Antimicrobial use and antimicrobial resistance in *Enterobacterales* species and *Enterococcus faecium*: a time series analysis.

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Keywords

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Summary

Irish and European antimicrobial resistance(AMR) surveillance data has highlighted increasing levels of resistance in *Enterobacterales* species and vancomycin resistance in *Enterococcus faecium*(VRE). Antimicrobial consumption(AC) in the Irish hospital setting is increasing.

Methods

A retrospective time series analysis(TSA) was conducted to evaluate the trends and possible relationship between AC of selected antimicrobials and the incidence of AMR in clinical isolates of *enterobacterales* species and *E. faecium* isolates resistant to vancomycin, from January 2017 to December 2020.

Results

Increased AC was seen in ceftriaxone(p= 0.0006), piperacillin/tazobactam(p = 0.03) and meropenem(p = 0.054) while ciprofloxacin and gentamicin use decreased.

The rates of AMR of *E.coli* were trending downwards, AMR rates of *K.pneumoniae* was stable or decreasing, while AMR rates for the other *enterobacterales* increased modestly with the greatest concern related to the increase in ertapenem resistance from 0.581% in 2017 to 5.19% in 2020 (p= 0.003). The rate of VRE decreased from 64% in 2017 to 53% in 2020 (p = 0.1).

TSA identified a correlation between piperacillin/tazobactam use and the decreasing rate of ceftriaxone resistance in *E.coli* isolates.

Conclusion

The decreasing or relatively stable rates of AMR found in e*nterobacterales* and VRE isolates in this study reflect the positive effect of the hospital AMS programme. The increasing incidence of CPE seen in this study should be addressed as part of the local AMS programme. The COVID-19 pandemic resulted in increased broad-spectrum antimicrobial use, however, it is too early to determine the impact of these changes on AMR rates.

Introduction

Antimicrobial resistance (AMR) is a significant threat to public health [1]. The increasing levels of resistance among gram negative *enterobacterales* organisms, and levels of

vancomycin-resistance among the gram positive *Enterococcus faecium* (VRE) organism are causing concern across Europe [2] and in Ireland [3].

The well-established link between inappropriate and excessive use of antimicrobials and selection for AMR [4] has contributed to the rise in AMR among the *enterobacterales* species [5]. Antimicrobial stewardship (AMS) interventions are an important element in tackling AMR and are well established in the Irish hospital setting [6]. However, the median overall rate of antimicrobial consumption (AC) in the Irish hospital setting has increased by 16% between 2009 and 2019 [7].

The COVID-19 pandemic associated with the novel coronavirus, SARS-CoV-2 [8], has had a significant impact on healthcare systems and delivery worldwide [9]. Many routine AMS activities have been reduced and the impact of this on the incidence of AMR are yet to be determined [10]. Furthermore, evidence suggests that there has been widespread and excessive prescription of antimicrobials due to the similarities between the clinical presentation, and difficulties in differentiating between COVID-19 and bacterial pneumonia, along with the absence of antiviral treatments with proven efficacy [11].

There is a lack of studies linking AC and AMR in the Irish hospital setting. Given the prevailing trends in AC and AMR in Ireland and the knowledge that AC is generally recognised as the primary driver of AMR, it is important to investigate how changes in AC influence bacterial susceptibility and at what time scale. This will assist in the development of policies to manage AMR particularly following the COVID-19 pandemic.

In this study we aim to investigate the trends and possible relationships between AC and AMR in *enterobacterales* species, and the proportion of *Enterococcus faecium* isolates resistant to vancomycin, between 2017-2020 in an Irish hospital setting. The AMR data will also be compared with EU and other Irish hospital data.

Methods

Hospital setting

The study hospital is a 271 bed, inner city, acute University Teaching Hospital, in the Republic of Ireland. The hospital is comprised of various medical and surgical specialities, a paediatric unit, and a general intensive care unit. The hospital established a formal AMS programme in 2007. Key AMS events and policies implemented prior to and during the study period are summarised in Supplementary data Table I.

Antimicrobial consumption

Inclusion criteria

Quarterly aggregated hospital AC data (dispensed to inpatients on all hospital wards) for antimicrobial agents indicated in the treatment of infections caused by the *enterobacterales* species were collated. This consisted of the following antimicrobial agents: Ceftriaxone, Ciprofloxacin, Levofloxacin, Ertapenem, Meropenem, Piperacillin/tazobactam, Gentamicin, Co-trimoxazole and Aztreonam. Data was also collated for Vancomycin.

Exclusion criteria

Antimicrobials dispensed to the out-patient setting.

Antimicrobials not indicated for the treatment of infections caused by the *enterobacterales* species.

The AC data was obtained from the hospital pharmacy electronic dispensing records for the study period (1/1/2017 to the 31/12/2020). The data was converted to the standardised WHO's Anatomical Therapeutic Chemical (ATC) Classification of defined daily dose (DDD and antibiotic usage was expressed as quarterly aggregated DDDs according to the 2018 ATC classification and normalised per 100 hospital/bed days used (BDU) [12].

AMR data

Inclusion criteria

Clinical microbiology specimens (blood, sterile fluid, sputum, urine, wound and anaerobic specimens) which identified the following organisms: *E.faecium*, *E.coli, K.pneumoniae* and other *enterobacterales* species processed from patients during an inpatient hospital stay during the study period.

Exclusion criteria

Duplicate isolates from the same patient [13].

Microbiological isolates from community samples or outpatient clinics.

