BMJ Open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-006596
Article Type:	Research
Date Submitted by the Author:	10-Sep-2014
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Public health, Research methods
Keywords:	Epidemiology < INFECTIOUS DISEASES, Infection control < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES, MICROBIOLOGY, STATISTICS & RESEARCH METHODS

Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis.

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Keywords:
Drug Resistance, Bacterial;
Infection Control;
Methicillin-Resistant *Staphylococcus* aureus;
Hand Hygiene;
Cohort Studies.

Word count:
Abstract = 2940 words
Background = 500 words
Methods = 935 words
Results = 1091 words
Discussion =1468 words
TOTAL (without abstract, tables, figures) = 3994 words

ABSTRACT

Objectives: To explore temporal associations between planned antibiotic stewardship and infection control interventions and the molecular epidemiology of Methicillin-Resistant *Staphylococcus* aureus (MRSA).

Design: Retrospective ecological study and time-series analysis integrating typing data from the Scottish MRSA reference laboratory.

Setting: Regional hospital and primary care in a Scottish Health Board.

Participants: General adult (N = 1,051,993) or intensive care (18,235) admissions and primary care registrations (460,000 inhabitants) between January 1997 and December 2012.

Interventions: Hand-hygiene campaign; MRSA admission screening; antibiotic stewardship limiting use of macrolides and '4Cs' (cephalosporins, co-amoxiclav, clindamycin and fluoroquinolones).

Outcome measures: Prevalence density of MRSA clonal complexes CC22, CC30 and CC5/*Other* in hospital (isolates/1000 occupied bed days, OBDs) and community (isolates/10,000 inhabitant-days).

Results: 67% of all clinical MRSA isolates (10,707/15,947) were typed. Regional MRSA population structure was dominated by hospital epidemic strains CC30, CC22 and CC45. Following declines in overall MRSA prevalence density, CC5 and other strains of community origin became increasingly important. Reductions in use of '4Cs' and macrolides anticipated declines in sub-lineages with higher levels of associated resistances. In multivariate time-series models (R^2 = 0.63 to 0.94) introduction of the hand-hygiene campaign, reductions in mean length of stay (when >4 days) and bed-occupancy (when >74 to 78%) predicted declines in CC22 and CC30, but not CC5/other strains. Lower importation pressures, expanded MRSA admission screening, and reductions in macrolide and 3rd generation cephalosporin use (thresholds for association: 135 to 141, and 48 to 81 Defined Daily Doses/1,000 OBDs, respectively) were followed by declines in all clonal complexes. Strain-specific associations with fluoroquinolones and clindamycin reflected resistance phenotypes of clonal complexes.

Conclusions: Infection control measures and changes in population antibiotic use were important predictors of MRSA strain dynamics in our region. Strategies to control MRSA should consider thresholds for effects and strain-specific impacts.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The internal and external validity of findings were strengthened by use of standardised data available over a long time horizon and for a geographically-defined population covered by a universal health system.
- By applying novel time-series analyses we demonstrated population interactions, strain-competition, and non-linear relationships with ecological determinants, convergent with understandings of the emergence and spread of resistance.
- An observational and ecological study design meant that associations may have been due to unidentified confounding variables, and may not have captured variation in molecular epidemiology explained by individual-level exposures.

INTRODUCTION

Staphylococcus aureus colonises around a third of humans, and is an important cause of infections in both hospital and community.[1] Resistance to penicillinase-resistant penicillins was first recognised more than 50 years ago,[2] and today MRSA is among the most commonly identified resistant nosocomial infections worldwide.[3] Resistance to β-lactam antibiotics is conferred by acquisition of a mobile genetic element: the *Staphylococcal* cassette chromosome (SCC*mec*).[4] This section of DNA contains the *mecA* gene, encoding for a modified penicillin binding protein; cassette chromosome recombinase genes, allowing for its excision and horizontal transfer; and variable elements encoding additional antibiotic resistances.[5,6] Rapid adaptation to selective pressures within a clonal genomic background facilitates clonal expansion and diversification, and this biodiversity allows MRSA to occupy a range of ecological niches.[7] Hospital-associated (HA-) strains typically contain SCC*mec* types I-III, encoding resistance to multiple antibiotics but also associated with slower growth and reduced toxin expression.[5] This fitness burden means HA-MRSA strains are typically limited to contexts of high-antibiotic pressure and high-density of vulnerable hosts. Community-associated (CA-) MRSA strains are characterised by SSC*mec* types IV–XI, carrying variable resistance to antibiotics and small fitness burdens.[5,8] These strains have a fitness advantage where selective pressures of antibiotic use fall below critical levels, and can infect healthy populations. Interactions of strains in hospital and community are increasingly recognised.[7, 9,10] The hospital epidemic strain EMRSA-15 is SSC*mec* IV, retaining some features consistent with its origin in the community.

The complex and evolving MRSA population structure creates challenges in the design and evaluation of control measures.[11] In the UK, national initiatives of infection control and antibiotic stewardship have been linked to a declining MRSA epidemic.[12-14] However, intervention effects may be strain-specific: the offset of fitness advantage and antibiotic resistance suggests that modifying ecological pressures could lead to clonal replacement.[10,11,15] Wyllie *et al.* have even suggested that declines in MRSA are attributable to spontaneous evolution within the MRSA population rather than impacts of infection control, and that health systems will continue to ride 'waves of trouble'.[16,17]

The ability to identify MRSA strains by molecular typing provides a tool for mapping their evolution and spread, and may inform more effective control strategies.[18] Europe studies have linked strain dominance to clinical context and antibiotic use,[15,19,20] with a particular focus on fluoroquinolones.[10,21-23] Advanced time-series analysis is well suited to investigating evolution in MRSA population structure, since it can distinguish the intrinsic progression of naturally occurring time-series from external influences of changes in ecological pressures.[24] While such analyses have explored associations between infection control measures and total MRSA rates,[25-29] we are not aware of any previous application to strain dynamics. Mathematical models have suggested critical thresholds in the impacts of ecological pressures, such as total antibiotic use, on resistance,[30,31] but to date empirical studies have only defined linear associations.

In this intervention study we used non-linear time-series analysis to investigate the extent to which national antibiotic stewardship and infection control strategies have determined the molecular epidemiology of MRSA across a Scottish health board between January 1997 and December 2012.

METHODS

Study Design

This retrospective observational study explored temporal associations between clinical burdens from MRSA clonal complexes and recent ecological exposures. Strain distribution and exposures were measured at monthly intervals over 16 years. This time-frame reflected the availability of routine typing data and covered a period of emergence, stabilisation, and decline in MRSA. It also allowed evaluation of the impacts of national infection control and antibiotic stewardship strategies, prompted by detection of high-rates of nosocomial infection in mandatory surveillance. Analysis controlled for natural progression within time-series of MRSA strain, strain-competition, and interactions between different clinical populations.

Setting and population

NHS Grampian is a large health board, serving 11% of Scotland's population. We investigated strain dynamics in three care settings: primary care (*community*), and general surgical/medical wards (*hospital*) or intensive care units (*ICU*) of the 1,000-bed regional referral hospital - Aberdeen Royal Infirmary (ARI). Less than 5% of admissions are transferred from other hospitals or regions. See table 1 for further details of participants.

Table 1: Study overview according to the ORION statement[32]

Setting: Community, hospital and intensive care unit (ICU) settings in North East Scotland. Infection prevention & control team (IPCT) including		**Dates:**1st Jan 1997 - 31st Dec 2012 (192 months)	**Population:** 480,000 adults registered in primary care; 1,091,250 admissions to general medical/surgical wards and 19,279 admissions to intensive care wards of Aberdeen Royal Infirmary (ARI). Mean (SD) age, 56 (1.2). Median (IQR) length-of-stay: 3.7 (3.5 to 4.1) Mean (SD) MRSA prevalence density in hospital and community = 1.91 (1.06) /1000 OBDs and 0.024 (0.017)/ 10,000 Inhabitant-days.
Antibiotic stewardship policy	January 1997 to April 2009: Annual reviews of hospital empirical antibiotic therapy guidelines. Very limited restrictive policies in place. Ongoing efforts to limit use of macrolides since Jan 2008. May 2009 to December 2012: Empirical guidelines recommended regimens avoiding '4C' antibiotics (Co-amoxiclav, cephalosporins, ciprofloxacin (all quinolones), clindamycin). Restricted supply of these antibiotics with use requiring prior authorisation from microbiology and pharmacy.		
General infection control measures	Alcohol gel introduced (Nov 2002)		
	National hand-hygiene campaign (Jan 2007)		
	National auditing of environmental cleaning (Apr 2006)		
	Healthcare Environment Inspectorate (HIE) inspection (Jan 2010)		
MRSA admission screening	Intensive Care Unit (ICU) Admission screening (May 2001)		
	Selective screening elective surgery & HDU (Jan 2006)		
	Universal admission screening (Aug 2008 to Mar 2011)		
	Targeted admission screening (March 2011 onwards) †		
Isolation and eradication policy	Isolation (single-room) or cohorting‡ of all patients with known MRSA or MRSA infected /colonised at admission. Decolonisation of all MRSA-positive patients with 5 days chlorhexidine body washes and intra-nasal mupirocin.		
Definitions and outcomes	Hospital-associated (HA-) MRSA cases	Non-duplicate MRSA isolates (1 per 14 days) from clinical specimens taken >48hrs after admission to hospital or ICU, excluding screening and infection control swabs.	
	Community-associated (CA-) MRSA case	Non-duplicate MRSA isolates from clinical specimens taken in the community or <48hrs of admission to hospital, excluding screening or infection control swabs.	
	Colonisation at admission	Isolation of MRSA from ≥1 admission screening swab, or known previous MRSA.	
	HA- or CA-MRSA Clonal Complex prevalence density	Hospital- or community-associated cases of MRSA attributable to a given clonal complex per 1000 OBDs (Hospital) or per 10,000 inhabitant-days (Community)	

† Recommended as a minimum standard by NHS Scotland following results of pathfinder study.[25]; OBDs = Occupied Bed Days; MRSA = Methicillin resistant *S.aureus.* SD = Standard Deviation.

Outcomes and exposures

The primary outcomes for the study were hospital- and community-associated prevalence densities of infections (de-duplicate clinical isolates) involving major clonal complexes grouped as CC22; CC30; and CC5/other strains. Data on prior healthcare exposures were not available so CA-MRSA included infections described elsewhere as healthcare-associated.

We considered a number of ecological exposures previously associated with MRSA burdens. Monthly population antibiotic use was measured in defined daily doses (DDDs)/1000 occupied bed days (OBDs) in hospital, or DDDs/1,000 inhabitant-days (IDs) in the community, and summarised according to the World Health Organisation Anatomical Therapeutic Chemical (WHO/ATC) classification.[33] Other covariates included: MRSA admission screening intensity (admissions screened/1000 OBDs); total and strain-specific importation pressures (admissions colonised or previous MRSA/1000 OBDs); mean length of stay (days) and bed-occupancy (%) in hospital populations. Consistent data on alcohol gel consumption and pre-intervention adherence with hand-hygiene or environmental cleaning standards were not available. We therefore introduced instrumental variables coding for changes in level (0 prior, 1 during intervention) and trend (autoregression*intervention) in strain prevalence densities associated with start of intervention.

Data Collection

Typing and antibiotic resistance phenotype data were derived from the Scottish MRSA Reference Laboratory (SMRSARL) for 10,707 MRSA clinical isolates and 4273 MRSA admission screening specimens from non-duplicate cases. Total antibiotic consumption in primary care was derived from the Prescribing Information System for Scotland (PRISMS). Remaining data was retrieved from regional health intelligence, pharmacy, microbiology, and infection control departments. Any individual or specimen level data were pseudo-anonymised by removal of identifiable personal information and replacement of unique personal or specimen numbers with matched study codes.

Laboratory methods

All *S.aureus* isolates were identified by agglutination, mainly with the Prolex™– Blue Staph Latex Kit (Pro-Lab). Antibiograms were determined using Clinical and Laboratory Standards Institute agar disk diffusion methods and, from 2008, by a Vitek™ instrument, using custom made *Staphylococcus* sensitivity cards (Biomerieux). EUCAST interpretative criteria were used from January 2012. MRSA screening swabs were cultured on MRSA selective medium, with use of chromogenic agar (Brilliance - Oxoid, UK) from 2006. Further details of methods utilised in the study period are available from previous publications.[25, 29]. All first patient clinical and screening isolates per year were sent to the reference lab until March 2011, after which only isolates from screening, blood cultures, outbreak investigations, or with unusual phenotypes were referred. Epidemiological typing of MRSA isolates into clonal complex was based on a combination of genotypic and phenotypic characteristics, matching >90% to known strains. Isolates were typed by the methods in use at the Reference Laboratory at the time of receipt. These varied during this study but always involved at least two independent methods. All isolates had their antibiotic resistance profile and biotype determined and at least one of phage typing, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR)-ribotyping or spa typing was also performed. When comparison with previously typed isolates did not allow a confident strain assignment, either the isolate was designated 'Other' or additional typing methods (usually multi-locus sequence typing, MLST) were used. No isolate was assigned to a lineage based on its

antibiotic resistance profile alone. Assignment to a sub-lineage was based on antibiotic resistance profile or SCC*mec* typing by PCR.

