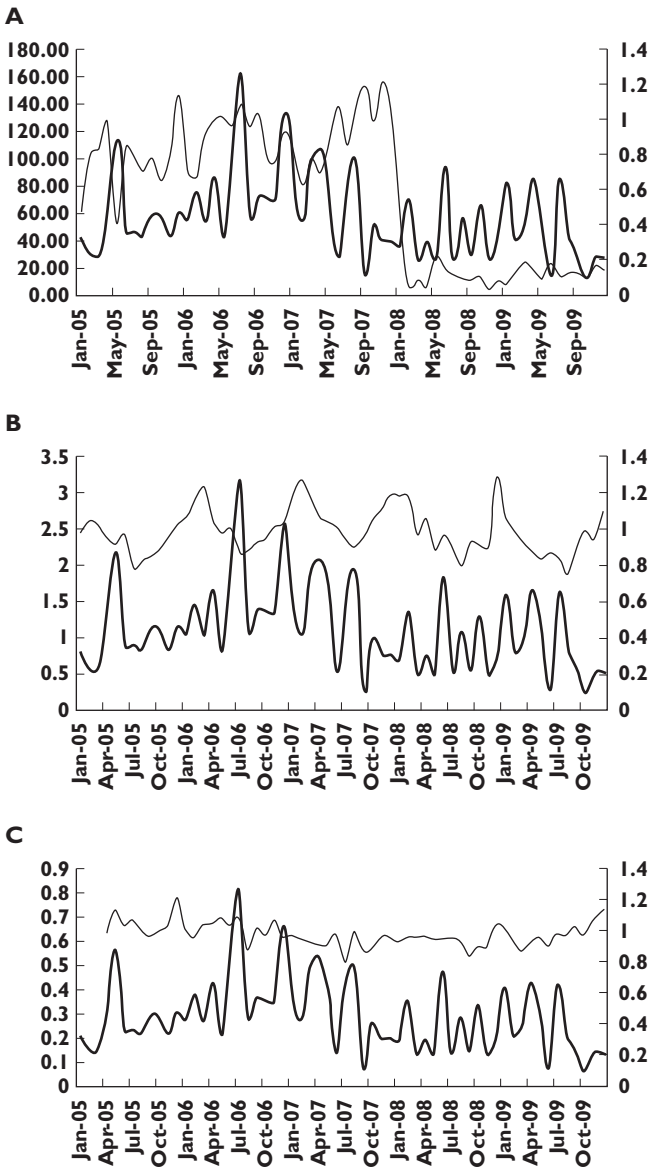

Figure 1

Monthly ESBL incidence *vs*. use of selected antibiotic classes or mean co-morbidity index, Antrim Area Hospital, January 2005–December 2009 (ESBLs, number of cases/1000 bed days, —— right y-axis, antimicrobial use and mean co-morbidity index, —— left y-axis). A) fluoroquinolones (DDDs/1000 bed days), B) community amoxicillin-clavulanic acid (DDDs/1000 inhabitants days) and C) mean co-morbidity index. Fluoroquinolone restriction (A) commenced in January 2008 ($P < 0.001$). Relationships between the presented antibiotics and ESBLs are presented in Table 2

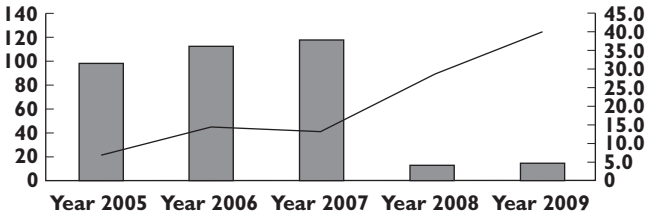

Figure 2

Yearly percentage of ciprofloxacin-susceptibility among ESBL-producing bacteria (right y-axis) *vs*. average yearly use of ciprofloxacin (DDDs/1000 bed days, left y-axis), Antrim Area Hospital (January 2005–Decemebr 2009). Fluoroquinolone restriction commenced in January 2008 ($P < 0.001$). Data were aggregated at the yearly level to ensure sufficient presentable data. Ciprofloxacin use (■); Ciprofloxacin-susceptibility (%) (——)