AMR data was collected from the hospital Microbiology laboratory database for the study period (1/1/2017 to the 31/12/2020). Bacterial identification and antibacterial susceptibility testing were performed in the clinical microbiology laboratory of the hospital using the Vitek®2 (bioMérieux) system according to the manufacturer's guidelines. Antimicrobial susceptibility was assessed according to the recommendations of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) antimicrobial susceptibility testing references and definitions [14]. Percentage resistance to a specified antimicrobial were calculated for the selected bacteria. The resistance rate was defined as the percentage of total isolates that were resistant to the selected agent. All non-susceptible isolates (i.e. resistant and intermediate) were considered resistant.

Additional AMR data

The European Antimicrobial Resistance Surveillance Network (EARS-Net) reports population-weighted EU/EEA wide AMR rates [3] using data submitted by medical microbiology laboratories including the study hospital. While there is some variability in reporting across Europe, data from Ireland is estimated to cover 96% of the population with a high geographical, hospital, patient and isolate representativeness. This AMR data is based on antimicrobial susceptibility testing generated using EUCAST methodology for invasive (blood or cerebrospinal fluid) isolates. Where groups of antimicrobials are reported, the reported figure is based on the result for the antimicrobial showing the highest level of resistance [3]. EU and National data is only available for VRE, *E.coli* and *K.pneumoniae*.

Statistical analysis

Initial analysis of the evolving trends in AC and AMR rates was conducted by linear regression analysis. Further analysis was conducted using time series analysis (TSA), which has been used previously to investigate possible correlations between AC and AMR where the data are measured repeatedly at equal intervals of time [15-17]. The Box–Jenkins method of TSA modelling was used to develop univariate autoregressive integrated moving

average (ARIMA) models of the AC and AMR data [18]. Following the development of univariate ARIMA models, a linear transfer function modelling method [16, 17] was used to investigate the dynamic relationship between antimicrobial use and the incidence of resistant isolates, considering possible time delays (lag times). These methods are described in detail in the supplemental data. All statistical analyses were performed with R version 4.0.3.

The study is reported according to the Strengthening the reporting of observational studies in Epidemiology (STROBE) statement: guidance for reporting observational studies[19] and the STROBE-AMS recommendations for reporting epidemiology studies of AMR and informing improvement in AMS [20].

Ethics

Ethical approval for this study was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (Reference numbers ECM4(p) and ECM3(xx)).

Results

Hospital trends in AC

The trends in AC of the individual antimicrobials can be seen in Figure 1. Increasing trends were seen in ceftriaxone consumption (p= 0.0006), Piperacillin/tazobactam consumption (p = 0.03) and meropenem consumption (p = 0.054). Decreasing trends were seen in ciprofloxacin consumption (p = 0.0012) and gentamicin consumption (p= 0.057). Further trend analysis of AC is contained in Supplemental data Table II.

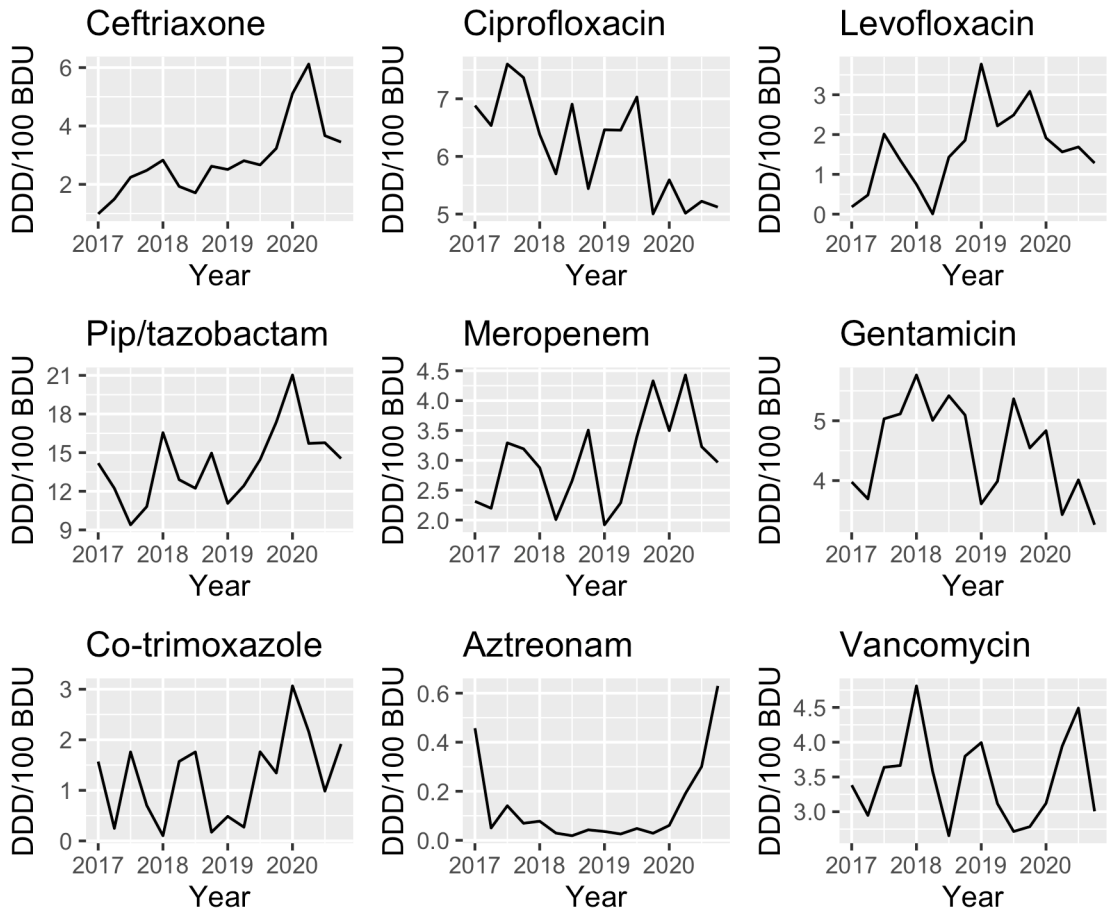

Figure 1 Quarterly antimicrobial consumption rates of selected antimicrobials from Q1 2017 to Q4 2020

Hospital trends in AMR

The rates of AMR for *E. coli, K.pneumoniae* and VRE incidence have been collated and are compared with Irish and European resistance figures in Table II.