Statistical analysis

Temporal trends in MRSA clonal complexes were estimated by applying the strain distributions (% typed isolates belonging to each clonal complex) to the total MRSA prevalence density in the same month in each clinical population. The distribution of antibiotic resistance phenotypes and sub-lineages by strain and quarter of year were summarised by heat-maps after excluding those appearing in ≤5 isolates in the study period. Autoregressive Integrated Moving Average (ARIMA) models were generated to explore temporal associations between hospital consumption of macrolides, ciprofloxacin, and clindamycin and associated resistances (% isolates) in each MRSA strain.[24]

To investigate the dissemination of clonal complexes through the regional healthcare network we considered temporal associations between strain prevalence density in ICU, hospital, and community and among those colonised with MRSA at admission. Granger causality tests were used to identify the direction of possible relationships (at lags 1-3 months). Long-run associations between time-series were defined by the Johansen cointegration test, and used to inform a Vector Error Correction model (lags 1-3 months) incorporating cointegration equations. Path diagrams were generated based on significant associations in these models, with connecting arrows proportional to the percentage of total variation in prevalence density explained by variation in other populations.

Finally, we used non-linear time-series analysis to explore significant predictors of strain prevalence density in hospital (full details are provided in supplemental file 1). Potentially significant non-linear associations were identified from visual inspection of the output from Generalised Additive Models (GAM). Candidate variables were entered into Multivariate Adaptive Regression Spline (MARS) models defining associations as a series of linear segments across ranges of the independent variables separated by thresholds (knots). Analyses were performed using SPSS 21.0 (IBM), Eviews 8.0 (IHS, California, USA) and SCA 8.1 (Scientific Computing Associates Corp. Illinois, US).

RESULTS

Trends in MRSA clonal complexes

Information on epidemiological typing was available for 60% (n = 4597/7727) of clinical isolates in the hospital population, 74% (5651/7647) of isolates in the community, and 80% (459/573) in the Intensive care unit (ICU) – figure 1a. Applying strain distributions (figure 1b) to the total MRSA prevalence densities in each population provided estimates of strain-specific prevalence densities - figure 1c.

A consistent secular trend in strain distribution was seen across all three populations. Between 1997 and 2003 CC30 (mostly UK-EMRSA-16) was the dominant strain. High prevalence densities of CC30 were seen in ICU before introduction of MRSA admission screening in this unit (May 2001), with little presence in the community. Between 2004 and 2008 the dominant strains were CC22 (UK-EMRSA-15) and, to a lesser extent, CC45 (limited to our region in Scotland), with large clinical burdens in all settings. Finally, from 2008 there was greater strain diversity, with CC5, CC8, CC1, and other clonal complexes of increasing importance. These strains explained 30% of HA-MRSA and 50% of CA- MRSA by 2012.

Trends in antibiotic resistance phenotypes and sub-lineages

Excluding resistance phenotypes represented by ≤5 isolates over the study period, MRSA isolates could be explained by 37 antibiograms – figure 2. 94% of CC30 and 90% of CC45 isolates were resistant to erythromycin, ciprofloxacin and clindamycin, and 78% of CC22 were characterised by resistance to erythromycin and ciprofloxacin. By contrast 92% of CC5 were susceptible to all three agents. Multi-drug resistance (≥3 antibiotic classes) was present in 88% (95% confidence interval (CI), 87 to 90%) of isolates before the third quarter of 2008, declining sharply thereafter to 60% (57 to 63%). Multi-drug resistance in CC22, increased from 6% when CC30 was dominant to 57% when CC22 was dominant (2004 to 2008), falling to 25% during antibiotic stewardship; Kruskal-Wallis test, $P = 0.002$. The most commonly acquired resistances in CC22 included trimethoprim (4% increasing to 66%; $P < 0.001$), tetracycline (1.4% to 10.7%; $P < 0.001$), clindamycin (1.3% to 3.9%); $P < 0.001$ for all comparisons. Concurrent increases in trimethoprim resistance were observed in CC30 (0.7% to 7.3%; $P < 0.001$), but not CC5/Other strains (10.5% to 4.8%; $P = 0.058$).

Changes in antibiotic resistance phenotypes of prevalent strains were predicted by trends in antibiotic consumption. During antibiotic stewardship resistance to erythromycin, ciprofloxacin and clindamycin declined in all strains – table 2 and figure 3.

Table 2: Temporal associations between hospital use of macrolides, fluoroquinolones and clindamycin and related antibiotic resistances within strains

Antibiotic and strain	ARIMA model† (p,d,q)(P,D,Q)	Model R^2	Lag	Coefficient (95% CI)‡	T ratio	*P* value
Macrolide use, DDDs/1000 OBDs						
CC22, % Erythromycin resistance	(1,0,1)(1,0,0)	0.291	0	0.088 (0.012 to 0.164)	2.25	0.026
CC30, % Erythromycin resistance	(2,0,2)(0,0,0)	0.432	5	0.098 (0.006 to 0.190)	2.08	0.039
CC5 & Other, % Erythromycin resistance	(1,0,0)(0,0,0)	0.109	0	0.110 (0.090 to 0.130)	11.51	<0.001
Fluoroquinolone use, DDDs/1000 OBDs						
CC22, % Ciprofloxacin resistance	(2,0,2)(1,0,0)	0.451	0	0.062 (0.027 to 0.097)	3.36	0.001
CC30, % Ciprofloxacin resistance	(2,0,2)(1,0,0)	0.331	0	0.128 (0.048 to 0.209)	3.14	0.002
CC5 & Other, % Ciprofloxacin resistance	(1,0,2)(0,0,0)	0.074	0	0.108 (0.076 to 0.140)	6.58	<0.001
Clindamycin use, DDDs/1000 OBDs						
CC22, % Clindamycin resistance	(1,0,1)(0,0,0)	0.298	0	0.173 (0.137 to 0.208)	9.76	<0.001
CC30, % Clindamycin resistance	(2,0,1)(0,0,0)	0.691	0	0.455 (0.067 to 0.843)	2.30	0.023
CC5 & Other, % Clindamycin resistance	(2,0,1)(0,0,0)	0.176	0	0.334 (0.175 to 0.493)	4.11	<0.001

† Autoregressive Integrated Moving Average models, in which: p = order (number) of non-seasonal autoregressive terms representing impact of previous values in time-series, d = order of differencing to achieve stationary time-series; q = order of non-seasonal moving average terms representing response to previous disturbances (residual error) in time-series; and P,D,Q reflect orders of seasonal (lag 12) autoregressive, differencing and moving average terms.

‡ Change in % resistance associated with a +1 DDD/1,000 OBDs increase in antibiotic use.

CI = Confidence Interval; DDDs = Defined Daily Doses; OBDs = Occupied Bed Days.

Changes in antibiotic resistance phenotypes within strains were partially explained by shifts in the distribution of sub-lineages – figure 4. Before antibiotic stewardship, hospital epidemic strains were dominated by sub-lineages with high rates of resistance to ciprofloxacin, erythromycin and clindamycin, including ST22-MRSA-IV (CC22), ST36-MRSA-II (CC30), and ST45-MRSA-II (CC45). During antibiotic stewardship higher proportions of isolates within these strains were from alternative sub-lineages, characterised by much lower rates of resistance to these three antibiotics. Conversely, within strains dominated by sub-lineages with low rates of resistance (including CC5 and CC8), alternative and more resistant sub-lineages declined during antibiotic stewardship. One exception was the increasing importance within CC8 of Panton-Valentine Leukocidin (PVL) positive isolates, resembling USA300.[6]

Interactions of MRSA population structure in different populations

Typing was available for 33% (4273/13,048) of non-duplicate MRSA admission screening isolates. Applying the strain distribution from this typing to the total MRSA positive admission swabs per month provided time-series for strain-specific importation pressures for general hospital and ICU environments. Trends in strain-specific importation pressures coincided with the strain-dynamics seen among clinical isolates.

Granger causality tests and Vector Error Correction (VEC) models confirmed significant temporal associations between prevalence density of strains in ICU, hospital and community populations, and strain-specific importations pressures – figure 5. Importation pressures followed trends in related hospital prevalence densities, with less consistent and sizeable associations with community or ICU trends. Community prevalence densities of CC22, CC30 and CC45 were strongly determined by prior rates in hospital and ICU. By contrast, hospital epidemiology of CC5/other was anticipated by rates in the community.

Multivariate time series analyses

Multivariate Adaptive Regression Spline (MARS) models explained 91%, 94% and 58% of variation in prevalence densities of CC22, CC30, and CC5/Other strains, respectively -table 3.

Table 3: Summary of Time Series Multivariate Adapative Regression Splines models

Explanatory variables (order of terms)	Lag (months)	Threshold†	Relation to threshold	Change in prevalence density (95% confidence interval)	T-ratio	P-value
(a) CC22 (R^2 = 0.912)						
AR(1)	1	1.06	Above	+0.474 (0.271 to 0.677)	+4.57	<0.001
AR(2)	1	2.18	Above	-0.530 (-0.941 to -0.119)	-2.52	0.023
CC30 prevalence density, cases/1000 OBDs	0	0.363	Above	-0.337 (-0.483 to -0.231)	-6.26	<0.001
Mean bed-occupancy, %	3	78.4	Above	+0.022 (0.006 to 0.038)	+2.66	0.017
Mean length of stay, days	2	4.06	Above	+0.694 (0.178 to 1.210)	+2.63	0.018
Hand-hygiene campaign*AR(1), trend effect.	6	0.26	Above	-0.143 (-0.231 to -0.055)	-3.16	0.006
Admissions screened for MRSA /1000 OBDs (1)	1	4.24	Above	+0.138 (0.088 to 0.188)	+5.38	<0.001
Admissions screened for MRSA /1000 OBDs (2)	1	7.87	Above	-0.137 (-0.188 to -0.086)	-5.26	<0.001
Admissions screened for MRSA /1000 OBDs (3)	1	69.7	Above	-0.007 (-0.012 to -0.002)	-2.42	0.028
MRSA+ at admission/1000 OBDs	0	0.145	Above	+0.178 (0.125 to 0.231)	+6.53	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(1)	2	78.6	Above	+0.033 (0.009 to 0.057)	+2.69	0.016
Fluoroquinolone use, DDDs /1000 OBDs(2)	2	72.8	Above	-0.032 (-0.055 to -0.009)	-2.62	0.019
Macrolide use, DDDs/1000 OBDs	1	135	Above	+0.009 (0.002 to 0.015)	+2.62	0.019
Co-amoxiclav use, DDDs/1000 OBDs	2	235	Above	+0.010 (0.004 to 0.016)	+3.10	0.007
3rd Gen. Cephalosporin use, DDDs/1000 OBDs	5	81.0	Below	-0.007 (-0.010 to -0.004)	-4.22	<0.001
(b) CC30 (R^2 = 0.940)						
AR(1)	1	1.189	Above	+6.40 (4.48 to 8.311)	+6.54	<0.001
AR(2)	1	1.273	Above	-6.62 (-8.85 to -4.40)	-5.84	<0.001
AR(3)	1	1.773	Above	+0.794 (0.240 to 1.349)	+2.80	0.010
CC22 prevalence density, cases/1000 OBDs (1)	0	0.157	Below	+4.34 (2.99 to 5.71)	+6.28	<0.001
CC22 prevalence density, cases/1000 OBDs (2)	0	0.157	Above	-0.207 (-0.288 to -0.126)	-5.01	<0.001
Mean bed-occupancy, %	1	73.7	Above	+0.021 (0.009 to 0.033)	+3.50	0.002
Mean length of stay, days	1	3.85	Above	+0.531 (0.274 to 0.787)	+4.05	<0.001
Admissions screened for MRSA /1000 OBDs	1	5.11	Above	-0.007 (-0.008 to -0.005)	-8.92	<0.001
MRSA+ at admission/1000 OBDs (1)	0	0.498	Below	-2.442 (-3.382 to -1.501)	-2.91	0.008
MRSA+ at admission/1000 OBDs (2)	0	0.498	Above	-2.492 (-4.596 to -1.247)	-2.91	0.008
MRSA+ at admission/1000 OBDs (3)	0	0.623	Above	+2.86 (1.15 to 4.56)	+3.27	0.003
MRSA+ at admission/1000 OBDs (4)	0	3.038	Above	-0.361 (-0.464 to -258)	-6.86	<0001
Fluoroquinolone use, DDDs /1000 OBDs(1)	4	49.4	Below	-0.049 (-0.071 to -0.027)	-4.38	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(2)	4	49.4	Above	+0.018 (0.017 to 0.019)	+3.92	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(3)	4	67.3	Above	-0.021 (-0.031 to -0.011)	-4.16	<0.001
Macrolide use, DDDs/1000 OBDs	1	141	Above	+0.022 (0.016 to 0.028)	+7.05	<0.001
Co-amoxiclav use, DDDs/1000 OBDs	5	160	Below	-0.005 (-0.008 to -0.002)	-3.35	0.003
Co-amoxiclav use, DDDs/1000 OBDs	5	160	Above	-0.003 (-0.005 to -0.001)	-3.82	<0.001
3rd gen. Cephalosporin use, DDDs/1000 OBDs		71.9	Below	-0.008 (-0.013 to -0.003)	-3.74	0.001
(c) CC5/Other strains (R^2 = 0.583)						
AR(1)	2	0.166	Below	-0.314 (-0.575 to -0.05)	-2.35	0.018
AR(2)	2	0.166	Above	-0.22 (-0.370 to -0.070)	-2.87	0.007
AR(3)	1	0.273	Above	-0.457 (-0.657 to -0.257)	-4.47	<0.001
Mean length of stay, days	1	3.98	Below	+0.177 (0.097 to 0.257)	+4.33	<0.001
Admissions screened for MRSA /1000 OBDs	0	110	Above	-0.011 (0.005 to 0.017)	-3.10	0.005
MRSA+ at admission/1000 OBDs (1)	3	4.565	Above	+0.041 (0.012 to 0.070)	+2.87	0.007
MRSA+ at admission/1000 OBDs (2)	5	6.235	Below	+0.184 (0.170 to 0.198)	+2.49	0.014
MRSA+ at admission/1000 OBDs (3)	5	6.235	Above	+0.971 (0.908 to 1.033)	+3.50	0.002
Macrolide use, DDDs/1000 OBDs	5	141	Above	+0.005 (0.002 to 0.008)	+3.59	0.002
Co-amoxiclav use, DDDs/1000 OBDs	5	241	Above	+0.008 (0.005 to 0.013)	+6.07	<0.001
3rd gen. Cephalosporin use, DDDs/1000 OBDs	5	47.1	Below	-0.004 (-0.006 to -0.002)	-3.69	<0.001

† Level of explanatory variable at which association appears. AR = Autoregressive term, reflecting impact of previous prevalence density in the same strain; DDDs = Defined Daily Doses; MRSA = Methicillin Resistance *Staphylococcus aureus;* OBDs = Occupied Bed Days.