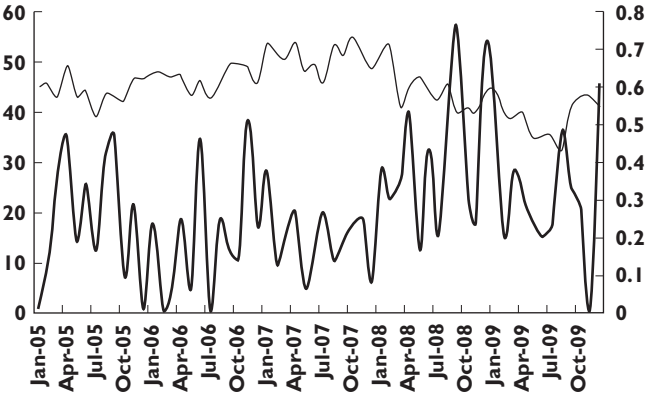

Figure 3

Monthly percentage ESBL ciprofloxacin-susceptibility rate (—— left y-axis) *vs*. monthly use of ciprofloxacin (—— right y-axis), the NHSCT area community (January 2005–December 2009). Fluoroquinolone restriction commenced in January 2008 ($P = 0.0007$) and this was associated with an improved susceptibility of ESBL-producing pathogens to ciprofloxacin ($P < 0.001$)

resistance in hospitals. The findings were consistent with the resistance patterns obtained from the AAH microbiology department, which showed that ESBLs were almost always resistant to amoxicillin-clavulanic acid (only 8% of ESBL isolates were susceptible). A possible explanation for such an interaction could be related to an increased hidden reservoir of resistant pathogens in the community (due to community exposure to antibiotic use), which may spread into hospitals on admission. Such findings confirm other few time series analysis reports in this area [9, 10] and may be of benefit to inform antibiotic stewardship in primary care settings. The non-observed relationship between the use of hospital amoxicillin-clavulanic acid and hospital ESBL-incidence rates should be interpreted with caution and be investigated with a larger sample size.

While much effort has been devoted towards the establishment of antibiotic stewardship procedures in primary and secondary healthcare settings, there is a lack of robust methodological support to guide informed decisions on optimizing antibiotic prescribing (e.g. antibiotic cycling/restriction policy). The analysis of time series is considered the strongest quasi-experimental design to evaluate longitudinal effects of healthcare interventions in the absence of a concurrent control group as stated by the Cochrane Effective Practice and Organization of Care Group (EPOC) [15]. Experience in healthcare Trusts in Northern Ireland showed the value of the latter methods

[17] in informing specific elements of an antibiotic stewardship programme in one healthcare Trust, which contributed to a reduction in the incidence of the CDI rates in that Trust [23]. Details on the possibility of the use of time series modelling in informing antibiotic cycling/restriction policies have been presented elsewhere [16, 17, 24].

In addition to antibiotic use, the model included a proxy measure for patient case mix characteristics i.e. Charlson co-morbidity index. To our knowledge, this is the first study which has included the Charlson co-morbidity index, to help adjust for patient case-mix, within time series analysis modelling. Measuring co-morbidity is considered an essential criterion in determining the disease burden, thus providing risk-adjustment criteria for case-mix purposes [25]. The role of the co-morbidity index in increasing the incidence of ESBL-producing pathogens is possibly associated with increased healthcare staff workload (in taking care of sicker patients) and the influence of this on compliance with hand hygiene and other infection control precautions. The co-morbidity index might be used to target the patient with enhanced clinical pharmacy and infection control services.

Restriction of certain classes of antibiotics has been proposed as one of the antibiotic-resistance control measures [26]. Whereas this could be practically implemented in hospital settings, the implementation of an antibiotic restriction policy in primary healthcare settings is more difficult. The latter was supported and maintained through continuous educational efforts as described earlier. In the present study, the findings showed that the fluoroquinolone restriction policy was successful with the mean use of fluoroquinolones (mainly ciprofloxacin) being significantly reduced in hospital and the community setting. Interestingly, this was associated with an improved ESBL-producing pathogen susceptibility to ciprofloxacin in both settings, with the ciprofloxacin susceptibility being improved from 16% to 28% in the community setting. The ciprofloxacin susceptibility was restored with immediate effect, as identified by the time series analysis technique. Moreover, the restriction of fluoroquinolone was associated with a reduction in the incidence of ESBL-producing pathogens in both the study site hospital and the surrounding community. However, the observed r^2 was relatively small for the surrounding community.