Table II: Percentage of clinical isolates of *E. coli, K. pneumoniae* and *E.faecium* resistant to selected antimicrobials from the study hospital (Hosp), Ireland and the EU 2017-2019 (Only study hospital data is available for 2020 at the time of writing)

Bacteria	Antimicrobial agent/Group	2017			2018			2019			2020
		Hosp	Ire	EU	Hosp	Ire	EU	Hosp	Ire	EU	Hosp
E. coli	Third generation cephalosporin*	21	12	14.9	15.8	12.9	15.1	13.2	12	15.1	14.2
	Carbapenems**	1.4	0	0.1	1.6	0	0.1	1.1	0	0.3	0
	Fluoroquinolones[#]	32.5	23.6	25.7	31.5	23.9	25.3	27.7	20.4	23.8	25.7
	Aminoglycosides	12.7	11.9	11.4	10.4	11.7	11.1	11.4	11.8	10.8	9.1
K. pneumoniae	Third generation cephalosporin*	19.6	14.9	31.2	17.8	14.5	31.7	11.9	17.6	31.3	10.8
	Carbapenems**	0	0.2	7.1	1.4	0.6	7.5	0	0.9	7.9	1.92
	Fluoroquinolones[#]	22.4	5.9	30.5	23.3	8.1	19.5	8	5.3	19.3	12.5
	Aminoglycosides	17.8	11.9	24.1	14.6	13	22.7	10	11	22.3	3.85
E.faecium	Vancomycin resistance	64.1	38.2	14.9	56.3	40.2	17.3	48.6	38.4	18.3	53

*EU and Ire data relates to cefotaxime/ceftriaxone/ceftazidime, study hospital data relates to ceftriaxone resistance

** EU and Ire data relates to imipenem/meropenem, study hospital data relates to ertapenem resistance

[#] EU and Ire data relates to ciprofloxacin/levofloxacin/ofloxacin, study hospital data relates to ciprofloxacin resistance

E.coli

The number of clinical isolates of *E.coli* per year ranged from 233 in 2017 to 211 in 2020. The evolving trends in *E.coli* resistance to selected antimicrobials can be seen in Figure 2. The rates of resistance to the listed antimicrobials in the clinical isolates of *E.coli* were all trending downwards e.g. (Ceftriaxone p=0.136, Ciprofloxacin p=0.138, Piperacillin/tazobactam p=0.143), except for co-trimoxazole(p=0.69). Fluoroquinolone resistance rates in the hospital have fallen since 2017 (p=0.138) but are consistently higher than national and EU rates. The presence of the ESBL resistance mechanism in the study hospital *E.coli* isolates ranged from 19.8% in 2017 to 14.1% in 2020 (p = 0.323). Further details on the annual rates of resistance are contained in Supplemental data Table III

