ORIGINAL ARTICLE

The dynamic relationship between antibiotic use and the incidence of vancomycin-resistant *Enterococcus*: time-series modelling of 7-year surveillance data in a tertiary-care hospital

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ABSTRACT

The role of antibiotics in the epidemiology of vancomycin-resistant *Enterococcus* (VRE) has been studied extensively, but controversies remain as to which, and to what extent, antibiotics facilitate the emergence and dissemination of VRE in hospitals. Aggregate data on the use of several antibiotic classes in terms of defined daily doses (DDD) per 100 patient-days (PD), and VRE incidence rates in terms of clinical isolates per 1000 PD, were evaluated during a 7-year period at a tertiary-care hospital. Time-series analysis (autoregressive integrated moving average (ARIMA) and transfer function models) was used to quantify the temporal effect of antibiotic use on VRE incidence and estimate effect-delays. The incidence rate of VRE observed in a specific bimester was found to be a function of its value during the preceding bimester and of prior changes in the volume of use of four antibiotic classes. In particular, an increase of one DDD/100 PD in the use of glycopeptides, fluoroquinolones, extended-spectrum cephalosporins and β-lactam–β-lactamase inhibitor combinations resulted, independently, in average changes of +0.024, +0.015, + 0.020 and −0.010 isolates per 1000 PD in the incidence of VRE, with average delays of 2, 4, 2 and 6 months, respectively, which explained 56% of the observed variation in VRE rates over time. Efforts to reduce VRE cross-transmission should be supplemented by targeted antibiotic control policies. The use of glycopeptides, broad-spectrum cephalosporins and fluoroquinolones in high amounts should be the targets of such policies. Penicillin–β-lactamase inhibitor combinations might be suitable substitutes for extended-spectrum cephalosporins.

Keywords   Antibiotic use, ARIMA, time series, transfer function model, vancomycin-resistant *Enterococcus*

INTRODUCTION

Vancomycin-resistant *Enterococcus* (VRE) has become established as a significant nosocomial pathogen since it was first reported 20 years ago [1]. A large body of research on the epidemiology of VRE has indicated that the appearance and dissemination of VRE within hospitals are dependent on multiple factors related to exposure to VRE reservoirs, host factors predisposing the patient to colonization or infection with VRE, and exposure to antibiotics [1–3].

The role of antibiotics in the epidemiology of VRE has been studied extensively, but controversies remain as to which, and to what extent, antibiotics facilitate the emergence and spread of VRE [1–4]. Increased risk of colonization or infection with VRE has been most frequently, but inconsistently, associated with exposure to extended-spectrum cephalosporins, fluoroquinolones, glycopeptides and anti-anaerobic agents such as metronidazole, clindamycin and imipenem [4]. In contrast, some evidence suggests that the use of β-lactam–β-lactamase-inhibitor combinations may be protective against VRE dissemination [4,5]. Moreover, antibiotic formulary interventions, most frequently employing restriction of vancomycin and broad-spectrum

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cephalosporins, have shown variable effects in controlling VRE [4,6].

In the 700-bed, tertiary-care University Hospital of Heraklion, we had the opportunity to observe the evolution of VRE since its initial emergence in 2000 [7]. Over the following years, VRE became endemic in the hospital despite infection control efforts [8]. In parallel, a significant, frequently excessive, increase in the use of antimicrobials was noticed in the hospital, including antibiotics commonly implicated in facilitating VRE colonization or infection [9].

The objective of this study was to investigate and quantify the temporal relationship between the use of several antibiotic classes and VRE incidence, using time-series analysis of hospital-wide surveillance data obtained over a 7-year period. The ultimate goal was to stimulate evidence-based antibiotic control interventions, targeted to those antibiotics that facilitate the emergence and spread of VRE in the hospital setting.

MATERIALS AND METHODS

Vancomycin-resistant enterococci

The number of VRE isolates from clinical cultures, excluding *Enterococcus gallinarum* and *E. casseliflavus*, during the period 2000–2006, was obtained from the microbiology laboratory database. Duplicate isolates, defined as those having the same resistance pattern as those recovered from the same patient during the same month, were excluded from analysis. Isolates obtained from colonization screening surveys (stool specimens) were also excluded [10]. The incidence density rate of VRE was calculated as the number of VRE isolates per 1000 patient-days (PD), at bimonthly and yearly time intervals and for the pool of the study period.