Prevalence densities of CC22 and CC30 were inversely related suggesting competition for the same ecological niche. Bed-occupancies above 74 to 78% and length-of-stay over 4 days, were associated with higher rates of CC22 and CC30 over the next 1 to 3 months (lags 1 to 3) – figure 6. The hand-hygiene campaign exerted a downward pressure on trends in CC22 strongest in months of high prevalence density. No association was noted with CC30 prevalence density which was already low at initiation of the campaign. In contrast, rates of CC5/other strains increased when length-of-stay was <4 days and were not related to hand-hygiene or bed-occupancy.

Importation pressure was important in determining nosocomial rates of CC22 and CC30 at almost all levels, whereas association with CC5/Other strains was mostly at high importation pressures (>6.24 MRSA+ admissions/1000 OBDs). Increased intensity of MRSA admission screening was followed by declines in prevalence density of CC30, CC22 and CC5/Other beyond thresholds of 5, 70 and 110 admissions screened per 1000 OBDs, respectively. The difference in threshold reflected the influence of earlier ICU screening on CC30, when overall inpatient screening levels were low.

Consistent non-linear associations were seen between inpatient macrolide or third generation cephalosporin use and prevalence density of all strains – figure 6. Macrolide consumption was positively associated with rates of CC30, CC22 and CC5, above a total use threshold of 125-141 DDDs /1000 OBDs. A 'ceiling' effect was noted for all associations with 3rd generation cephalosporin use, with reductions in consumption below 71-81 DDDs/1000 OBDs associated with lower prevalence densities, but no relationship seen above this threshold. A threshold effect was also observed with Co-amoxiclav use above 235-241 DDDs/1,000 OBDs being followed by similar increases in CC22 and CC5/other prevalence density, but a positive association with CC30 was only seen at lower levels of consumption (up to 160 DDDs/1,000 OBDs).

Other strain-specific associations reflected the resistance phenotype of the strain. Clindamycin consumption above 25 DDDs/1,000 OBDs was positively associated with rates of CC30, but was not significantly related to CC22 or CC5/other strains at any level of use. Increases in CC30 prevalence density were seen at levels of fluoroquinolone use up to 68 DDDs/1,000 OBDs (lag 4). Consumption above this level was inversely associated with CC30 but positively associated with CC22, suggesting selective advantage of CC22 under higher antibiotic pressure.

Where antibiotic consumption was positively associated with strain prevalence density, the median (range) % isolates within strains with related resistances was 98.1% (40% to 100%), compared to 3.7% (3.5% to 32%) where no association was identified (Mann-Whitney U test, P = 0.004). Consumption of other antibiotics in hospital or community were not significantly related to strain dynamics.

DISCUSSION

This 16-year retrospective study represents the first ever application of non-linear time-series analysis to investigate ecological determinants of MRSA strain dynamics. Following recent declines in hospital-associated epidemic strains such as CC22 and CC30, clonal complexes arising from the community, including CC5, became increasingly important in the region. Large shifts in strain distribution were underpinned by more subtle changes in sub-lineages and antibiotic phenotypes associated with changes in selective pressure from antibiotic use. Even after accounting for interactions between clinical populations, and natural progression within time series, we demonstrated that changes in infection control and antibiotic use were important predictors of this evolving MRSA population structure. Improved hand-hygiene, and reductions in bed-occupancy or length-of-stay, were followed by declining inpatient burdens from hospital-associated epidemic strains but had little or opposite effects on community strains. The hospital-associated prevalence density of all clonal complexes declined with increasing intensity of admission screening, but thresholds for association were strain-specific. Responses to consumption of antibiotics reflected the resistance phenotype of the strain and were subject to total use thresholds.

This study had several limitations. An observational and ecological design meant that associations may not be causal, may be explained by unidentified confounding variables, and may not reflect variation in molecular epidemiology due to individual-level exposures. However, although retrospective in nature, use of routinely collected data from electronic databases and standardised microbiological and clinical definitions minimised risks of information bias. Between 6% and 42% of variation in strain prevalence densities was not explained by multivariate models, suggesting unidentified determinants. We were unable to obtain consistent data on: staffing-levels;[35] transfer-rates;[23] isolation and decolonisation performance;[36] and compliance with hand-hygiene and environmental cleaning before initiation of national strategies.[37] External validity was strengthened by exploring strain-dynamics in a geographically-defined population covered by a universal health system, and in various levels of care. However, our findings also highlight the importance of regional conditions in shaping strain-dynamics, limiting generalizability beyond the UK.

Previous evidence on associations between infection control measures or antibiotic use and MRSA strain-dynamics has largely been from in-vitro or animal experiments,[21,38] and mathematical models.[39] While such studies have demonstrated important concepts of stain competition and strain-specific impacts of manipulating selective pressures, examining the evolution of MRSA in real-life contexts provides greater ecological and population validity. Wyllie and colleagues have highlighted the importance of considering internal strain-dynamics when evaluating the contribution of national infection control strategies to recent declines in MRSA within the UK.[16] In a large observational study, these authors explored the evolution of MRSA and two epidemic strains (CC30 and CC22) in Oxfordshire hospitals alongside infection control strategies.[17] They concluded that recent falls in MRSA rates were more likely attributable to spontaneous strain dynamics than interventions since: declines were seen before intensification of infection control; and decline in CC30 was much steeper than that in CC22. Elsewhere, in a 10 year study of an MRSA population in a London hospital, Knight *et al.* noted a similar shift in dominant strain from CC30 to CC22, and attributed it to fitness advantage in CC22 after acquisition of additional resistances.[22] This evolution was independent of ecological pressures, but fluoroquinolone resistance was a key feature of successful hospital strains and overall MRSA declined after restriction of these antibiotics. These investigations made limited attempts to

model impacts of interventions and changing antibiotic use, adjust for expected progression of time-series, or consider population interactions. In overcoming these methodological weaknesses, our study helps reconcile conflicting evidence.

Firstly, results of multivariate models suggest that even those infection control measures expected to have general effects can have strain-specific impacts due to differences in the temporal and spatial distribution of clonal complexes. The possibility of a threshold effect in hand-hygiene has been suggested previously.[40] Our findings also suggest that impacts of a national initiative to improve hand-hygiene were dependent upon background prevalence densities of CC22 and CC30 during the campaign.[40] Greater impact during period of high prevalence density is consistent with the role of hand-hygiene in reducing transmission, and of diminishing returns at lower prevalence density.[41] Several time-series analyses have demonstrated the importance of bed-occupancy in determining rates of MRSA,[42] with both guidelines[43] and research[35] suggesting safety thresholds between 82 and 90%. We found highly consistent associations between bed-occupancy and rates of CC22 and CC30 above thresholds of 74-78%: much lower than average bed-occupancies of 82-88% across the UK.[44] Congruent with hospital burdens from CC5/other strains being driven by importation from the community, no associations were seen with hand-hygiene or bed-occupancy. Similarly while lower average length-of-stay anticipated declines in CC22 and CC30, it was associated with increases in hospital burdens from CC5/other strains. Given that antibiotic-resistant infections lead to longer length-of-stay a complex bidirectional relationship is likely.[45] We noted the threshold of hospital-wide MRSA admission screening at which declines were seen varied considerably between strains, probably reflecting the roll-out among different clinical populations, and background rates of strains.[46] Population interaction models suggested that ICU was a key environmental niche for CC30, consistent with a highly drug resistance phenotype. Early introduction of admission screening in this unit (May 2001) resulted in an abrupt and permanent decline in total MRSA rates,[47] which this study suggests was attributable to control of CC30. Responsiveness may also reflect much more frequent carriage of qacA, encoding for chlorhexidine resistance, in CC22 compared to CC30.[48] However, we have not identified increasing chlorhexidine resistance in the ICU. Declines in CC22 and CC5/other strains were limited to months when hospital-wide screening exceeded 70 and 110 admissions screens/1,000 OBDs: a level only seen during expansion to HDU/surgical and universal admission screening, respectively. On the basis of cost-effectiveness,[49] risk-factor based (targeted) screening is advocated in Scotland. However, since community strains can appear in patients without traditional risk-factors for MRSA, this approach may be insufficient to prevent invasion into hospitals.[50]

We further demonstrated the importance of selective pressures from population antibiotic use in determining the molecular epidemiology of MRSA. Alongside non-linear associations strongly related to the typical resistance profiles of strains, declining use of '4C' and macrolide antibiotics coincided with changes in antibiotic resistance phenotypes and shift towards more susceptible sub-lineages within all clonal complexes. Total antibiotic use thresholds may represent 'tipping points' at which ability to adapt to different selective pressure determines strain success within environmental niches. The rapidity of change within strains during antibiotic stewardship contrasts with theoretical and mathematical models suggesting that rapidly acquired resistances may be lost slowly due to compensatory evolution minimising fitness costs of resistance.[31, 51] Studies in France have described secular trends towards strains and resistance phenotypes with susceptibility to macrolides and gentamicin despite a lack of change in antibiotic consumption.[15,19] However, use of

macrolides in these areas was around 40 DDDs/1,000 OBDs, and well below the thresholds for association with strain prevalence in our study. The studies also highlighted the selective advantage of strains carrying SCC type IV, associated with high genetic plasticity mediated by the frequent transfer of[42] mobile genetic elements.[15] Consistent with Knight *et al.* we noted that dominance of CC22-IV in hospital coincided with acquisition of multiple antibiotic resistances.[22] We have previously noted increasing trimethoprim resistance in major epidemic strains associated with regional use in MRSA throat decolonisation.[52] Our finding that CC22 outcompeted CC30 at higher intensity of fluoroquinolone (FQ) use is congruent with lower fitness costs of FQ resistance in CC22,[21] and its critical role in the dissemination of CC22 through the UK health system.[23]

Our findings suggest that implementation and evaluation of interventions to control MRSA can be improved by consideration of non-linear and strain-specific impacts. Recognising critical thresholds in modifiable ecological pressures may enhance cost-effectiveness by determining optimal levels of intervention and identifying areas where impacts are unlikely.[53] Limiting population antibiotic use to below critical levels may provide a powerful means to balance immediate clinical need with avoidance of resistance and sustainability of use.[30] Further applications of our approach in other populations and clinical contexts is required to elucidate factors modifying thresholds for association with ecological variables, and to adapt antibiotic stewardship or infection control policies to local scenarios. These factors may include: age and comorbidities in the clinical population; baseline rates of MRSA; existing strain distributions; importation pressures;[50] and interactions with other populations.[23] Previous investigations have demonstrated complex within-host strain dynamics. Multi-level analyses could quantify the relative contribution of individual and population level exposures to acquisition or infection with specific strains.[54] The relative weakness of existing hospital-based infection control measures in controlling CC5/other strains seen in this study suggests a pressing need for strategies to control burdens from clonal complexes arising in the community.[55]

In conclusion, this study found evidence that changes in infection control and population antibiotic use have contributed to MRSA strain dynamics in Scotland over the past 16 years. Declines in overall clinical burdens from MRSA were convergent with intensified hospital infection control and antibiotic stewardship strategies removing selective pressures favouring hospital epidemic strains. Future efforts to control MRSA, and in particular evolving community strains, should consider thresholds for effects and strain-specific impacts.

ACKNOWLEDGEMENTS The authors wish to thank the Scottish MRSA Reference Laboratory, and health intelligence, medical microbiology and pharmacy departments of NHS Grampian, for their help in obtaining data. TL, IMG, JMML and CN designed the study, strain-typing was coordinated by GE, data-collection was by TL, GE, GM and RSS, and analysis by TL, JMML and CN. All authors reviewed and approved the final manuscript.

COMPETING INTERESTS TL, CRJD, GM, RSS, CN , JMML and GE have no conflicts of interest to declare. IMG consults and lectures for various pharmaceutical and diagnostic companies specialising in control of MRSA.

FUNDING This study was part of the TSARINAS (Time Series Analysis of Resistance, Infection control, and Antibiotic Stewardship) project, supported by grants from the NHS Grampian Clinical Microbiology Endowment Fund. No external funding was attached to this project.

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FIGURE LEGENDS

Figure 1: Epidemiological typing of clinical MRSA isolates, and distribution of clonal complexes† as cumulative % typed isolates or prevalence density by populations. † 'Other' clonal complexes included CC7, CC15, CC59, CC88, CC93 and C239.

Figure 2: Heat map of antibiotic resistance phenotypes including total number in study period, % of isolates in each strain, and % of all isolates per quarter of year

Figure 3: Percentage of isolates within strains resistant to Erythromycin, Ciprofloxacin or Clindamycin and consumption of related antibiotics from univariate ARIMA time-series models (3m moving averages)

Figure 4: Heat map describing relative frequency (% total isolates in strain per quarter) of sub-lineages of the five most prevalent clonal complexes

Figure 5: Flow charts of temporal associations between prevalence density of MRSA strains in different clinical populations, as derived from Vector Error Correction (VEC) models. Boxes represent patient populations, arrows the direction of temporal association, and numbers (months) the delay in associated changes. Arrow width is proportional to the % of total variation in response time-series (population prevalence density) explained by input time-series.