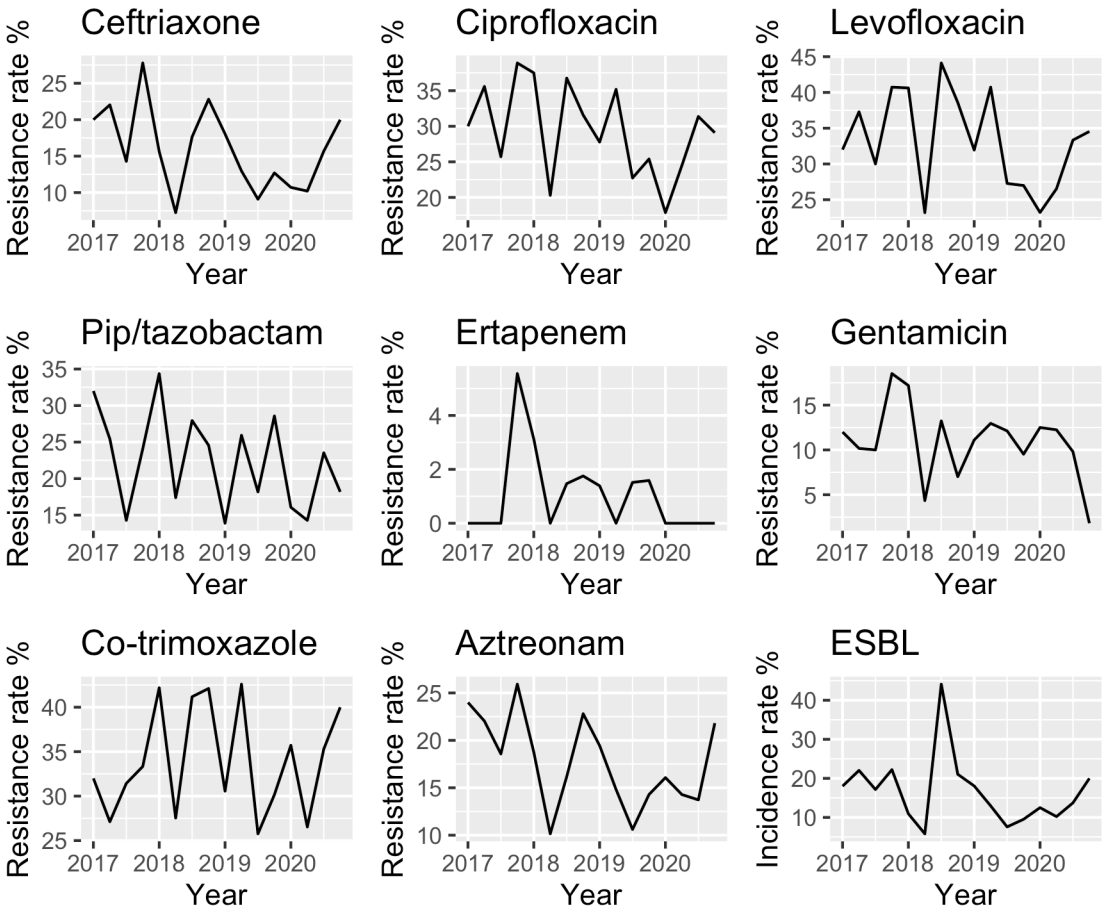

Figure 2 Quarterly E.coli resistance rates to selected antimicrobials from Q1 2017 to Q4 2020

K. pneumoniae

The number of clinical isolates of *K.pneumoniae* per year ranged from 72 in 2017 to 59 in 2020. The evolving trends in *K.pneumoniae resistance* to selected antimicrobials can be seen in Figure 3. The rates of resistance to the listed antimicrobials in the clinical isolates of *K.pneumoniae* were stable or trending downwards. Third generation cephalosporin resistance was considerably higher in the EU than in Ireland, and in the study hospital. Carbapenem resistance rates were higher in the EU, but were trending slowly upwards in Ireland, and fluctuating in the study hospital based on ertapenem resistance rates. Fluoroquinolone resistance was higher in 2017 in the study hospital (22%) than the EU (30%); the hospital rate has fallen below the EU rate but remains above the national rate in the following years as can be seen in the ciprofloxacin and levofloxacin rates in Table 2 and Figure 3. The presence of the ESBL resistance mechanism in *K.pneumoniae* isolates ranged from 19.8% in 2017 to 8.88% in 2020 ($p = 0.0254$) in the study hospital. Further details on the annual rates of resistance are contained in Supplemental data Table IV.

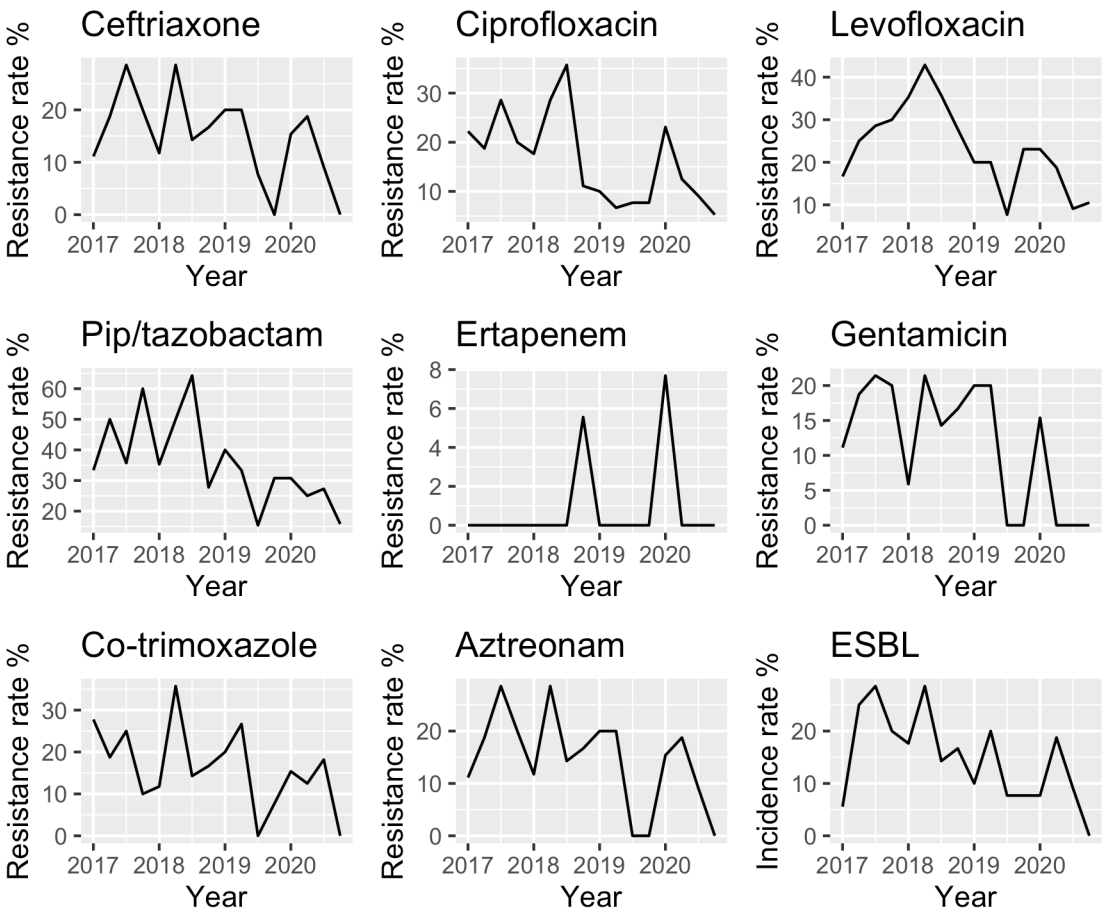

Figure 3 Quarterly K.pneumoniae resistance rates to selected antimicrobials from Q1 2017 to Q4 2020

Other *Enterobacterales* species (excluding *K. pneumoniae* and *E. coli*)

The number of clinical isolates of other *enterobacterales* species per year ranged from 165 in 2017 to 133 in 2020. The most frequently isolated other *enterobacterales* species were: *Citrobacter, Enterobacter, Proteus* and *Serratia.* The evolving resistance trends in the other *enterobacterales* species to selected antimicrobials can be seen in Figure 4. While there were some increases in the rates of resistance to most antimicrobials during the study period the trend of greatest concern was in ertapenem resistance which increased from 0.581% in 2017 to 5.19% in 2020 (p-value= 0.003).