Antibiotic use

Antibiotics were classified according to the anatomic therapeutic chemical (ATC) classification system. Volume data, obtained from the pharmacy dispensing records for the period 2000–2006, were converted into defined daily doses (DDD) according to the 2006 version of the ATC/DDD system, and were standardized into usage density rates per 100 PD (DDD/100 PD) at bimonthly and yearly intervals [11]. Evaluation of use was limited to antibiotic classes that have been commonly implicated as either risk factors or protective factors for VRE occurrence [4]. Groups included in the analysis were β-lactam–β-lactamase inhibitor combinations (ampicillin–sulbactam, amoxicillin–clavulanate, ticarcillin–clavulanate, and piperacillin–tazobactam), second-generation cephalosporins (cefuroxime, ceforanide, cefaclor, cefamandole, and cefoxitin), extended-spectrum cephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefixime, and cefepime), fluoroquinolones (ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin,

and norfloxacin), glycopeptides (vancomycin and teicoplanin), carbapenems (imipenem and meropenem), imidazoles (metronidazole and ornidazole) and lincosamides (clindamycin).

Statistical analysis

Linear trends of bimonthly antibiotic use rates were described by the p-value and the sign of the slope coefficient in linear regression on time. The Box and Jenkins (autoregressive integrated moving average (ARIMA)) modelling approach was employed to analyze the temporal behaviour of bimonthly VRE rates, based on its previous values, trends and abrupt changes in the recent past. Linear transfer function (LTF) modelling was used to quantify the dynamic relationship between the use of several antibiotic groups and VRE incidence, taking into account possible delays of effects. These approaches have been used previously to study relationships between antimicrobial use and resistance [12–15], and have been described in detail by Monnet *et al.* [16].

The optimum LTF model was developed as a polynomial distributed lag regression model, through the use of the 'general-to-specific' approach [13]. In particular, all series of bimonthly antibiotic use rates were included in an initial model. First-order differencing was used for all input series to obtain stationarity, and lags of up to two time-points were considered (up to 6-month delays, accounting for the differencing). The initial model was the following:

$$\nabla VRE(t) = c + \sum_k \sum_{i=0}^{2} w_{ki} \nabla X_k(t - i) + e(t),$$

where VRE(t) denotes the series of bimonthly VRE incidence rates, ∇ is the first-order differencing operator (e.g. $\nabla VRE(t) = VRE(t) - VRE(t-1)$), $X_k(t)$ denotes the input series of antibiotic use rates, and e(t) is the residual series, which can be seen as describing the effect of all factors other than those included in the model. The residual series was initially approached using an autoregressive model of order 1. This initial model was fitted to the data series to obtain estimates for parameters c and w_{ki}. The estimation results suggested that some parameters were zero and should be eliminated from the model. In this way, the initial model was progressively simplified. The simplification process involved three iterative steps: (i) identification of a tentative model by eliminating unnecessary lags and non-significant terms, and specification of a tentative ARIMA model for the residuals; (ii) estimation of the parameters of the identified model; and (iii) use of diagnostic checks to examine model adequacy, including the *T*-ratio test for the statistical significance of parameters, and the Ljung–Box statistic to verify that residuals had no autocorrelation pattern and corresponded to white noise. The determination coefficient, R^2, corresponding to the percentage of the variance of the observed time series of VRE rates explained by the model, was calculated for the LTF model and for the ARIMA model solely on the basis of past VRE rates. Data were analyzed using the Trends module of SPSS for Windows, version 15.

RESULTS

During the study period, 113 non-duplicate, non-screening VRE strains were isolated, including 80

(71%) *E. faecium* and 33 (29%) *E. faecalis* strains, from patients hospitalized in 21/32 hospital wards. The most common clinical specimens cultured were urine (36%), blood (19%) and pus (10%). Various other samples (cerebrospinal fluid, peritoneal fluid, pleural fluid, soft tissue sample, etc.) accounted for less than 5% each. Among the vancomycin-resistant *E. faecium* isolates, 74 (93%) had the VanA phenotype and six (7%) the VanB phenotype of resistance. All *E. faecalis* isolates had the VanA resistance phenotype.