It is scientifically plausible that decreasing antimicrobial use may lead to a reversal of antimicrobial resistance. However, this may vary with different pathogens and different antibiotic classes [27]. According to the line of evidence proposed by Lipsitch [27], the impact of reducing antibiotic prescribing on reversing antibiotic resistance is mainly driven by the 'fitness cost' of resistance to bacteria. Antimicrobial resistance considerably impairs the growth rate or infectiousness of some bacteria, thereby limiting the ability of resistant infections to spread [27]. Few studies have assessed the relationship between reducing the use of specific antibiotics and the subsequent restoration of antibiotic susceptibility [13]. Our findings confirm the latter investigation and strongly suggest that changes in ciprofloxacin susceptibility of ESBL-producing pathogens may be improved, following successful restriction, within a short timescale.

The study design has several strengths, including the use of time series analysis techniques which allowed for the accurate determination of the significant variables, their size effects, and the average delays to observe an effect. In addition, the study involved all patients hospitalized during the study period, with the exception of paediatric patients who were excluded since the WHO DDDs system is not applicable for this group of patients. Furthermore, data were collected as part of routine hospital practice and independently from the study. Thus, selection and information bias are unlikely. The study has also some limitations. Firstly, associations demonstrated by quasi-experimental studies at the population level may not correlate with associations at the level of individual patients [28]. Secondly, it was not possible to adjust for the effect of infection control practices, which were re-enforced during a major CDI outbreak that occurred in the AAH [14], on incidence of ESBL-producing pathogens during the study period. Such parameters may explain the 62% of the variance which was not explained by our model. Similarly, the evaluation of the impact of the restriction policy involved modelling the restriction policy as dummy variables, i.e. other possible predictors (e.g. infection control, patient characteristics, veterinary antibiotic usage) were not assessed. Such possible variables may be involved in the percentage which was not explained by the presented models.

Thirdly, measuring Charlson Index comorbidity was performed utilizing data (i.e. primary and secondary diagnoses) that were obtained from the Hospital Episode Statistics (HES) in the AAH, which may have underestimated the co-morbidity burden. However, the coding was undertaken by clinical coders, with significant coding experiences, thus, contributing to greater record accuracy and completeness. Finally, the identification of ESBL-producing pathogens is challenging due to single isolates producing multiple different beta-lactamase types, varying substrate affinities and inoculum effect. This may result in the occurrence of both false positive and false negative results with all phenotypic confirmatory tests. However, the sensitivity and specificity of the Vitek 2 system, which was employed in the present study, is in excess of 90% and eliminates errors due to subjective interpretation [29].

In conclusion**,** the present research attempted to clarify relationships between antibiotic use and incidence of ESBL-producing pathogens in hospitals, while assessing the impact of an antibiotic restriction policy (in both primary and secondary healthcare settings) on ESBL incidence rates. The findings of this study support the role of fluoroquinolone use and amoxicillin-clavulanic acid in increasing the incidence of ESBL-producing pathogens in

hospitals. Fluoroquinolone restriction reversed ciprofloxacin resistance and reduced ESBL-producing bacteria incidence rates in primary and secondary healthcare settings.

Importantly, restricting specific classes may result in an increase in the use of other classes [30], which may act as risk factors for the development of other pathogen resistance [16, 17, 30], thus highlighting the importance of monitoring antibiotic policies and promoting the informed use of non-restricted agents. The results showed a positive association between co-morbidity index and ESBLs incidence rates. Finally, measuring the size of effects and delays to observe an effect, which was possible using time series analysis, may facilitate designing efficient antibiotic stewardship.

Competing Interests

There are no competing interests to declare.

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