The presence of ESBL positive *enterobacterales* isolates was insignificant (1.43% in 2017 to 0% in 2020, [p =0.013]). Further details on the annual rates of resistance are contained in Supplemental data Table V.

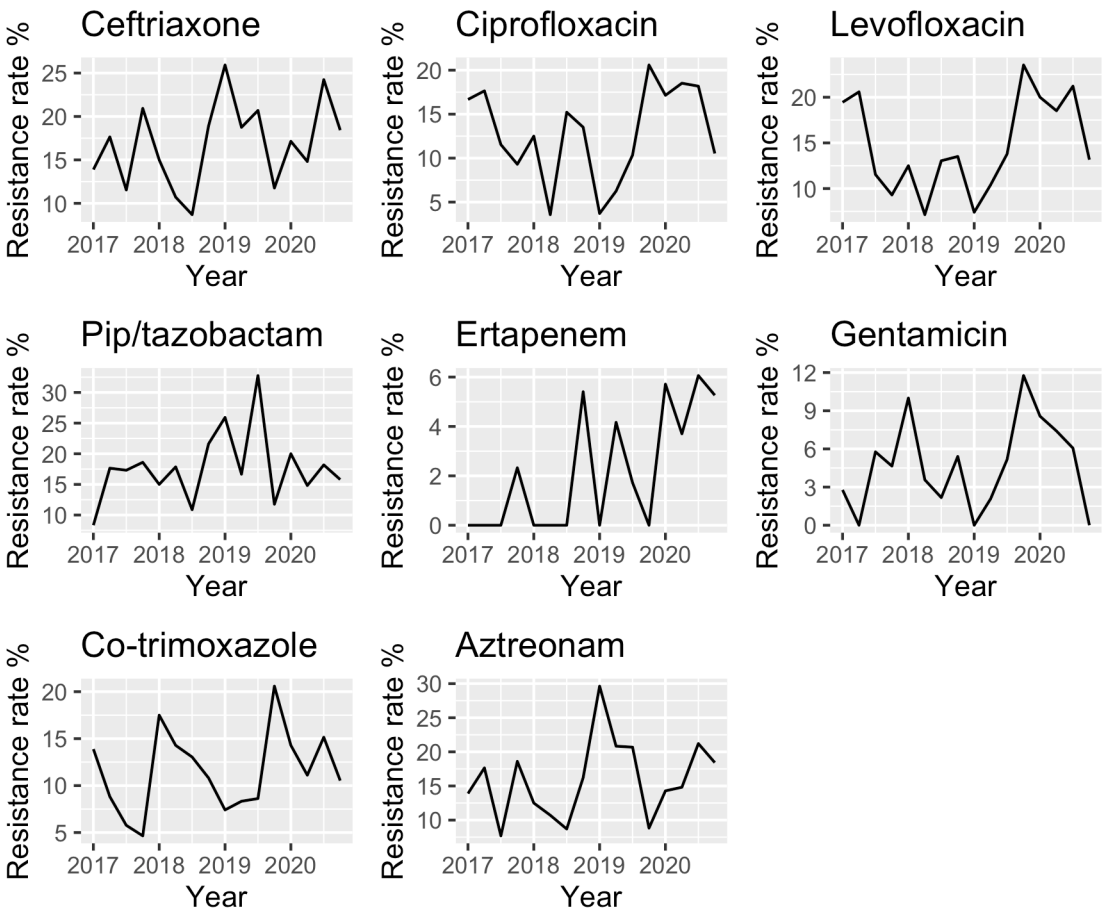

Figure 4 Quarterly resistance rates for other Enterobacterales species to selected antimicrobials from Q1 2017 to Q4 2020

Enterococcus faecium

The number of clinical isolates of *E.faecium* per year ranged from 116 in 2017 to 124 in 2020. In the study hospital the rate of VRE has decreased from 64% in 2017 to 53% in 2020 ($p = 0.1$) but is considerably higher than seen both nationally and in the remainder of the EU.

Incidence and dynamic regression of *E.coli* resistance to ceftriaxone and the influence of piperacillin/tazobactam

For ceftriaxone resistance in *E.coli* we identified an ARIMA model [18] with one significant moving average term of order 2. The transfer function model was developed which explained 86% (R^2=86) of the variation in incidence with piperacillin/tazobactam consumption as a statistically significant explanatory variable for ceftriaxone resistance in *E.coli*. A 1% increase in piperacillin/tazobactam use would result in a 1.33% decrease in ceftriaxone resistance immediately and a further 0.488 % decrease in 3 months. The transfer function model also included the moving average term of the resistance rate itself with a lag of 6 months. Further details are contained in Supplemental data Table V.

Discussion

This study contains an analysis of the rates of AC and AMR in an Irish teaching hospital using TSA. The findings show that while overall AC rates and broad-spectrum antimicrobial (ceftriaxone, piperacillin/tazobactam and meropenem) use increased over the study period there was not a corresponding increase in rates of AMR, in fact the rates of resistance decreased or remained stable with a few exceptions. These findings suggest that hospital AC is just one of several factors that influence the rates of AMR seen in the hospital setting. Analysis of AMR trends in this study showed hospital rates of resistance in *E.coli* and *K.pneumoniae* were decreasing or stable. The rates of AMR in the other *enterobacterales* isolates were relatively stable or slowly increasing. The rate of ertapenem resistance (CPE) in *K.pneumoniae* and other *enterobacterales* isolates is increasing, following a national trend of increasing incidence of CPE and the diagnosis of invasive CPE infections [21]. The rate of VRE in the study hospital has fallen from 64% in 2017 to 53% in 2020 but is higher than national and EU figures.