The overall incidence density of VRE, pooled over the study period, was 0.090 isolates per 1000 PD, varying from 0.044 in surgical wards and 0.067 in medical wards, to 0.199 in haemato-oncology wards and 0.227 in intensive-care units. Increasing annual rates of VRE were observed in all patient-care areas throughout the study period (Fig. 1).

The hospital-wide antimicrobial use rate, pooled over the study period, was 105.7 DDD/ 100 PD. A significant trend towards increasing overall use was observed from the beginning to the end of the study period (p <0.001), with use in 2006 being 18.5% higher than in 2000. The individual classes studied accounted for 70.5% of total antibiotic use (Fig. 2). Cephalosporins comprised the most widely used group (23.7% of total use; pooled rate, 25.0 DDD/100 PD), and use throughout the study period was constant (p 0.572). Second in rank of use was the group of β-lactam–β-lactamase inhibitor combinations,

Fig. 2. Annual rates of hospital-wide use of main antimicrobial groups, measured in defined daily doses (DDD) per 100 patient-days (PD), University Hospital of Heraklion, 2000–2006.

which accounted for 18.0% of total antibiotic use (pooled rate, 19.0 DDD/100 PD) and showed an increasing linear trend during the study period (p <0.001), with an annual average increase of 0.53 DDD/100 PD. Fluoroquinolones (13.3% of total use; pooled rate, 14.0 DDD/100 PD) showed a significantly increasing trend (p <0.001), with a marked annual average increase of 1.89 DDD/ 100 PD. Glycopeptides accounted for 4.5% of total antibiotic use (pooled rate, 4.8 DDD/100 PD) and their use significantly increased during the study period (p 0.005), with an annual average increase of 0.17 DDD/100 PD. Carbapenems (3.9% of total use; pooled rate, 4.1 DDD/100 PD) also increased in use (p <0.001), with an annual average increase of 0.54 DDD/100 PD. The use of imidazoles accounted for 3.6% of total antibiotic use (pooled rate, 3.8 DDD/100 PD), and had an increasing linear trend (annual average increase, 0.31 DDD/100 PD; p 0.007). Clindamycin accounted for 3.5% of total antibiotic use (pooled rate, 3.7 DDD/100 PD), and showed a decreasing linear trend during the study period (annual average decrease, 0.07 DDD/100 PD; p 0.001).

Using the Box–Jenkins method, an ARIMA model was developed for the time series of bimonthly VRE incidence rates. The series became stationary after first-order differencing. The
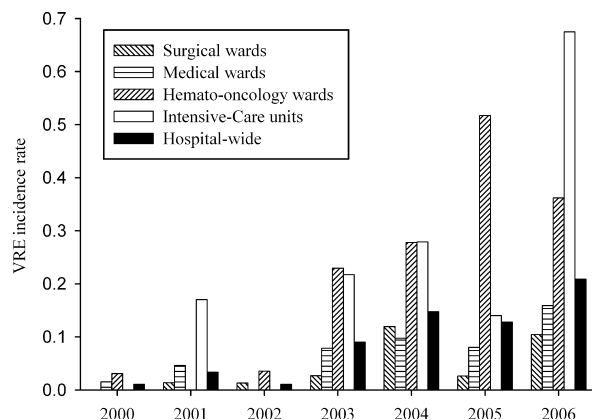
Fig. 1. Annual incidence rates of vancomycin-resistant enterococci (VRE) (number of VanA and VanB isolates per 1000 patient-days) for different patient-care areas, University Hospital of Heraklion, 2000–2006.
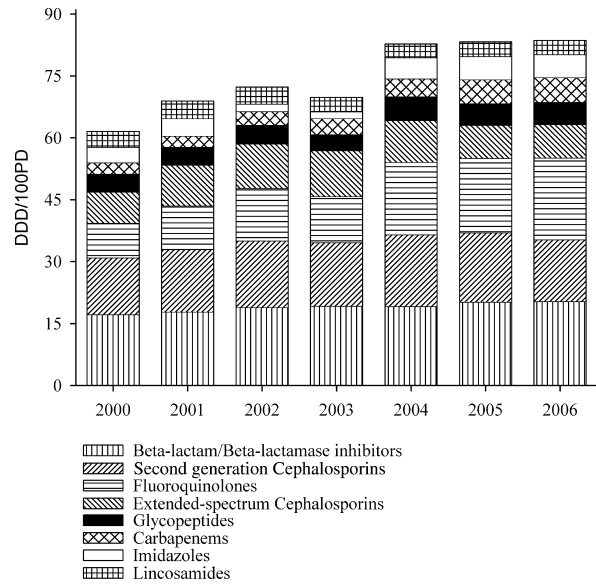
model was of the form ARIMA (0, 1, 1), having only one significant first-order moving average term. Table 1 (part A) presents the univariate analysis model, which can also be represented by the equation $VRE(t) = VRE(t - 1) + e(t) - 0.667 e(t - 1)$, where the term $VRE(t)$ denotes the incidence of vancomycin-resistant enterococci at time t (measured in 2-month intervals), and the term $e(t)$ denotes a random error at time t describing the effects of all factors other than $VRE(t - 1)$ on $VRE(t)$. According to this model, the incidence of VRE at time t is related to the same incidence observed at the previous time-point $t - 1$, plus a moving average term representing disturbances or abrupt changes of VRE rates. The determination coefficient (R^2) was 0.49, indicating that the model explained 49% of the variations in VRE rates over time.