Figure 6: Contribution charts illustrating non-linear associations between explanatory variables and prevalence density of CC22, CC30, CC5/other strains. Lines represent the change in (Δ) prevalence density (y-axis) associated with changes in explanatory variables over their observed range (see boxplots). Thresholds ('knots') are represented by a change in direction in the line. Where y = 0 there is no association with the explanatory variable. A dotted line represents an area of uncertainty within which the actual threshold is likely to be located.

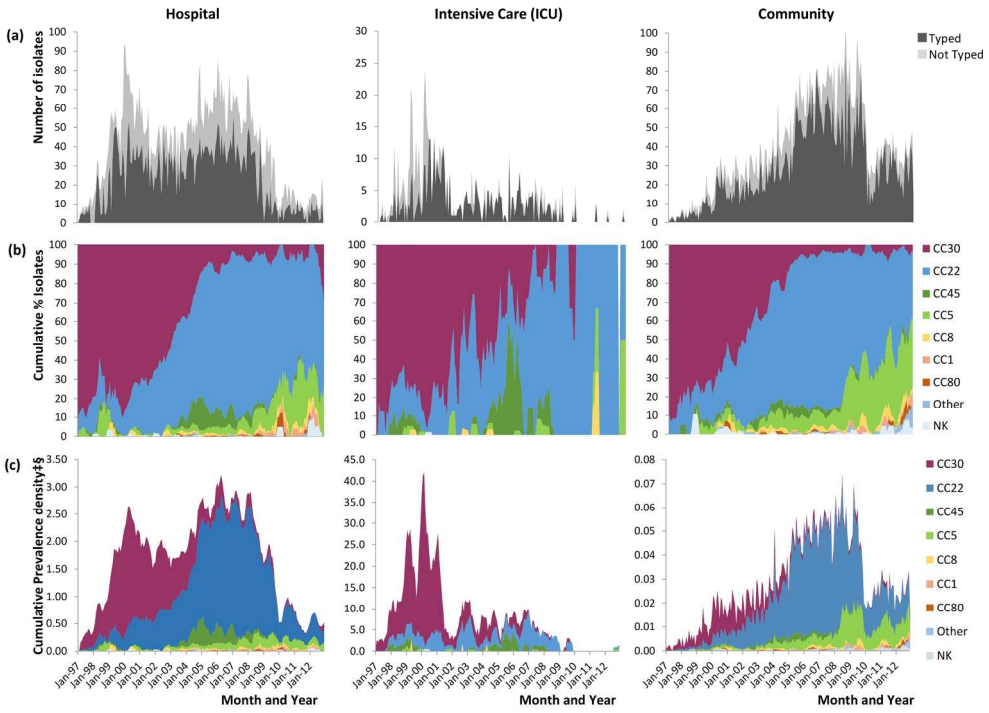

Figure 1: Epidemiological typing of clinical MRSA isolates, and distribution of clonal complexes† as cumulative % typed isolates or prevalence density by populations.<\b> † 'Other' clonal complexes included CC7, CC15, CC59, CC88, CC93 and C239.
190x134mm (300 x 300 DPI)

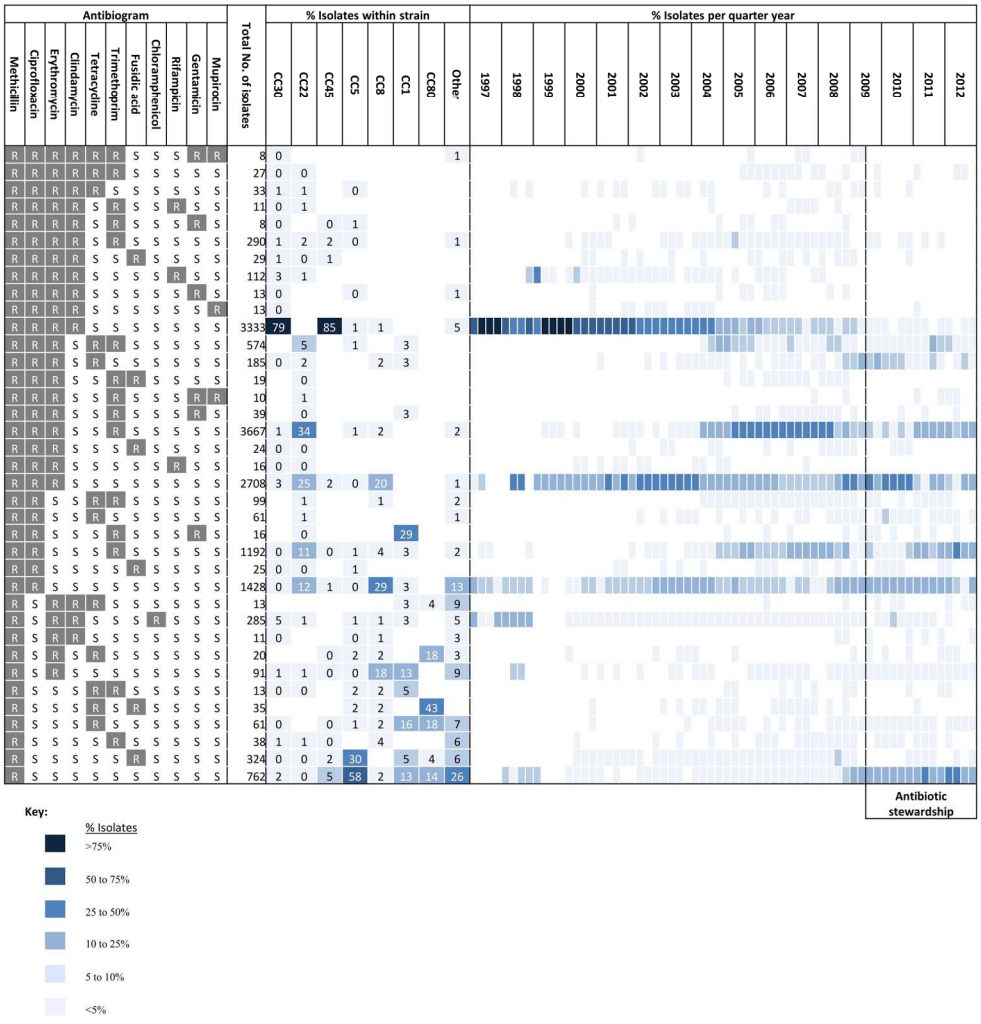

Figure 2: Heat map of antibiotic resistance phenotypes including total number in study period, % of isolates in each strain, and % of all isolates per quarter of year<\b>
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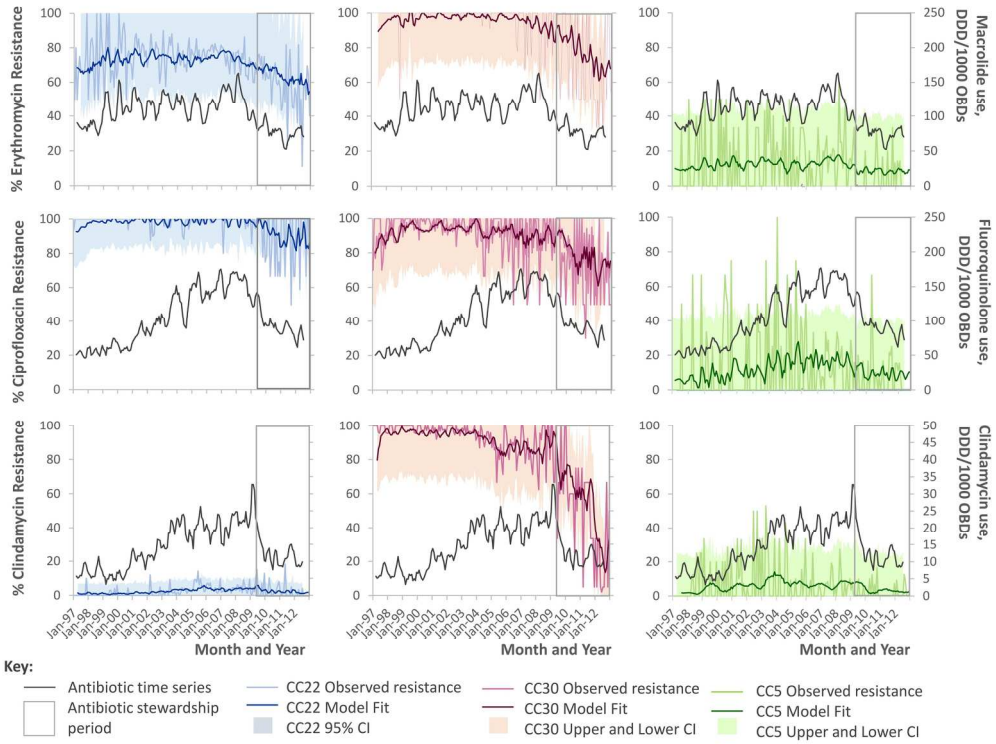


Figure 3: Percentage of isolates within strains resistant to Erythromycin, Ciprofloxacin or Clindamycin and consumption of related antibiotics from univariate ARIMA time-series models (3m moving averages)
190x143mm (300 x 300 DPI)

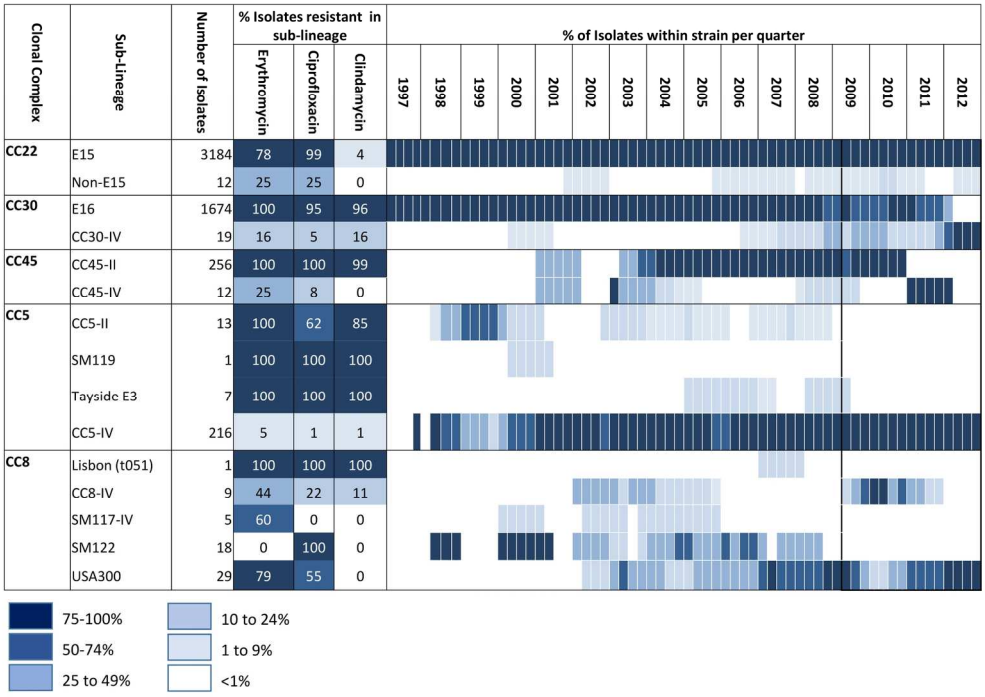


Figure 4: Heat map describing relative frequency (% total isolates in strain per quarter) of sub-lineages of the five most prevalent clonal complexes<\b>
177x125mm (300 x 300 DPI)

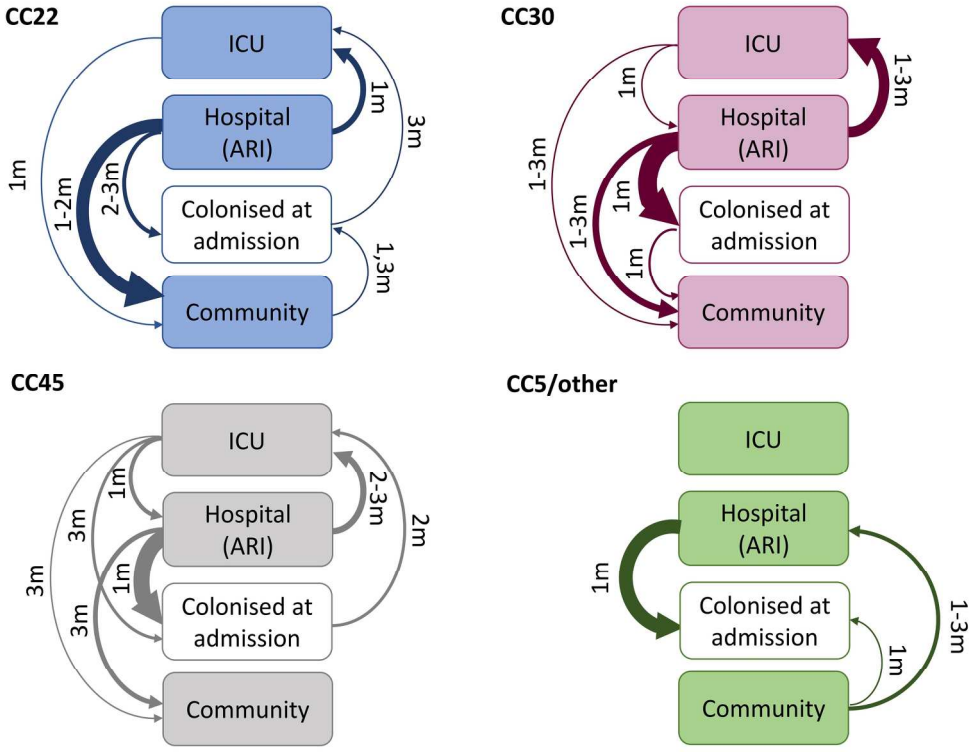


Figure 5: Flow charts of temporal associations between prevalence density of MRSA strains in different clinical populations, as derived from Vector Error Correction (VEC) models<\b> Boxes represent patient populations, arrows the direction of temporal association, and numbers (months) the delay in associated changes. Arrow width is proportional to the % of total variation in response time-series (population prevalence density) explained by input time-series. 190x149mm (300 x 300 DPI)