The decreasing or stabilising rates of AMR among *enterobacterales* isolates seen in this study is encouraging and suggests the hospital AMS programme is having a positive impact on gram negative AMR rates [22]. A recent analysis of AC and AMR data from the EU has identified similar findings in terms of stabilisation of AMR rates in *E.coli* and *K.pneumoniae*, which was associated with the positive effects of AMS initiatives [23]. The decrease in the rate of VRE in this study is also encouraging as historically, AMS programmes have been less effective in reducing the incidence rates of VRE [22]. It is important that the increasing incidence of CPE seen in this study is addressed as part of the local AMS programme. The most effective AMS interventions to target CPE are those which address carbapenem use and incorporate education and restrictive measures [24, 25]. The reduction in AMS activities [10] seen during the COVID-19 pandemic is likely to have contributed to the increased carbapenem use seen during this study.

When each of the AC-AMR combinations (e.g. AC and ertapenem resistance, AC and VRE rates) were cross-correlated using linear regression of the ARIMA model residuals, only one significant correlation between antimicrobial use and AMR was identified. This may have been because it is difficult to demonstrate a statistically significant correlation due to the complex and evolving nature of AMR despite the link between AMR and AC being well

established [26]. A recent study from the Netherlands in the outpatient setting found that the association between antimicrobial use and resistance was weak [27]. Suggested factors for the lack of correlation included patient related factors (e.g. age, sex), individual patient antimicrobial exposure, resistance mechanisms to antimicrobials between different bacteria [27] and the interaction with the use of other antimicrobials [16].

In this study a correlation between piperacillin/tazobactam use and the rate of resistance to ceftriaxone in *E.coli* was observed using TSA. Over the study period the rate of resistance to ceftriaxone in *E.coli* decreased while the use of ceftriaxone increased particularly in 2020. The increased consumption of ceftriaxone during 2020 did not appear to have an immediate impact on the rate of ceftriaxone resistance but this should be monitored due to a potential lag in the influence of the increased use on rates of resistance. The use of piperacillin/tazobactam also increased during the study period but using TSA a correlation was identified with the decrease in ceftriaxone resistance in *E.coli.* This effect has also been seen in a study involving the substitution of piperacillin/tazobactam for a broad-spectrum cephalosporin (ceftazidime) which resulted in decreasing levels of ceftazidime resistance in other *enterobacterales* species *(K.pneumoniae* and *Proteus mirabilis)* [28]. Resistance strain dynamics can play an important role in changes in AMR rates in *E.coli* species and are influenced by antimicrobial consumption changes [17]. In situations where there are high levels of resistance to third generation cephalosporins such as ceftriaxone as seen in the study hospital, efforts to substitute ceftriaxone with piperacillin/tazobactam should be considered.

The consumption of broad spectrum antimicrobials ceftriaxone, piperacillin/tazobactam and meropenem, increased over the course of the study with a particularly large increase in 2020, during the COVID-19 pandemic. As expected, the increased consumption of these antimicrobials was associated with the treatment of suspected pneumonia in patients with suspected or proven COVID-19 following national recommendations for AMS strategies during the pandemic [29]. Studies have estimated that up to 72% of hospitalised COVID-19 patients received antimicrobials while the rate of bacterial co-infection ranged from 6% [30] to 11% [31]. Such instances of poor AMS highlight the challenges faced in the delivery of AMS programmes during the pandemic [31] and the negative impact it has had on AMS activities [10]. The increases in the prescribing of broad-spectrum antimicrobials and decreased AMS activities has led to concerns that AMR may proliferate in hospitals because

of COVID 19. However, the COVID-19 pandemic does not appear to have resulted in significant changes to AMR rates seen in the study hospital in 2020, or elsewhere to date [32].

One potential benefit of the COVID-19 pandemic was the enhancement of Infection Prevention and Control (IPC) [33] practices. AMS programmes are more effective when implemented alongside IPC measures [22] as antimicrobial use selects for AMR, it's dissemination is facilitated by suboptimal IPC practices and AC. While some AMS activities decreased during the COVID-19 pandemic, IPC was brought to the fore, and an increased recognition of the importance of IPC in reducing the transmission of infection resulted.

The focus of this study was on AC and AMR in the hospital setting but it is important to acknowledge the impact of community antimicrobial use has on AMR, as antimicrobial use in one setting can impact AMR in the other. This 'spill-over' effect has previously been suggested as a reason for not detecting AC and AMR correlations particularly in smaller patient populations [34]. The response to the COVID-19 pandemic are also likely to impact AC and AMR in the community, decreased international travel, social distancing and mask wearing all reducing the transmission of infection [32]. The implications of changes to community AC and other changes in response to the COVID 19 pandemic will be important to explore.

Limitations of the study

The study was ecological in nature and did not analyse AMR and AC data at the patient level and nor did not control for patient-related factors. The study was unable to determine if individual patients identified with a resistant organism were exposed to the most relevant antimicrobial, other antimicrobials or risk-factors conventionally associated with resistance which means we cannot exclude confounding factors that may be responsible for the observed relationships.

Patient demographic data were only available for AMR and not AC, limiting the value of trying to include variables such as age, sex and location in our analysis. However, given the large sample size, effects of sampling variation in patient-level characteristics should theoretically be negligible.