Following the LTF modelling approach, the series of bimonthly antibiotic use rates were introduced into the model. This resulted in the multivariate model shown in Table 1 (part B), having the series of bimonthly usage rates of four antibiotic classes as significant predictors. This model can be represented by the equation $VRE(t) = VRE(t - 1) + 0.024(XA(t) - XA(t - 1)) + 0.015(MA(t - 1) - MA(t - 2)) + 0.020(DD(t) - DD(t - 1)) - 0.010(CR(t - 2) - CR(t - 3)) + e(t)$, where the incidence rate of VRE observed in a specific bimester is a function of the same incidence observed during the preceding bimester and of the change that occurred in the use of glycopeptides (XA), fluoroquinolones (MA), extended-spectrum cephalosporins (DD) and β-lactamase inhibitors (CR), plus an error $e(t)$

representing the effects of all factors other than those included in the model.

According to the LTF model, the use of glycopeptides had a positive relationship with the incidence of VRE: an increase of 1 DDD/100 PD of glycopeptide use results, with an average delay of 2 months, in an average increase of 0.024 VRE isolates per 1000 PD, after taking into account the current level of VRE incidence and the changes in the use of the other antibiotic classes. Similar interpretations hold for the other classes of antibiotics in the model. An increase of 1 DDD/100 PD in the use of extended-spectrum cephalosporins and fluoroquinolones results, independently, in average increases of 0.020 and 0.015 in the incidence of VRE, with average delays of 2 and 4 months, respectively. In contrast, β-lactam–β-lactamase inhibitor combinations showed an inverse relationship with VRE incidence: an increase of 1 DDD/100 PD in their use results, with an average delay of 6 months, in an average decrease of 0.010 in the incidence of VRE. Other antimicrobial groups, including second-generation cephalosporins, carbapenems, imidazoles and lincosamides, did not enter the model as significant predictors.

The determination coefficient of the LTF model was 0.56, implying that the model explained 56% of the observed variation in the VRE rates over time. In Fig. 3, the actual VRE rates observed during the study period have been plotted against the rates predicted by the LTF model, showing that the model mimics very well the actual evolution of VRE rates over time.