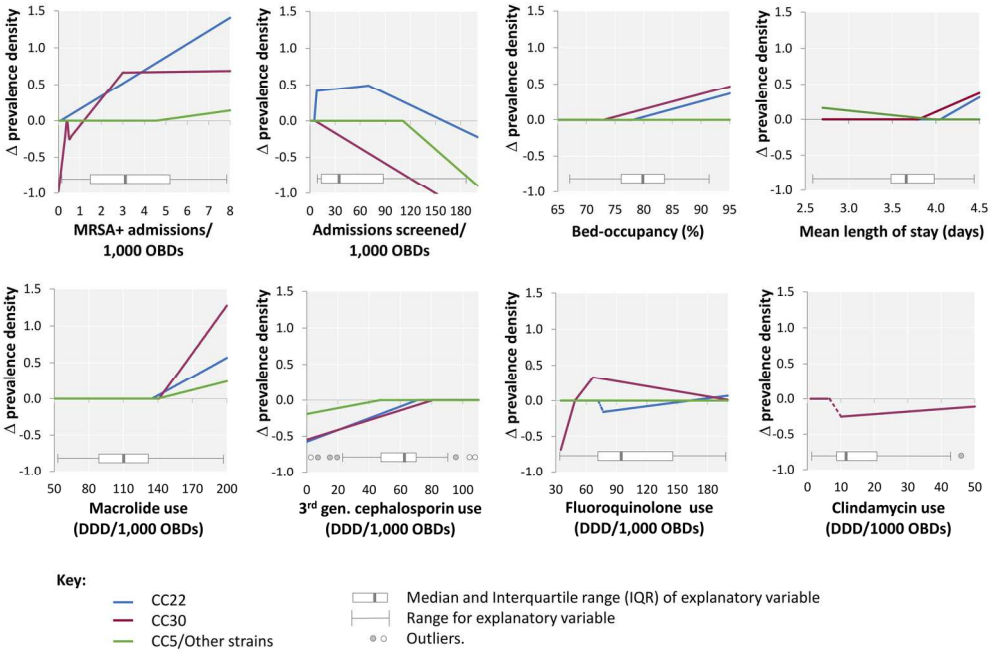


Figure 6: Contribution charts illustrating non-linear associations between explanatory variables and prevalence density of CC22, CC30, and CC5/other strains.<\b> Lines represent the change in (Δ) prevalence density (y-axis) associated with changes in explanatory variables over their observed range (see boxplots). Thresholds ('knots') are represented by a change in direction in the line. Where y = 0 there is no association with the explanatory variable. A dotted line represents an area of uncertainty within which the actual threshold is likely to be located. 168x113mm (300 x 300 DPI)

Supplemental File 1: Statistical Appendix detailing non-linear time-series analysis method

In the present article, we applied a novel time-series analysis (TSA) method to detect non-linear relationships between methicillin resistant *Staphylococcus aureus* (MRSA) and ecological exposures, including antibiotic use and infection control measures. We intend to publish a more detailed review of this methodology elsewhere, but present here a summary for those wishing to replicate our approach.

Non-linear TSA provides a more general form of the linear transfer-function (TF) models based on the Autoregressive Integrated Moving Average (ARIMA) approach. In linear TF models an outcome time-series (e.g. rate of resistant infection) is predicted as a linear function of contemporaneous or recent (lagged) ecological exposures and terms defining stochastic elements of natural time-series, including autoregression (response to prior values of the outcome time-series), moving average (response to prior 'shocks' (deviation from trend) in the outcome time-series) and integration of long-term trends (differencing of outcome time-series).

Mirroring the approach suggested by Box and Jenkins (1976) for ARIMA analysis,[1] we conducted non-linear TSA by a '3-step' process:

1. Identification

Firstly, we identified potentially significant (non-linear) associations between ecological exposures and resistance prevalence densities via inspection of the output from a General Additive Model (GAM) procedure.[2,3]. The GAM procedure is useful when we suspect the relationships between predictor variables ($x_{1\text{-}k}$) and dependent variable or outcome time-series (y) are nonlinear. A model of the form $y = f(x_1, x_2, ..., x_k)$ in a GAM can be written as a sum of smooth standardized functions $\alpha_j(.)$ as follows

$$E(y \mid x_1, x_2, ..., x_k) = \alpha_0 + \sum_{j=1}^{k} \alpha_j(x_j) + e$$

Such that expected values from functions of independent variables are equal to zero:

$$E[\alpha_j(x_j)] = 0 \quad \forall j = 1, ..., k$$

In GAM each function is defined by a forward stepwise estimation using a scatterplot smoother. Each time-series is centered to zero and a spline series added to form a smoothed series.

$$x_i^* = (x_i - \bar{x}_i) + s_i$$

The new function with splines can be estimated by the Ordinary Least Square approach:

$y^* = \beta_0^{gam} + \sum_{i=1}^{k} \beta_i^{gam} x_i + e_i$ where we have removed the nonlinearities from y ($y^* = y - \sum_{i=1}^{k} s_i$).

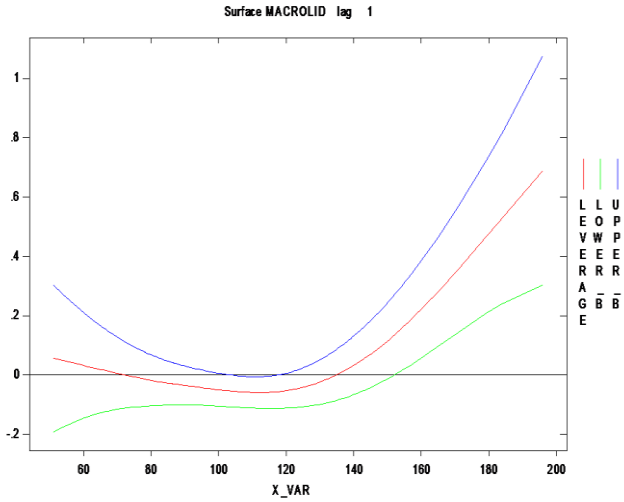
Predicted values for y can be recovered as $\hat{y} = \hat{y}^* + \sum_{i=1}^{k} s_i$

The forward stepwise estimation procedure uses a diagnostic test based on the residual sum square (RSS) differences between enhanced and restricted estimation.

$\frac{(RSS_R - RSS_E)}{RSS_E / (n-p)}$. Where; n = number of observations and p = parameters

Parameters (p) are added until level (k) where no significant improvements can be made to the estimate. GAM also provides upper and a lower confidence limits for the nonlinear relationship. Graphical illustration of the model estimate and confidence limits provides a means to identify independent variables (and lags) demonstrating potentially significant non-linear associations with the outcome (dependent) variable – figure i.

Figure i: Example of output from a GAM. A significant non-linear relationship is found between population macrolide consumption (x-axis) and resistance (y-axis represents change in prevalence density) 1 month later (lag 1). The central red graph line (labelled 'leverage') represents the model estimate for the change in rate of antibiotic resistant infection across all observed values of macrolide use (c. 50 to 200 DDDs/1,000 OBDs). Lines above and below represent upper and lower 95% confidence limits, respectively. Where a line at y = 0 (no change in prevalence density) falls within the 95% confidence limits no association between macrolide use and prevalence density is likely. Where the model estimate and 95% confidence limits deviate substantially from this line (the y=0 line falls outside the 95% CI) a significant association is likely. Based on visual inspection we expect a 'threshold' for association between the 95% Confidence limits (c. 120 to 150 DDDs/1,000 OBDs here). Below this threshold no association exists between macrolides and resistance. Above this threshold a positive association is seen, with increasing use of macrolide associated with increasing rate of resistance.



Intervention variables and Intervention analysis

The above holds for continuous time-series variables (e.g. antibiotic use in DDDs/1,000 OBDs) but in some instances only the date of the intervention +/- some idea of the shape of effect may be known. In intervention analysis (IA) it may be the explicit aim of the researcher to identify the total effect of the introduction of a new strategy. In both instances it is necessary to construct transfer functions for intervention variables describing (i) change in level (ii) change in slope (or trend).

To estimate a transfer function including intervention variables and other covariates we can proceed as follows:

Let us consider a transfer function model of the general form: $y_t = \alpha_0 + \sum_{j=1}^{p} \phi_j y_{t-j} + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$

Where; $\sum_{j=1}^{p} \phi_j y_{t-j}$ = sum of p-order autoregression terms (y_{t-j} = (y) in previous time-periods)

$\sum_{i=1}^{k} \rho_i x_{t-i}$ = the sum of transfer functions between explanatory variables (x_t) and (y_t).

Now, we can add dummy variables related to an intervention started at period τ such that:

$$d_t, \begin{cases} d_t = 0 \text{ for } t < \tau \\ d_t = 1 \text{ for } t \geq \tau \end{cases}$$

Our transfer function model, incorporating an intervention then consists of:

$$y_t = \alpha_0 + \alpha_0^I d_t + \sum_{j=1}^{p} (\phi_j + \phi_j^I d_{t-j}) y_{t-j} + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$$

Where; α_0^I is the parameter for the immediate effect on y_t (level effect)

ϕ_j^I is the parameter for the effect on the j^{th} autoregression term (slope effect)

The model can be rewritten as: $y_t = \alpha_0 + \alpha_0^I d_t + \sum_{j=1}^{p} \phi_j y_{t-j} + \sum_{j=1}^{p} \phi_j^I (d_{t-j} y_{t-j}) + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$

Where; $d_{t-j} y_{t-j}$ = interaction between the intervention dummy (d_{t-j}) and an autoregressive (y_{t-j}).

The total impact of an intervention is the sum of:

i. The level effect ($a_0^I d_t$)
ii. The slope effect, reflected in changes in autoregressive terms $\sum_{j=1}^{p} \phi_j^I (d_{t-j} y_{t-j})$

2. Estimation

After identifying significant non-linear associations by the GAM procedure, we then enter candidate variables (and lags) into a Multivariate Adaptive Regression Spline (MARS) model which is able to define thresholds in the relationships between independent and dependent variables. This procedure provides a systematic nonlinear estimation strategy that fit splines according to the seminal work of Friedman (1991).[4] It can detect and fit models in situations where there are distinct break points in associations, such as a result of a change in the underlying probability density function of the coefficients, i.e. a change in the slope.

As in GAM, we assume a nonlinear model $y = f(x_1,\cdots$ involving *N* observations for variables $x_1,\cdots$.
The MARS procedure attempts to approximate the nonlinear function with the addition of a weighted basis function: $\hat{f}(X) = \sum_{j=1}^{s} c_j K_j(X)$

Where; each $\{K_j(X)\}_{j=1}^{s}$ is associated with *s* sub-regions $\{R_j\}_{j=1}^{s}$ in the range of values of the independent variable.
and c_j is the coefficient for the j^{th} product basis function.

OLS is a particular case of a MARS procedure in which a single function defines the relationship between explanatory and outcome variables across all sub-regions from the total range of an independent variable.

MARS procedure can identify the sub-regions in which the coefficients are stable (approximately linear) and other regions when they are zero. For a function with two sub-regions defined by different slopes,

$$\begin{cases} y = \alpha + \beta_1 x + e & \text{for } x > 100 \\ y = \alpha + \beta_2 x + e & \text{for } x < 100 \end{cases}$$

MARS specification can be written as $y = \alpha' + c_1 \max(x - \tau^*, 0) - c_2 \max(\tau^* - x, 0) + e$

Where; the knot value (τ^*) = 100
and each max( ) is a truncated spline function, so $c_1 \equiv \beta_1$ and $c_2 \equiv \beta_2$.

It is worth to notice that it correspond a OLS estimation with a transformed independent variable (z):

$$y = \alpha' + c_1 z_1 - c_2 z_2 + e$$
$$z_1 = \max(x - \tau^*, 0)$$
$$z_2 = \max(\tau^* - x, 0)$$

To reach convergence in the MARS procedure Friedman (1991) suggested using a modified form of the generalized cross validation criterion (MGCV): $MGCV = [(1/N)\sum_{i=1}^{N}(y_i - \hat{f}(X))^2] / [1 - [C(M)^* / N]^2]$

Where; N is the number of observations,
$\hat{f}(X_i) \equiv \hat{y}_i$ (so $(y_i - \hat{f}(X))$ is the error for observation number i); and
$C(M)^*$ is a complexity penalty.