This study only included clinical isolates and not routine surveillance cultures leading to a risk of selection bias due to an underestimate of the incidence of resistance. It is possible that not all patients with infections in the hospital may have been identified, some diagnosed infections may not have had specimens sent to the microbiology laboratory for investigations, and some specimens from infected patients may not have grown a microorganism to identify and submit for antimicrobial susceptibility tests.
This was a single centre retrospective study which makes it difficult to explore the complex relationship further. Future studies involving multi-centres and community practice would allow the examination of the relationships more fully.
Other studies have suggested more frequent observations should be used when conducting TSA [15] however this was not practical for this study. The use of a longer reporting period should be considered in future studies with the use of monthly data to increase sensitivity to identify possible correlations.

Conclusion

The decreasing or relatively stable rates of AMR found in the *enterobacterales* and VRE isolates in this study are a positive response to the hospital AMS programme. Broad spectrum AC increased over the course of the study with a particularly large increase in response to the COVID-19 pandemic. Much of the increase during the COVID-19 pandemic may have been unnecessary and occurred at a time of decreased AMS programme activities but has not resulted in changes to AMR rates to date. IPC practices have improved in response to the COVID-19 pandemic and continue to contribute to the effectiveness of AMS programmes.
Future research should explore the possible link between AC and AMR in the local community as well as the hospital setting, and patient level AC and AMR data would be a significant advantage for this. The use of TSA to analyse routine AC and AMR data as part of AMS programmes should also be considered, as it would allow for the identification and analysis of correlations such as that between piperacillin/tazobactam and ceftriaxone resistance in *E.coli* identified in this study to be investigated further.

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Conflicts of interests statement

Nothing to declare

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Supplemental data

Box-Jenkins method

The Box-Jenkins method consisted of the following steps:

Each time series was checked for stationary requirements (constant mean, variance and autocorrelation through time), with the unit root test (augmented Dickey–Fuller test); Some series did not need any transformation while some series required suitable differencing or other types of transformations, e.g., logarithmic transformation, to obtain a stationary series; Once the series was stationary the sample autocorrelation function (ACF) and partial autocorrelation function (PACF) were used to identify the auto-regressive (AR), moving-average (MA) or mixed behaviour (ARMA). The ARIMA (p,d,q) model notation consists of *p,* the order of AR terms, *d,* the order of non-seasonal differencing operations, and *q,* the order of MA terms. Having constructed separate models for each antibiotic and resistance time series, we then diagnosed them for acceptability using the Akaike Information Criterion (AIK) and the Ljung- Box statistic for white noise for residuals.

Following the development of univariate ARIMA models, a linear transfer function modelling method[16, 17] was used to investigate the dynamic relationship between antibiotic use and the incidence of resistant isolates, considering possible time delays (lag times). In this study the output or response was the percentage of AMR and the explanatory variable was AC. The cross-correlation function (CCF) between the residuals of the two ARIMA models was used to determine the correlations between the antibiotic use series and the incidence of resistant isolates. The transfer function model was then estimated with significance tests for parameter estimates at a p value of <0.05 used to eliminate unnecessary terms. The most parsimonious model was chosen, i.e. the model with the fewest parameters and highest biological plausibility. All final model residuals passed a 'white noise' test (based on the Ljung–Box statistics). For each model, the R^2 coefficient was calculated as goodness-of-fit measure, expressing the fraction of the variance of the dependent variable explained by the dynamic regression model. For the purposes of this manuscript only the significant findings are reported.

Table I: Annual antimicrobial consumption trends for in-patient antibiotic use in the study hospital 2017-2020 (Mean annual DDD/100 bed days)

Antimicrobial	2017	2018	2019	2020	Trend	p-value of trend
Third Generation cephalosporin						
Ceftriaxone	1.8	2.27	2.8	4.59	Increasing	0.0006
Carbapenems						
Ertapenem	0	0	0.0497	0.212	Increasing	0.011
Meropenem	2.75	2.76	2.98	3.53	Increasing	0.054
Fluroquinolones						
Ciprofloxacin	7.1	6.11	6.24	5.24	Decreasing	0.0012
Levofloxacin	1.01	1.01	2.89	1.61	Increasing	0.076
Aminoglycosides						
Gentamicin	4.45	5.32	4.38	3.88	Decreasing	0.057
Broad spectrum penicillin combination						
Piperacillin-tazobactam	11.7	14.2	13.8	16.8	Increasing	0.03
Other antimicrobials						
Aztreonam	0.18	0.0422	0.0347	0.296	Increasing	0.371
Co-trimoxazole	1.07	0.902	0.966	2.03	Increasing	0.15
Vancomycin	3.41	3.71	3.15	3.64	Stable	0.86

Table II: Percentage of non-duplicate clinical isolates of *E.coli* antibiotic resistance trends in the MUH (Annual mean quarterly resistance %)

Antibiotics	2017	2018	2019	2020	Trend	p-value of trend
Number of Isolates	233	258	255	211		
Ceftriaxone	21	15.8	13.2	14.2	Decreasing	0.136
Ciprofloxacin	32.5	31.5	27.8	25.7	Decreasing	0.138
Levofloxacin	35	36.6	31.7	29.4	Decreasing	0.233
Co-trimoxazole	31	38.3	32.3	34.4	Stable	0.69
Ertapenem	1.39	1.59	1.12	0	Stable	0.32
Meropenem	0	0.39	0	0	Stable	0.467
Piperacillin-tazobactam	23.9	26.1	21.6	18	Stable	0.143
Gentamicin	12.7	10.4	11.4	9.09	Stable	0.206
Aztreonam	22.6	17	14.8	16.5	Decreasing	0.07