Table 1. Autoregressive integrated moving average (ARIMA) and linear transfer function (LTF) models used to estimate the dynamic relationship between the hospital-wide incidence of vancomycin-resistant *Enterococcus* (VRE) and antibiotic use, University Hospital of Heraklion, 2000–2006

Term[a]	Delay[b]	Parameter estimation			Ljung–Box Statistic[d]		R^2 coefficient
		Value (SE)[c]	*T*-ratio	p	Value	p	
A. ARIMA model for the incidence of VRE							
Moving average	1	+0.667 (0.121)	5.52	<0.001	20.5	0.251	0.49
B. LTF model for VRE incidence, accounting for antibiotic use							
Glycopeptide use	1	+0.024 (0.009)	2.80	0.008	18.5	0.424	0.56
Fluoroquinolone use	2	+0.015 (0.005)	2.74	0.010			
Third- and fourth-generation cephalosporins	1	+0.020 (0.009)	2.22	0.033			
Combinations of penicillins with BLI	3	−0.010 (0.005)	−2.10	0.043			

SE, standard error; BLI, β-lactamase inhibitors.
[a]First-order differencing was used for all time series involved in the models.
[b]Delay before effect is observed (measured in 2-month intervals), accounting for the order of series differencing.
[c]Size and direction of the effect.
[d]Diagnostic check for model residuals. A significant value implies that there is a structure in the observed series that is not accounted for by the model.
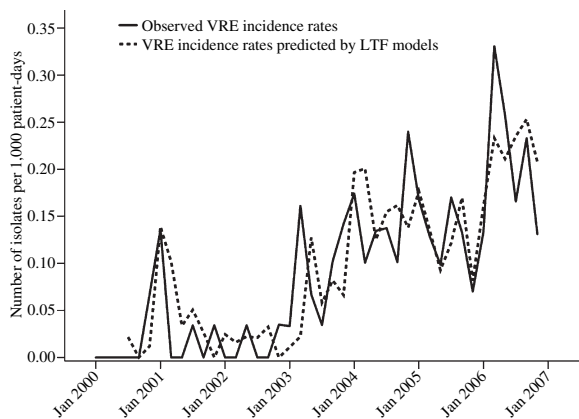
Fig. 3. Observed bimonthly incidence of vancomycin-resistant enterococci (VRE) as compared to incidence predicted by the linear transfer function model (LTF), University Hospital of Heraklion, 2000–2006.

DISCUSSION

Antibiotic use has been ascribed a crucial role in the nosocomial epidemiology of VRE. Antibiotic exposure may select for VRE in colonized patients, may decrease resistance against colonization of the gastrointestinal tract of patients previously free of VRE, and may facilitate VRE transmission by causing stool incontinence, thus increasing the risk of environmental contamination [17,18].

However, findings implicating specific antibiotics in the emergence and spread of VRE and corresponding effect estimates have been inconsistent across published studies [4]. For instance, the effect of vancomycin use has been controversial. In a meta-analysis of patient-level studies, Carmeli *et al.* [19] concluded that the association between vancomycin treatment and the risk of VRE colonization or infection that has been reported in the literature may be due to inappropriate control group selection and confounding by time at risk, and noted that studies that accounted for these factors did not reveal significant associations. However, most of these studies did not examine the interactions of several antibiotic classes, and used binary (yes/no) antibiotic exposure variables during the limited risk period of hospital stay, and thus did not account for dose dependencies and time effects [4]. Moreover, only a few studies assessed the influence of contact patterns with VRE carriers or overall colonization pressure [20]; therefore, factors central to VRE transmission have not been taken into account in reported associations [4].

Population-based studies have also been employed to investigate the role of vancomycin use in the epidemiology of VRE at the level of a ward or hospital. A large study conducted at 126 US intensive-care units showed that vancomycin exposure was the most significant modifiable risk factor for VRE colonization [10]. Yet, in a recent systematic review focusing on population-level associations, it was not possible to conclusively determine a potential role for reductions in vancomycin use in controlling VRE [6].

Aside from confounding and misclassification biases that may explain divergence in individual-level and ecological-level associations [21], another possible explanation involves the violation of the assumptions of independence and dynamic linearity required by classical statistical techniques [22]. Cross-transmission of VRE may create non-linear population dynamics, as exposure to antibiotics may increase the risk of acquisition, not only for the exposed individual, but also for close contacts [21]. When there is dependence of outcome among individuals, neither regression coefficients from ecological studies nor risk measures from patient-level studies can accurately reflect how much change in VRE incidence can be expected from a given change in antibiotic exposure [22]. Classical regression techniques cannot account for the population dynamics of the relationship, i.e. the effect of past VRE rates on current rates and the necessary delay before a change of antibiotic use affects VRE incidence [12].