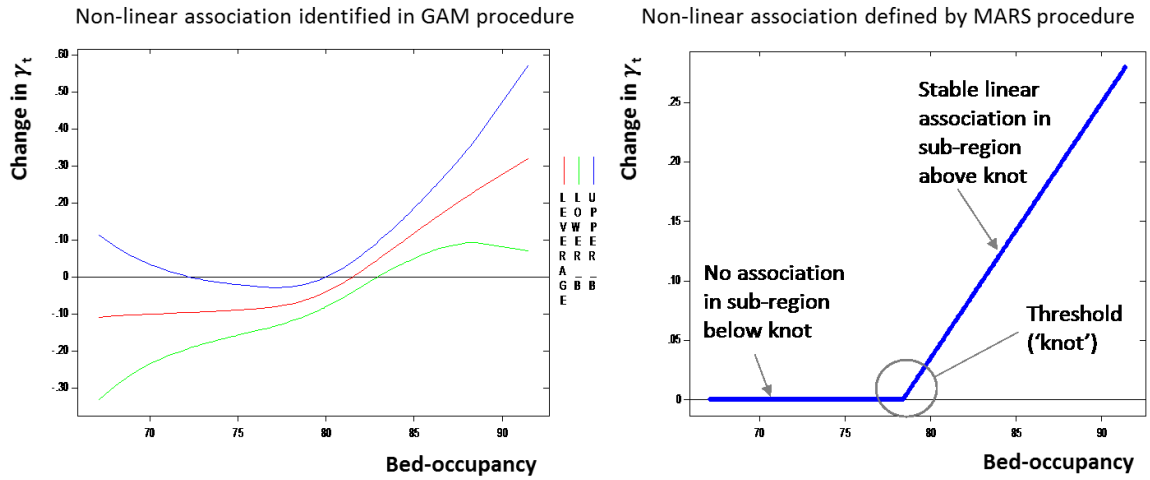
The default is to set $C(M)^*$ equal to a function of the effective no. of parameters: $C(M)^* = C(M) + \delta M$

Where; δ can be set by the user (Friedman suggests a value of 3).(Friedman 1991).
$C(M)$ is the number of parameters being fit; and
M is the number of non-constant basis functions in the model.

Minimizing the MGCV value controls how many parameters will finally remain in the model and can be used to form an estimate of the relative importance of each x_i variable. Once we include in MARS all those relevant variables detected by GAM convergence works in an approximation of the econometric general to specific approach, removing non-significant variables.

For each model, contribution charts show the nonlinear relationship of independent and dependent variables. Slopes are estimated c_i in MARS specification, and changes in slopes are knots τ^* (figure ii.)

Figure ii. Example of contribution chart from MARS output (right) with associated non-linear association identified in GAM.



3. Diagnosis:

A number of checks are made to ensure adequacy of model fit, as follows:

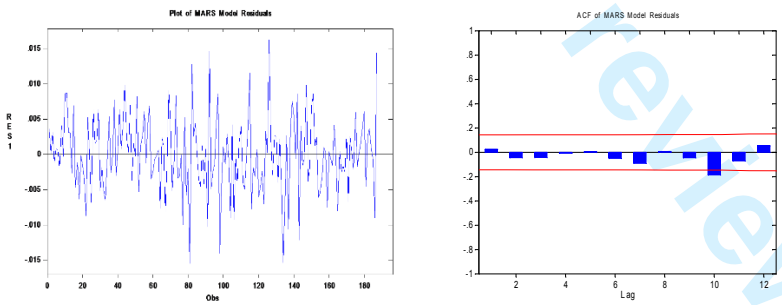
i. Residuals should correspond to 'white noise' (be normally distributed, with homogeneous variance, and mean = 0)

ii. A Hinich test is used to identify any non-linearities not detected by the model.

iii. Autocorrelation functions (ACF) display values not significantly different from zero.

Figure iii. Diagnostic checks of MARS model (a) residuals by observation (b) ACF of residuals

(a) **(b)**



Model performance is compared by:

i. R^2, representing the % of total variance in the outcome variable predicted by the model.

ii. Mean Absolute Percentage Error (MAPE) and Root Mean Squared Error (RMSE), provide measures of forecasting error. Improvement in fit is identified by smaller MAPE and RMSE.

Software:

GAM and MARS procedures can be run in a number of free or commercially available software packages. In the current paper we used SCA 8.1 (Scientific Computing Associates Corp. Illinois, US).

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[1] Box G, Jenkins G. Time series analysis: Forecasting and control, San Francisco, CA. Holden-Day 1970.

[2] Hastie T, and Tibshirani R. *Generalized Additive Models*. Chapman & Hall 1990.

[3] Faraway J. *Extending the Linear Model with R*. New York: Chapman & Hall/CRC 2006.

[4] Friedman J. Multivariate Adaptive Regression Splines. *Annals of Statistics* 1991;19 (1):1-67

[5] Hinich M. Testing for Gaussianity and Linearity of a Stationary Time Series. *Journal of Time Series Analysis* 1982;3:169-176.

[6] Stokes, Houston H. Specifying and Diagnostically Testing Econometric Models, ed. 2. New York, NY. Quorum Books 1997.

ORION Checklist for Intervention Studies or Outbreak Reports of Nosocomial Infection. (Stone et al. 2007)

Item	Descriptor (*If possible; ** If relevant)	Author's evaluation	Comment
1. Title & Abstract	Is paper described as an outbreak report (OR) or intervention study (IS)?	Yes	See "Objectives" in abstract.
	Is design of IS described?	Yes	Retrospective ecological study and time-series analysis
	Is Intervention & main outcomes described?	Yes	See "Interventions" and "Outcome measures" in abstract
INTRODUCTION			
2. Background	Are background and rationale for IS/OR explained?	Yes	See background
	Is organism described as epidemic, endemic?	Yes	"In the UK, national initiatives of infection control and antibiotic stewardship have been linked to a declining MRSA epidemic"
3. Type of paper	Is paper described as IS or OR?	Yes	See last paragraph of background.
	If OR, is number of outbreaks given?	N/A	N/A
4. Dates	Are start & finish dates of IS or OR given?	Yes	See last paragraph of background.
5. Objectives	Are objectives stated for OR? Are hypotheses stated for IS?	Yes	See last paragraph of background.
METHODS			
6. Design	Is study design described? How?	Yes	Retrospective ecological study and time-series analysis
	Is study described as retrospective, prospective or ambidirectional?	Yes	Retrospective.
	Does it state if decision to report or intervene prompted by any outcome data or not?	Yes	...national infection control and antibiotic stewardship strategies, prompted by detection of high-rates of nosocomial infection in mandatory surveillance
	Is it stated if study formally implemented or not, with protocol & endpoints?	Yes	Retrospective observational study (Not formally implemented).
7. Participants	Is number patients admitted given?	Yes	See table 1.
	Is age & length of stay given?	Yes	See table 1.
	Are eligibility criteria for IS or case definitions for OR given?	Yes	See table 1.
	Is % inter/intra-hospital transfers or admissions from care homes given?*	Yes	< 5% of admissions are transferred from other hospitals or regions
	Are potential risk factors for acquisition organism given?**	Yes	See "Outcomes and exposures"
8. Setting	Is unit, ward or hospital (and its units) described?.	Yes	See table 1 and "population and setting"
	Are number of beds, presence and staffing of infection control team given?	Yes	See table 1 and "population and setting"
9. Interventions	Are phases defined by major change in specific infection control practice?	Yes	See table 1.

	Is a summary table given, with details of interventions, their delivery and timing given?	Yes	See table 1.
10. Culturing & Typing	Are details of culture media, antibiograms and/or typing given?	Yes	See Laboratory methods
	Are details environmental sampling given?*	N/A	N/A
11. Infection-related outcomes	Are there clearly defined primary& secondary outcomes?	Yes	See table 1. and "Outcomes and exposures"
	Are they given at regular time intervals ?	Yes	Monthly over 16 years.
	Are there sufficient time points per phase? (see ORION author's checklist)?	Yes	192 time-points, including 148 before antibiotic stewardship
	Are denominators given (eg admissions, discharges, bed days)?	Yes	Occupied Bed Days (hospital); Inhabitant-days (community)
	Is all cause mortality given?	N/A	Not a study objective or outcome.
	Is prevalence organism, or incidence of colonisation on admission at same time intervals*?	Yes	Importation pressure and overall MRSA prevalence density measured at same time-intervals.
	In a short IS or OR is a chart used with duration patient stay & dates detection of organism ? (see author checklist)	N/A	N/A
12. Economic outcomes	Is this a formal economic study?	N/A	N/A
	If so, are outcomes defined? Are resources (for interventions) described? Are costs in basic units? Are assumptions stated?.	N/A	N/A
13. Potential Threats to internal validity	Which potential confounders were considered, recorded or adjusted for?(eg: length of stay, case mix, occupancy, staffing levels, hand-hygiene,, antibiotic use, strain type, processing of isolates, seasonality).	Yes	See "Outcomes and exposures"
	Are measures to avoid bias described? (eg blinding; standardisation outcome assessment/provision of care).	Yes	Informational bias reduced by electronic records and microbiologically defined outcome measures.
14. Sample size	Are power calculations given? (if appropriate)	N/A	Justifiation given in "Study Design" for time-period.
15. Statistical methods	Are statistical methods to compare groups or phases described?	Yes	"Statistical methods" and supplemental file 1.
	Do these account for dependencies in outcome data?	Yes	Explicitly measured in autoregression.
	Do they adjust where necessary for confounders?	Yes	Multivariate time-series analysis adjusted for ecological variables
	Are methods for subgroup or adjusted analyses described? Are they planned or not (exploratory)?	Yes	Variables used in adjusted analysis (multivariate time-series analysis) determined *a priori.*
	Is statistical analysis of an OR appropriate/necessary?	N/A	N/A
RESULTS			
16. Recruitment	Are the dates defining periods of recruitment & follow up given**?	N/A	N/A
	Is there a flow diagram**?	N/A	N/A

17. Outcomes and estimation	Is the estimated effect size & its precision given for main outcomes?	Yes	Coefficients (+ 95% CI) given in table 3.
	Is there a graphical summary of outcomes (for dependent data and most time series)?	Yes	Figure 6 provides summary of relationships between antibiotic use, infection control measures and strain prevalence densities
18. Ancillary analysis	Are subgroup analyses reported?	N/A	
	Are possible confounders adjusted for?	N/A	
19. Harms	Are these pre-specified in each group or phase?	N/A	Not a study objective or outcome.
DISCUSSION			
20. Interpretation	IS: is evidence for/against hypotheses assessed?	Yes	Hypothesis that antibiotic stewardship and infection control measures affect MRSA strain-dynamics discussed.
	Are plausible alternative explanations considered, including regression to mean &reporting bias?	Yes	Study limitations and threats to internal validity discusssed.
	OR: Is clinical significance of observations considered?	N/A	N/A
	Are explanatory hypotheses generated?	Yes	Concept of 'critical thresholds' in total antibiotic use.
21. Generalisability	Is there discussion of how results may generalise to different target populations or settings?	Yes	Comment made on external validity, and likely dependence of non-linear associations on clinical context
	Is feasibility of interventions considered?	Yes	Need to balance clinical priority with control of resistance noted as key to antibiotic stewardship. Limits to current infection control noted.
22. Overall evidence	Are results interpreted in context of current evidence?	Yes	Evaluated in light of previous evidence on MRSA strain dynamics

BMJ Open

Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-006596.R1
Article Type:	Research
Date Submitted by the Author:	17-Feb-2015
Complete List of Authors:	Lawes, Timothy; Royal Aberdeen Children's Hospital, Paediatrics López-Lozano, José-Maria; Hospital Vega Baja, Medicine Preventive-Infection Control Team Nebot, César; Centro Universitario de la Defensa (CUD) de San Javier, Econometrics Macartney, Gillian; Aberdeen Royal Infirmary, Antibiotic Pharmacy Subbarao-Sharma, Rashmi; Aberdeen Royal Infirmary, Pharmacy Dept Dare, Ceri; Aberdeen Royal Infirmary, Medical Microbiology Edwards, Giles; Scottish MRSA Reference Laboratory (SMRSARL), Gould, Ian; Aberdeen Royal Infirmary, Medical Microbiology
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Epidemiology, Public health, Research methods
Keywords:	Epidemiology < INFECTIOUS DISEASES, Infection control < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES, MICROBIOLOGY, STATISTICS & RESEARCH METHODS

Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis.

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Keywords:
Drug Resistance, Bacterial;
Infection Control;
Methicillin-Resistant *Staphylococcus* aureus;
Hand Hygiene;
Cohort Studies.

Word count:
Abstract = 2940 words
Background = 500 words
Methods = 935 words
Results = 1091 words
Discussion =1468 words
TOTAL (without abstract, tables, figures) = 3994 words

ABSTRACT

Objectives: To explore temporal associations between planned antibiotic stewardship and infection control interventions and the molecular epidemiology of Methicillin-Resistant *Staphylococcus* aureus (MRSA).

Design: Retrospective ecological study and time-series analysis integrating typing data from the Scottish MRSA reference laboratory.

Setting: Regional hospital and primary care in a Scottish Health Board.

Participants: General adult (N = 1,051,993) or intensive care (18,235) admissions and primary care registrations (460,000 inhabitants) between January 1997 and December 2012.

Interventions: Hand-hygiene campaign; MRSA admission screening; antibiotic stewardship limiting use of macrolides and '4Cs' (cephalosporins, co-amoxiclav, clindamycin and fluoroquinolones).

Outcome measures: Prevalence density of MRSA clonal complexes CC22, CC30 and CC5/*Other* in hospital (isolates/1000 occupied bed days, OBDs) and community (isolates/10,000 inhabitant-days).

Results: 67% of all clinical MRSA isolates (10,707/15,947) were typed. Regional MRSA population structure was dominated by hospital epidemic strains CC30, CC22 and CC45. Following declines in overall MRSA prevalence density, CC5 and other strains of community origin became increasingly important. Reductions in use of '4Cs' and macrolides anticipated declines in sub-lineages with higher levels of associated resistances. In multivariate time-series models (R^2 = 0.63 to 0.94) introduction of the hand-hygiene campaign, reductions in mean length of stay (when >4 days) and bed-occupancy (when >74 to 78%) predicted declines in CC22 and CC30, but not CC5/other strains. Lower importation pressures, expanded MRSA admission screening, and reductions in macrolide and 3rd generation cephalosporin use (thresholds for association: 135 to 141, and 48 to 81 Defined Daily Doses/1,000 OBDs, respectively) were followed by declines in all clonal complexes. Strain-specific associations with fluoroquinolones and clindamycin reflected resistance phenotypes of clonal complexes.