Table III: Percentage of non-duplicate clinical isolates of *Klebsiella pneumoniae* antibiotic resistance trends (Annual mean quarterly resistance %)

Antibiotics	**2017**	**2018**	**2019**	**2020**	**Trend**	**p-value of trend**
Number of Isolates	72	63	51	59		
Ceftriaxone	19.6	17.8	11.9	10.8	Decreasing	0.043
Ciprofloxacin	22.4	23.3	8.01	12.5	Decreasing	0.014
Levofloxacin	25.1	35.4	17.7	15.4	Decreasing	0.0255
Co-trimoxazole	20.4	19.6	13.6	11.5	Decreasing	0.053
Ertapenem	0	1.39	0	1.92	Increasing	0.471
Meropenem	0	1.39	0	1.92	Increasing	0.471
Piperacillin-tazobactam	44.8	44.3	29.9	24.7	Decreasing	0.012
Gentamicin	17.8	14.6	10	3.85	Decreasing	<0.01
Aztreonam	19.6	17.8	10	10.8	Decreasing	0.0462

The most frequently isolated other *Enterobacterales* species were:
2017 –165 isolates (Citrobacter (21), Enterobacter(55), Proteus(28), Serratia(21))
2018-151 isolates (Citrobacter (20), Enterobacter(39), Proteus(35), Serratia(20))
2019-167 isolates (Citrobacter (16), Enterobacter(59), Proteus(30), Serratia(22))
2020-133 isolates (Citrobacter (12), Enterobacter(34), Proteus(36), Serratia(21))

Table IV: Study hospital percentage of non-duplicate clinical isolates of other *Enterobacterales* species antibiotic resistance trends (Annual mean quarterly resistance %)

Antibiotics	2017	2018	2019	2020	Trend	p-value of trend
Number of Isolates	165	151	167	133		
Ceftriaxone	16	13.3	19.3	18.7	Stable	0.231
Ciprofloxacin	13.8	11.2	10.2	16.1	Fluctuating	0.571
Levofloxacin	15.2	11.5	13.8	18.2	Fluctuating	0.353
Co-trimoxazole	8.28	13.9	11.2	12.8	Increasing	0.353
Ertapenem	0.581	1.35	1.47	5.19	Increasing	0.003
Meropenem	0	0.676	0	0	Stable	0.918
Piperacillin-tazobactam	15.5	16.3	21.8	17.2	Increasing	0.426
Gentamicin	3.3	5.29	4.76	5.51	Increasing	0.44
Aztreonam	14.5	12	20	17.2	Fluctuating	0.3

Table V: Multivariate transfer function model of piperacillin/tazobactam use and temporal relationship with the incidence of non-duplicate clinical isolates of *E.coli* resistant to ceftriaxone

	(1)
(Intercept)	53.930 *** [9.003]
Piperacillin/tazobactam lag 0	-1.333 ** [-4.564]
Piperacillin/tazobactam lag 1	-0.488 [-2.246]
Moving average term 2	-0.839 *** [-6.509]
N	12
R squared	0.864
logLik	-23.135
AIC	56.270

*** p < 0.001; ** p < 0.01; * p < 0.05. T statistics in brackets.

Arima modelling

Antibiotic time series	Operation to make stationary	Arima model (p,d,q)
Vancomycin	Difference and log	(0,1,1)
Total antimicrobial use	log	(0,0,1)
Ceftriaxone	Difference and log	(0,1,1)
Piperacillin/tazobactam	Difference and log	(0,1,1)
Ciprofloxacin	Difference	(1,1,1)
Levofloxacin	Difference and log	(0,1,2)
Meropenem	Difference	(0,1,1)
Ertapenem	Difference	(0,1,1)
Aztreonam	Difference	(0,1,0)
Gentamicin	Difference and log	(0,1,1)
Co-trimoxazole	log	(0,0,0)

E.coli time series

Antibiotic resistance	Operation to make stationary	Arima model (p,d,q)
Ciprofloxacin	Log	(0,0,0)
Levofloxacin	Log	(0,0,0)
Meropenem	None	(0,0,0)
Ertapenem	Difference	(0,1,1)
Gentamicin	Difference	(0,1,1)
Ceftriaxone	Difference and log	(0,1,2)
ESBL positive	Difference and log	(0,1,1)
Piperacillin/tazobactam	Log	(0,0,0)
Co-trimoxazole	Log	(0,0,1)
Aztreonam	Difference	(0,1,0)

K.Pneumoniae

Antibiotic resistance	Operation to make stationary	Arima model (p,d,q)
Ciprofloxacin	Difference	(0,1,2)
Levofloxacin	Difference	(0,1,1)
Meropenem	None	(0,0,0)
Ertapenem	None	(0,0,0)
Gentamicin	Difference	(0,1,2)
Ceftriaxone	Difference	(0,1,1)
ESBL positive	Difference	(0,1,2)
Piperacillin/tazobactam	Difference	(2,1,0)
Co-trimoxazole	None	(0,0,0)
Aztreonam	Difference	(0,1,1)

Enterobacteriaceae

Antibiotic resistance	Operation to make stationary	Arima model (p,d,q)
Ciprofloxacin	Difference	(0,1,1)
Levofloxacin	Difference	(0,1,1)
Meropenem	Not required	
Ertapenem	Difference	(2,1,1)
Aztreonam	Difference	(0,1,2)
Gentamicin	Difference	(0,1,0)
Ceftriaxone	Difference	(0,1,1)
ESBL positive	Not required	
Piperacillin/tazobactam	None	(0,0,2)
Co-trimoxazole	Difference	(0,1,1)
Aztreonam	Difference	(0,1,2)

VRE
Difference and Arima model (0,1,2)