In this study, time-series analysis was used to create a model of the incidence of VRE over time, as a function of its retrospective behaviour and prior usage rates of several antibiotic classes. Unlike usual statistical methods, this approach takes into account the correlation between consecutive observations in order to describe concomitant variations, i.e. changes in antibiotic use followed by changes in VRE rates [16]. Using this approach, it was possible to quantify the relationship between variations in use of glycopeptides and subsequent variations in VRE rates, after controlling for past levels of VRE incidence and use of other antibiotics. This model showed that an increase of 1 DDD/100 PD of glycopeptide use results, independently, in a subsequent average increase of 0.024 VRE isolates per

1000 PD. Therefore, it suggests that glycopeptide use contributes to VRE occurrence at the population level and should be a target of policies aiming at controlling VRE, as recommended by current guidelines [23].

This analysis also demonstrated a positive temporal relationship between VRE rates and the use of extended-spectrum cephalosporins and fluoroquinolones. In particular, increases of 1 DDD/100 PD in the use of extended-spectrum cephalosporins and fluoroquinolones resulted, independently, in subsequent average increases of 0.020 and 0.015 in VRE incidence, respectively. These findings are consistent with a meta-analysis of patient-level studies, which showed significant associations with the risk of VRE colonization or infection for broad-spectrum cephalosporins (OR, 3.44; $p < 0.001$) and fluoroquinolones (OR, 2.33; $p < 0.001$) [4].

Evidence of a negative effect against the spread of VRE for β-lactam–β-lactamase inhibitor combinations has been reported in patient-level studies [4,5]. Moreover, formulary substitution of β-lactam–β-lactamase inhibitors for broad-spectrum cephalosporins has been associated with reductions in VRE rates [24–26]. This model produced results in agreement with these observations, by showing an inverse temporal correlation between β-lactam–β-lactamase combinations and VRE rates.

A significant increase in overall antibiotic use was noted during the study period (18.5%), including the groups of antibiotics that were implicated in the analysis as independent predictors of VRE incidence. The results reveal up to three-fold higher rates of antibiotic use than those reported by individual hospitals or multicentre studies in other European countries [9,11], indicating that there was considerable inappropriate use of antibiotics in the study setting. The analysis suggested that restricting the use of glycopeptides, broad-spectrum cephalosporins and fluoroquinolones may help to control the increasing incidence of VRE. This is, however, a difficult and complex task in today's era of multidrug resistance. In the study setting, the proportion of enterococci exhibiting acquired resistance to vancomycin remains less than 10% [7,8], suggesting that a shift towards routine use of linezolid or daptomycin, and decreased use of vancomycin, would not be recommended. In contrast, efforts should be focused on diminishing unnecessary use and ensuring prudent use of vancomycin by harmonizing current practices with prevailing guidelines [23]. Moreover, any effort to limit the use of fluoroquinolones and extended-spectrum cephalosporins should be made with care, because it may lead to an increase in the use of other groups of antibiotics (e.g. carbapenems), with a resulting selection of different resistant organisms, perhaps more virulent than VRE. These results suggested that β-lactam–inhibitor combinations might be suitable substitutes for broad-spectrum cephalosporins in order to control VRE, but it is also important that current practices in the use of cephalosporins are examined thoroughly to determine targets for limiting unnecessary or inappropriate use.

Using time-series modelling, it was also possible to estimate the time-lags between variations in antibiotic use and subsequent variations in VRE incidence. Effect-delays ranged between 2 and 4 months for the three antibiotic groups found to be positively correlated with VRE rates, whereas the effect-delay was longer (6 months) for β-lactam–β-lactamase inhibitors, which exhibited a negative relationship with VRE rates. These findings are in line with the predictions of mathematical models concerning the effects of various patterns of antibiotic treatment at the population level, which have suggested that the spread of resistance due to antibiotic use will be faster than its decline when selection pressure is removed [27].