Conclusions: Infection control measures and changes in population antibiotic use were important predictors of MRSA strain dynamics in our region. Strategies to control MRSA should consider thresholds for effects and strain-specific impacts.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The internal and external validity of findings were strengthened by use of standardised data available over a long time horizon and for a geographically-defined population covered by a universal health system.
- By applying novel time-series analyses we demonstrated population interactions, strain-competition, and non-linear relationships with ecological determinants, convergent with understandings of the emergence and spread of resistance.
- An observational and ecological study design meant that associations may have been due to unidentified confounding variables, and may not have captured variation in molecular epidemiology explained by individual-level exposures.

INTRODUCTION

Staphylococcus aureus colonises around a third of humans, and is an important cause of infections in both hospital and community.[1] Resistance to penicillinase-resistant penicillins was first recognised more than 50 years ago,[2] and today MRSA is among the most commonly identified resistant nosocomial infections worldwide.[3] Resistance to β-lactam antibiotics is conferred by acquisition of a mobile genetic element: the *Staphylococcal* cassette chromosome (SCC*mec*).[4] This section of DNA contains the *mecA* gene, encoding for a modified penicillin binding protein; cassette chromosome recombinase genes, allowing for its excision and horizontal transfer; and variable elements encoding additional antibiotic resistances.[5,6] Rapid adaptation to selective pressures within a clonal genomic background facilitates clonal expansion and diversification, and this biodiversity allows MRSA to occupy a range of ecological niches.[7] Hospital-associated (HA-) strains typically contain SCC*mec* types I-III, encoding resistance to multiple antibiotics but also associated with slower growth and reduced toxin expression.[5] This fitness burden means HA-MRSA strains are typically limited to contexts of high-antibiotic pressure and high-density of vulnerable hosts. Community-associated (CA-) MRSA strains are characterised by SSC*mec* types IV–XI, carrying variable resistance to antibiotics and small fitness burdens.[5,8] These strains have a fitness advantage where selective pressures of antibiotic use fall below critical levels, and can infect healthy populations. Interactions of strains in hospital and community are increasingly recognised.[7, 9,10] The hospital epidemic strain EMRSA-15 is SSC*mec* IV, retaining some features consistent with its origin in the community.

The complex and evolving MRSA population structure creates challenges in the design and evaluation of control measures.[11] In the UK, national initiatives of infection control and antibiotic stewardship have been linked to a declining MRSA epidemic.[12-14] However, intervention effects may be strain-specific: the offset of fitness advantage and antibiotic resistance suggests that modifying ecological pressures could lead to clonal replacement.[10,11,15] Wyllie *et al.* have even suggested that declines in MRSA are attributable to spontaneous evolution within the MRSA population rather than impacts of infection control, and that health systems will continue to ride 'waves of trouble'.[16,17]

The ability to identify MRSA strains by molecular typing provides a tool for mapping their evolution and spread, and may inform more effective control strategies.[18] European studies have linked strain dominance to clinical context and antibiotic use,[15,19,20] with a particular focus on fluoroquinolones.[10,21-23] Advanced time-series analysis is well suited to investigating evolution in MRSA population structure, since it can distinguish the intrinsic progression of naturally occurring time-series from external influences of changes in ecological pressures.[24] While such analyses have explored associations between infection control measures and total MRSA rates,[25-29] we are not aware of any previous application to strain dynamics. Mathematical models have suggested critical thresholds in the impacts of ecological pressures, such as total antibiotic use, on resistance,[30,31] but to date empirical studies have only defined linear associations.

In this intervention study we used non-linear time-series analysis to investigate the extent to which national antibiotic stewardship and infection control strategies have determined the molecular epidemiology of MRSA across a Scottish health board between January 1997 and December 2012.

METHODS

Study Design

This retrospective observational study explored temporal associations between clinical burdens from MRSA clonal complexes and recent ecological exposures. Strain distribution and exposures were measured at monthly intervals over 16 years. This time-frame reflected the availability of routine typing data and covered a period of emergence, stabilisation, and decline in MRSA. It also allowed evaluation of the impacts of national infection control and antibiotic stewardship strategies, prompted by detection of high-rates of nosocomial infection in mandatory surveillance. Analysis controlled for natural progression within time-series of MRSA strain, strain-competition, and interactions between different clinical populations.

Setting and population

NHS Grampian is a large health board, serving 11% of Scotland's population. We investigated strain dynamics in three care settings: primary care (*community*), and general surgical/medical wards (*hospital*) or intensive care units (*ICU*) of the 1,000-bed regional referral hospital - Aberdeen Royal Infirmary (ARI). Less than 5% of admissions are transferred from other hospitals or regions. See table 1 for further details of participants.

Table 1: Study overview according to the ORION statement[32]

Setting: Community, hospital and intensive care unit (ICU) settings in North East Scotland. Infection prevention & control team (IPCT) including		**Dates:**1st Jan 1997 - 31st Dec 2012 (192 months)	**Population:** 480,000 adults registered in primary care; 1,091,250 admissions to general medical/surgical wards and 19,279 admissions to intensive care wards of Aberdeen Royal Infirmary (ARI). Mean (SD) age, 56 (1.2). Median (IQR) length-of-stay: 3.7 (3.5 to 4.1) Mean (SD) MRSA prevalence density in hospital and community = 1.91 (1.06) /1000 OBDs and 0.024 (0.017)/ 10,000 Inhabitant-days.
Antibiotic stewardship policy	January 1997 to April 2009: Annual reviews of hospital empirical antibiotic therapy guidelines. Very limited restrictive policies in place. Ongoing efforts to limit use of macrolides since Jan 2008. May 2009 to December 2012: Empirical guidelines recommended regimens avoiding '4C' antibiotics (Co-amoxiclav, cephalosporins, ciprofloxacin (all quinolones), clindamycin). Restricted supply of these antibiotics with use requiring prior authorisation from microbiology and pharmacy.		
General infection control measures	Alcohol gel introduced (Nov 2002)		
	National hand-hygiene campaign (Jan 2007)		
	National auditing of environmental cleaning (Apr 2006)		
	Healthcare Environment Inspectorate (HIE) inspection (Jan 2010)		
MRSA admission screening	Intensive Care Unit (ICU) Admission screening (May 2001)		
	Selective screening elective surgery & HDU (Jan 2006)		
	Universal admission screening (Aug 2008 to Mar 2011)		
	Targeted admission screening (March 2011 onwards) †		
Isolation and eradication policy	Isolation (single-room) or cohorting‡ of all patients with known MRSA or MRSA infected /colonised at admission. Decolonisation of all MRSA-positive patients with 5 days chlorhexidine body washes and intra-nasal mupirocin.		
Definitions and outcomes	Hospital-associated (HA-) MRSA cases	Non-duplicate MRSA isolates (1 per 14 days) from clinical specimens taken >48hrs after admission to hospital or ICU, excluding screening and infection control swabs.	
	Community-associated (CA-) MRSA case	Non-duplicate MRSA isolates from clinical specimens taken in the community or <48hrs of admission to hospital, excluding screening or infection control swabs.	
	Colonisation at admission	Isolation of MRSA from ≥1 admission screening swab, or known previous MRSA.	
	HA- or CA-MRSA Clonal Complex prevalence density	Hospital- or community-associated cases of MRSA attributable to a given clonal complex per 1000 OBDs (Hospital) or per 10,000 inhabitant-days (Community)	

† Recommended as a minimum standard by NHS Scotland following results of pathfinder study.[25]; OBDs = Occupied Bed Days; MRSA = Methicillin resistant *S.aureus.* SD = Standard Deviation.

Outcomes and exposures

The primary outcomes for the study were hospital- and community-associated prevalence densities of infections (de-duplicate clinical isolates) involving major clonal complexes grouped as CC22; CC30; and CC5/other strains. Data on prior healthcare exposures were not available so CA-MRSA included infections described elsewhere as healthcare-associated.

We considered a number of ecological exposures previously associated with MRSA burdens. Monthly population antibiotic use was measured in defined daily doses (DDDs)/1000 occupied bed days (OBDs) in hospital, or DDDs/1,000 inhabitant-days (IDs) in the community, and summarised according to the World Health Organisation Anatomical Therapeutic Chemical (WHO/ATC) classification.[33] Other covariates included: MRSA admission screening intensity (admissions screened/1000 OBDs); total and strain-specific importation pressures (admissions colonised or previous MRSA/1000 OBDs); mean length of stay (days) and bed-occupancy (%) in hospital populations. Consistent data on alcohol gel consumption and pre-intervention adherence with hand-hygiene or environmental cleaning standards were not available. We therefore introduced instrumental variables coding for changes in level (0 prior, 1 during intervention) and trend (autoregression*intervention) in strain prevalence densities associated with start of intervention.

Data Collection

Typing and antibiotic resistance phenotype data were derived from the Scottish MRSA Reference Laboratory (SMRSARL) for 10,707 MRSA clinical isolates and 4273 MRSA admission screening specimens from non-duplicate cases. Total antibiotic consumption in primary care was derived from the Prescribing Information System for Scotland (PRISMS). Remaining data was retrieved from regional health intelligence, pharmacy, microbiology, and infection control departments. Any individual or specimen level data were pseudo-anonymised by removal of identifiable personal information and replacement of unique personal or specimen numbers with matched study codes.

Laboratory methods

All *S.aureus* isolates were identified by agglutination, mainly with the Prolex™– Blue Staph Latex Kit (Pro-Lab). Antibiograms were determined using Clinical and Laboratory Standards Institute agar disk diffusion methods and, from 2008, by a Vitek™ instrument, using custom made *Staphylococcus* sensitivity cards (Biomerieux). EUCAST interpretative criteria were used from January 2012. MRSA screening swabs were cultured on MRSA selective medium, with use of chromogenic agar (Brilliance - Oxoid, UK) from 2006. Further details of methods utilised in the study period are available from previous publications.[25, 29]. All first patient clinical and screening isolates per year were sent to the reference lab until March 2011, after which only isolates from screening, blood cultures, outbreak investigations, or with unusual phenotypes were referred. Epidemiological typing of MRSA isolates into clonal complex was based on a combination of genotypic and phenotypic characteristics, matching >90% to known strains. Isolates were typed by the methods in use at the Reference Laboratory at the time of receipt. These varied during this study but always involved at least two independent methods. All isolates had their antibiotic resistance profile and biotype determined and at least one of phage typing, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR)-ribotyping or spa typing was also performed. If the resistance pattern or biotype was not one commonly associated in Scotland with the determined lineage then additional typing methods (usually multi-locus sequence typing, MLST) were used or, rarely, the strain was designated 'Other'. This means that that, despite

the multiplicity of typing methods used during the period of the study, there is high confidence in the typing result for those isolates ascribed to a specific lineage. No isolate was assigned to a lineage based on its antibiotic resistance profile alone. Assignment to a sub-lineage was based on antibiotic resistance profile or SCC*mec* typing by PCR.

Statistical analysis

Temporal trends in MRSA clonal complexes were estimated by applying the strain distributions (% typed isolates belonging to each clonal complex) to the total MRSA prevalence density in the same month in each clinical population. The distribution of antibiotic resistance phenotypes and sub-lineages by strain and quarter of year were summarised by heat-maps after excluding those appearing in ≤5 isolates in the study period. Autoregressive Integrated Moving Average (ARIMA) models were generated to explore temporal associations between hospital consumption of macrolides, ciprofloxacin, and clindamycin and associated resistances (% isolates) in each MRSA strain.[24]

To investigate the dissemination of clonal complexes through the regional healthcare network we considered temporal associations between strain prevalence density in ICU, hospital, and community and among those colonised with MRSA at admission. Granger causality tests were used to identify the direction of possible relationships (at lags 1-3 months). Long-run associations between time-series were defined by the Johansen cointegration test, and used to inform a Vector Error Correction model (lags 1-3 months) incorporating cointegration equations. Path diagrams were generated based on significant associations in these models, with connecting arrows proportional to the percentage of total variation in prevalence density explained by variation in other populations.

Finally, we used non-linear time-series analysis to explore significant predictors of strain prevalence density in hospital (full details are provided in supplemental file 1). Potentially significant non-linear associations were identified from visual inspection of the output from Generalised Additive Models (GAM). Candidate variables were entered into Multivariate Adaptive Regression Spline (MARS) models defining associations as a series of linear segments across ranges of the independent variables separated by thresholds (knots). Analyses were performed using SPSS 21.0 (IBM), Eviews 8.0 (IHS, California, USA) and SCA 8.1 (Scientific Computing Associates Corp. Illinois, US).

RESULTS

Trends in MRSA clonal complexes

Information on epidemiological typing was available for 60% (n = 4597/7727) of clinical isolates in the hospital population, 74% (5651/7647) of isolates in the community, and 80% (459/573) in the Intensive care unit (ICU) – figure 1a. Applying strain distributions (figure 1b) to the total MRSA prevalence densities in each population provided estimates of strain-specific prevalence densities - figure 1c.

A consistent secular trend in strain distribution was seen across all three populations. Between 1997 and 2003 CC30 (mostly UK-EMRSA-16) was the dominant strain. High prevalence densities of CC30 were seen in ICU before introduction of MRSA admission screening in this unit (May 2001), with little presence in the community. Between 2004 and 2008 the dominant strains were CC22 (UK-EMRSA-15) and, to a lesser extent, CC45 (limited to our region in Scotland), with large clinical burdens in all settings. Finally, from 2008 there was greater strain diversity, with CC5, CC8, CC1, and other clonal complexes of increasing importance. These strains explained 30% of HA-MRSA and 50% of CA- MRSA by 2012.