Past levels of VRE incidence are the main force driving current VRE rates according to the study model, and by itself, the retrospective behaviour of VRE incidence explained 49% of the variation in its values over time. This reflects the importance of colonization pressure in the hospital [20], and emphasizes that efforts to reduce cross-transmission are the cornerstone of any intervention to control VRE [28]. However, it has been noted that attempts to prevent or reduce VRE occurrence by interventions in infection control and antimicrobial use as separate entities may be too simplistic. In a mathematical model describing the population-level relationship between antibiotic use and VRE, Austin *et al.* [29] found infection control measures to be closely linked with control of antibiotic use, and suggested that the impact of infection control efforts may be negated by inappropriate antibiotic use. This analysis supports these arguments, because the

introduction of antibiotic use rates into the model increased the proportion of the variance in VRE rates explained by the model to 56%.

The remaining 44% of the variation in VRE rates can be attributed to other factors, including variations in infection control measures, cross-transmission frequency and susceptibility of the patient population. Therefore, the confounding effect of these factors on the relationships observed in this study is unknown. Infection control measures, including prompt detection and reporting of VRE by the microbiology laboratory, periodically performed prevalence surveys in high-risk units to detect VRE-colonized patients, isolation precautions for colonized patients and education of hospital staff regarding the problem of vancomycin resistance, have been undertaken in our hospital since VRE first emerged [7], but data regarding the degree of compliance with these efforts over time were not recorded. Presumably, if longitudinal data on factors such as compliance rates for hand washing and glove use, surrogates for hospital hygiene (e.g. volumes of medicated soaps and alcoholic hand rubs) or indicators of workload and overcrowding were available, they could be included in the model and could possibly increase the proportion of the variance in VRE rates explained by the model. Despite these limitations, the time-series analysis of hospital-wide surveillance data from the hospital pharmacy and clinical microbiology laboratory allowed us to quantify a temporal effect of the use of several antibiotic classes on the incidence of VRE, which in turn provided targets for antibiotic control interventions.

In conclusion, this study has illustrated that traditional infection control efforts to reduce VRE cross-transmission should be supplemented by targeted antibiotic control policies. Use of glycopeptides, broad-spectrum cephalosporins and fluoroquinolones in high amounts should be the targets of such policies. Penicillin–β-lactamase inhibitor combinations might be suitable substitutes for extended-spectrum cephalosporins.

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TRANSPARENCY DECLARATION

REFERENCES

1. Mascini EM, Bonten MJ. Vancomycin-resistant entero-cocci: consequences for therapy and infection control. *Clin Microbiol Infect* 2005; **11** (suppl): 43–56.
2. Chavers LS, Moser SA, Benjamin WH *et al.* Vancomycin-resistant enterococci: 15 years and counting. *J Hosp Infect* 2003; **53**: 159–171.
3. Shepard BD, Gilmore MS. Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. *Microbes Infect* 2002; **4**: 215–224.
4. Harbarth S, Cosgrove S, Carmeli Y. Effects of antibiotics on nosocomial epidemiology of vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 2002; **46**: 1619–1628.
5. Patterson JE. Antibiotic utilization: is there an effect on antimicrobial resistance? *Chest* 2001; **119** (suppl): 426–430.
6. de Bruin MA, Riley LW. Does vancomycin prescribing intervention affect vancomycin-resistant *enterococcus* infection and colonization in hospitals? A systematic review. *BMC Infect Dis* 2007; **7**: 24.
7. Christidou A, Gikas A, Scoulica E *et al.* Emergence of vancomycin-resistant enterococci in a tertiary hospital in Crete, Greece: a cluster of cases and prevalence study on intestinal colonisation. *Clin Microbiol Infect* 2004; **10**: 999–1005.
8. Gikas A, Christidou A, Scoulica E *et al.* Epidemiology and molecular analysis of intestinal colonization by vancomycin-resistant enterococci in Greek hospitals. *J Clin Microbiol* 2005; **43**: 5796–5799.
9. Kritsotakis EI, Assithianakis P, Kanellos P *et al.* Surveillance of monthly antimicrobial consumption rates stratified by patient-care area: a tool for triggering and targeting antibiotic policy changes in the hospital. *J Chemother* 2006; **18**: 394–401.
10. Fridkin SK, Edwards JR, Courval JM *et al.* The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant enterococci in 126 U.S. adult intensive care units. *Ann Intern Med* 2001; **135**: 175–183.
11. Kritsotakis EI, Gikas A. Surveillance of antibiotic use in hospitals: methods, trends and targets. *Clin Microbiol Infect* 2006; **12**: 701–704.
12. Lopez-Lozano JM, Monnet DL, Yague A *et al.* Modelling and forecasting antimicrobial resistance and its dynamic relationship to antimicrobial use: a time series analysis. *Int J Antimicrob Agents* 2000; **14**: 21–31.
13. Monnet DL, MacKenzie FM, Lopez-Lozano JM *et al.* Antimicrobial drug use and methicillin-resistant *Staphylococcus aureus*, Aberdeen, 1996–2000. *Emerg Infect Dis* 2004; **10**: 1432–1441.
14. Muller A, Lopez-Lozano JM, Bertrand X *et al.* Relationship between ceftriaxone use and resistance to third-generation cephalosporins among clinical strains of *Enterobacter cloacae*. *J Antimicrob Chemother* 2004; **54**: 173–177.
15. Mahamat A, Lavigne JP, Fabbro-Peray P *et al.* Evolution of fluoroquinolone resistance among *Escherichia coli* urinary

tract isolates from a French university hospital: application of the dynamic regression model. *Clin Microbiol Infect* 2005; **11**: 301–306.

16. Monnet DL, Lopez-Lozano JM, Campillos P *et al.* Making sense of antimicrobial use and resistance surveillance data: application of ARIMA and transfer function models. *Clin Microbiol Infect* 2001; **7** (suppl): 29–36.

17. Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med* 2000; **342**: 710–721.

18. Rice LB. Emergence of vancomycin-resistant enterococci. *Emerg Infect Dis* 2001; **7**: 183–187.

19. Carmeli Y, Samore MH, Huskins C. The association between antecedent vancomycin treatment and hospital-acquired vancomycin-resistant enterococci: a meta-analysis. *Arch Intern Med* 1999; **159**: 2461–2468.

20. Bonten MJ, Slaughter S, Ambergen AW *et al.* The role of 'colonization pressure' in the spread of vancomycin-resistant enterococci: an important infection control variable. *Arch Intern Med* 1998; **158**: 1127–1132.

21. Harbarth S, Harris AD, Carmeli Y, Samore MH. Parallel analysis of individual and aggregated data on antibiotic exposure and resistance in gram-negative bacilli. *Clin Infect Dis* 2001; **33**: 1462–1468.

22. Koopman JS, Longini IM. The ecological effects of individual exposures and nonlinear disease dynamics in populations. *Am J Public Health* 1994; **84**: 836–842.

23. Hospital Infection Control Practices Advisory Committee (HICPAC). Recommendations for preventing the spread of vancomycin resistance. *Am J Infect Control* 1995; **23**: 87–94.

24. Quale J, Landman D, Saurina G *et al.* Manipulation of a hospital antimicrobial formulary to control an outbreak of vancomycin-resistant enterococci. *Clin Infect Dis* 1996; **23**: 1020–1025.

25. Bradley SJ, Wilson AL, Allen MC *et al.* The control of hyperendemic glycopeptide-resistant *Enterococcus* spp. on a haematology unit by changing antibiotic usage. *J Antimicrob Chemother* 1999; **43**: 261–266.

26. May AK, Melton SM, McGwin G *et al.* Reduction of vancomycin-resistant enterococcal infections by limitation of broad-spectrum cephalosporin use in a trauma and burn intensive care unit. *Shock* 2000; **14**: 259–264.

27. Bonhoeffer S, Lipsitch M, Levin BR. Evaluating treatment protocols to prevent antibiotic resistance. *Proc Natl Acad Sci USA* 1997; **94**: 12106–12111.

28. Armeanu E, Bonten MJ. Control of vancomycin-resistant enterococci: one size fits all? *Clin Infect Dis* 2005; **41**: 210–216.

29. Austin DJ, Bonten MJ, Weinstein RA *et al.* Vancomycin-resistant enterococci in intensive-care hospital settings: transmission dynamics, persistence, and the impact of infection control programs. *Proc Natl Acad Sci USA* 1999; **96**: 6908–6913.