Trends in antibiotic resistance phenotypes and sub-lineages

Excluding resistance phenotypes represented by ≤5 isolates over the study period, MRSA isolates could be explained by 37 antibiograms – figure 2. 94% of CC30 and 90% of CC45 isolates were resistant to erythromycin, ciprofloxacin and clindamycin, and 78% of CC22 were characterised by resistance to erythromycin and ciprofloxacin. By contrast 92% of CC5 were susceptible to all three agents. Multi-drug resistance (≥3 antibiotic classes) was present in 88% (95% confidence interval (CI), 87 to 90%) of isolates before the third quarter of 2008, declining sharply thereafter to 60% (57 to 63%). Multi-drug resistance in CC22, increased from 6% when CC30 was dominant to 57% when CC22 was dominant (2004 to 2008), falling to 25% during antibiotic stewardship; Kruskal-Wallis test, $P = 0.002$. The most commonly acquired resistances in CC22 included trimethoprim (4% increasing to 66%; $P < 0.001$), tetracycline (1.4% to 10.7%; $P < 0.001$), clindamycin (1.3% to 3.9%); $P < 0.001$ for all comparisons. Concurrent increases in trimethoprim resistance were observed in CC30 (0.7% to 7.3%; $P < 0.001$), but not CC5/Other strains (10.5% to 4.8%; $P = 0.058$).

Changes in antibiotic resistance phenotypes of prevalent strains were predicted by trends in antibiotic consumption. During antibiotic stewardship resistance to erythromycin, ciprofloxacin and clindamycin declined in all strains – table 2 and figure 3.

Table 2: Temporal associations between hospital use of macrolides, fluoroquinolones and clindamycin and related antibiotic resistances within strains

Antibiotic and strain	ARIMA model† (p,d,q)(P,D,Q)	Model R^2	Lag	Coefficient (95% CI)‡	T ratio	*P* value
Macrolide use, DDDs/1000 OBDs						
CC22, % Erythromycin resistance	(1,0,1)(1,0,0)	0.291	0	0.088 (0.012 to 0.164)	2.25	0.026
CC30, % Erythromycin resistance	(2,0,2)(0,0,0)	0.432	5	0.098 (0.006 to 0.190)	2.08	0.039
CC5 & Other, % Erythromycin resistance	(1,0,0)(0,0,0)	0.109	0	0.110 (0.090 to 0.130)	11.51	<0.001
Fluoroquinolone use, DDDs/1000 OBDs						
CC22, % Ciprofloxacin resistance	(2,0,2)(1,0,0)	0.451	0	0.062 (0.027 to 0.097)	3.36	0.001
CC30, % Ciprofloxacin resistance	(2,0,2)(1,0,0)	0.331	0	0.128 (0.048 to 0.209)	3.14	0.002
CC5 & Other, % Ciprofloxacin resistance	(1,0,2)(0,0,0)	0.074	0	0.108 (0.076 to 0.140)	6.58	<0.001
Clindamycin use, DDDs/1000 OBDs						
CC22, % Clindamycin resistance	(1,0,1)(0,0,0)	0.298	0	0.173 (0.137 to 0.208)	9.76	<0.001
CC30, % Clindamycin resistance	(2,0,1)(0,0,0)	0.691	0	0.455 (0.067 to 0.843)	2.30	0.023
CC5 & Other, % Clindamycin resistance	(2,0,1)(0,0,0)	0.176	0	0.334 (0.175 to 0.493)	4.11	<0.001

† Autoregressive Integrated Moving Average models, in which: p = order (number) of non-seasonal autoregressive terms representing impact of previous values in time-series, d = order of differencing to achieve stationary time-series; q = order of non-seasonal moving average terms representing response to previous disturbances (residual error) in time-series; and P,D,Q reflect orders of seasonal (lag 12) autoregressive, differencing and moving average terms.

‡ Change in % resistance associated with a +1 DDD/1,000 OBDs increase in antibiotic use.

CI = Confidence Interval; DDDs = Defined Daily Doses; OBDs = Occupied Bed Days.

Changes in antibiotic resistance phenotypes within strains were partially explained by shifts in the distribution of sub-lineages – figure 4. Before antibiotic stewardship, hospital epidemic strains were dominated by sub-lineages with high rates of resistance to ciprofloxacin, erythromycin and clindamycin, including ST22-MRSA-IV (E15), ST36-MRSA-II (E16), and ST45-MRSA-II. During antibiotic stewardship higher proportions of isolates within these strains were from alternative sub-lineages, characterised by much lower rates of resistance to these three antibiotics. Conversely, within strains dominated by sub-lineages with low rates of resistance (including CC5 and CC8), alternative and more resistant sub-lineages, such as SM119, Tayside E3 and CC5-II, declined during antibiotic stewardship. One exception was the increasing importance within CC8 of Panton-Valentine Leukocidin (PVL) positive isolates, resembling USA300.[6]

Interactions of MRSA population structure in different populations

Typing was available for 33% (4273/13,048) of non-duplicate MRSA admission screening isolates. Applying the strain distribution from this typing to the total MRSA positive admission swabs per month provided time-series for strain-specific importation pressures for general hospital and ICU environments. Trends in strain-specific importation pressures coincided with the strain-dynamics seen among clinical isolates.

Granger causality tests and Vector Error Correction (VEC) models confirmed significant temporal associations between prevalence density of strains in ICU, hospital and community populations, and strain-specific importations pressures – figure 5. Importation pressures followed trends in related hospital prevalence densities, with less consistent and sizeable associations with community or ICU trends. Community prevalence densities of CC22, CC30 and CC45 were strongly determined by prior rates in hospital and ICU. By contrast, hospital epidemiology of CC5/other was anticipated by rates in the community.

Multivariate time series analyses

Multivariate Adaptive Regression Spline (MARS) models explained 91%, 94% and 58% of variation in prevalence densities of CC22, CC30, and CC5/Other strains, respectively -table 3.

Table 3: Summary of Time Series Multivariate Adapative Regression Splines models

Explanatory variables (order of terms)	Lag (months)	Threshold†	Relation to threshold	Change in prevalence density (95% confidence interval)	T-ratio	P-value
(a) CC22 (R^2 = 0.912)						
AR(1)	1	1.06	Above	+0.474 (0.271 to 0.677)	+4.57	<0.001
AR(2)	1	2.18	Above	-0.530 (-0.941 to -0.119)	-2.52	0.023
CC30 prevalence density, cases/1000 OBDs	0	0.363	Above	-0.337 (-0.483 to -0.231)	-6.26	<0.001
Mean bed-occupancy, %	3	78.4	Above	+0.022 (0.006 to 0.038)	+2.66	0.017
Mean length of stay, days	2	4.06	Above	+0.694 (0.178 to 1.210)	+2.63	0.018
Hand-hygiene campaign*AR(1), trend effect.	6	0.26	Above	-0.143 (-0.231 to -0.055)	-3.16	0.006
Admissions screened for MRSA /1000 OBDs (1)	1	4.24	Above	+0.138 (0.088 to 0.188)	+5.38	<0.001
Admissions screened for MRSA /1000 OBDs (2)	1	7.87	Above	-0.137 (-0.188 to -0.086)	-5.26	<0.001
Admissions screened for MRSA /1000 OBDs (3)	1	69.7	Above	-0.007 (-0.012 to -0.002)	-2.42	0.028
MRSA+ at admission/1000 OBDs	0	0.145	Above	+0.178 (0.125 to 0.231)	+6.53	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(1)	2	78.6	Above	+0.033 (0.009 to 0.057)	+2.69	0.016
Fluoroquinolone use, DDDs /1000 OBDs(2)	2	72.8	Above	-0.032 (-0.055 to -0.009)	-2.62	0.019
Macrolide use, DDDs/1000 OBDs	1	135	Above	+0.009 (0.002 to 0.015)	+2.62	0.019
Co-amoxiclav use, DDDs/1000 OBDs	2	235	Above	+0.010 (0.004 to 0.016)	+3.10	0.007
3rd Gen. Cephalosporin use, DDDs/1000 OBDs	5	81.0	Below	-0.007 (-0.010 to -0.004)	-4.22	<0.001
(b) CC30 (R^2 = 0.940)						
AR(1)	1	1.189	Above	+6.40 (4.48 to 8.311)	+6.54	<0.001
AR(2)	1	1.273	Above	-6.62 (-8.85 to -4.40)	-5.84	<0.001
AR(3)	1	1.773	Above	+0.794 (0.240 to 1.349)	+2.80	0.010
CC22 prevalence density, cases/1000 OBDs (1)	0	0.157	Below	+4.34 (2.99 to 5.71)	+6.28	<0.001
CC22 prevalence density, cases/1000 OBDs (2)	0	0.157	Above	-0.207 (-0.288 to -0.126)	-5.01	<0.001
Mean bed-occupancy, %	1	73.7	Above	+0.021 (0.009 to 0.033)	+3.50	0.002
Mean length of stay, days	1	3.85	Above	+0.531 (0.274 to 0.787)	+4.05	<0.001
Admissions screened for MRSA /1000 OBDs	1	5.11	Above	-0.007 (-0.008 to -0.005)	-8.92	<0.001
MRSA+ at admission/1000 OBDs (1)	0	0.498	Below	-2.442 (-3.382 to -1.501)	-2.91	0.008
MRSA+ at admission/1000 OBDs (2)	0	0.498	Above	-2.492 (-4.596 to -1.247)	-2.91	0.008
MRSA+ at admission/1000 OBDs (3)	0	0.623	Above	+2.86 (1.15 to 4.56)	+3.27	0.003
MRSA+ at admission/1000 OBDs (4)	0	3.038	Above	-0.361 (-0.464 to -258)	-6.86	<0001
Fluoroquinolone use, DDDs /1000 OBDs(1)	4	49.4	Below	-0.049 (-0.071 to -0.027)	-4.38	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(2)	4	49.4	Above	+0.018 (0.017 to 0.019)	+3.92	<0.001
Fluoroquinolone use, DDDs /1000 OBDs(3)	4	67.3	Above	-0.021 (-0.031 to -0.011)	-4.16	<0.001
Macrolide use, DDDs/1000 OBDs	1	141	Above	+0.022 (0.016 to 0.028)	+7.05	<0.001
Co-amoxiclav use, DDDs/1000 OBDs	5	160	Below	-0.005 (-0.008 to -0.002)	-3.35	0.003
Co-amoxiclav use, DDDs/1000 OBDs	5	160	Above	-0.003 (-0.005 to -0.001)	-3.82	<0.001
3rd gen. Cephalosporin use, DDDs/1000 OBDs	5	71.9	Below	-0.008 (-0.013 to -0.003)	-3.74	0.001
(c) CC5/Other strains (R^2 = 0.583)						
AR(1)	2	0.166	Below	-0.314 (-0.575 to -0.05)	-2.35	0.018
AR(2)	2	0.166	Above	-0.22 (-0.370 to -0.070)	-2.87	0.007
AR(3)	1	0.273	Above	-0.457 (-0.657 to -0.257)	-4.47	<0.001
Mean length of stay, days	1	3.98	Below	+0.177 (0.097 to 0.257)	+4.33	<0.001
Admissions screened for MRSA /1000 OBDs	0	110	Above	-0.011 (0.005 to 0.017)	-3.10	0.005
MRSA+ at admission/1000 OBDs (1)	3	4.565	Above	+0.041 (0.012 to 0.070)	+2.87	0.007
MRSA+ at admission/1000 OBDs (2)	5	6.235	Below	+0.184 (0.170 to 0.198)	+2.49	0.014
MRSA+ at admission/1000 OBDs (3)	5	6.235	Above	+0.971 (0.908 to 1.033)	+3.50	0.002
Macrolide use, DDDs/1000 OBDs	5	141	Above	+0.005 (0.002 to 0.008)	+3.59	0.002
Co-amoxiclav use, DDDs/1000 OBDs	5	241	Above	+0.008 (0.005 to 0.013)	+6.07	<0.001
3rd gen. Cephalosporin use, DDDs/1000 OBDs	5	47.1	Below	-0.004 (-0.006 to -0.002)	-3.69	<0.001

† Level of explanatory variable at which association appears. AR = Autoregressive term, reflecting impact of previous prevalence density in the same strain; DDDs = Defined Daily Doses; MRSA = Methicillin Resistance *Staphylococcus aureus;* OBDs = Occupied Bed Days.

Prevalence densities of CC22 and CC30 were inversely related suggesting competition for the same ecological niche. Bed-occupancies above 74 to 78% and length-of-stay over 4 days, were associated with higher rates of CC22 and CC30 over the next 1 to 3 months (lags 1 to 3) – figure 6. The negative coefficient for the interaction term hand-hygiene*AR(1) suggests the hand-hygiene campaign exerted a downward pressure on CC22 strongest in months of high prevalence density (where values of AR(1) were high). No association was noted with CC30 prevalence density which was already low at initiation of the campaign. In contrast, rates of CC5/other strains increased when length-of-stay was <4 days and were not related to hand-hygiene or bed-occupancy.

Importation pressure was important in determining nosocomial rates of CC22 and CC30 at almost all levels, whereas association with CC5/Other strains was mostly at high importation pressures (>6.24 MRSA+ admissions/1000 OBDs). Increased intensity of MRSA admission screening was followed by declines in prevalence density of CC30, CC22 and CC5/Other beyond thresholds of 5, 70 and 110 admissions screened per 1000 OBDs, respectively. The difference in threshold reflected the influence of earlier ICU screening on CC30, when overall inpatient screening levels were low.

Consistent non-linear associations were seen between inpatient macrolide or third generation cephalosporin use and prevalence density of all strains – figure 6. Macrolide consumption was positively associated with rates of CC30, CC22 and CC5, above a total use threshold of 125-141 DDDs /1000 OBDs. A 'ceiling' effect was noted for all associations with 3rd generation cephalosporin use, with reductions in consumption below 71-81 DDDs/1000 OBDs associated with lower prevalence densities, but no relationship seen above this threshold. A threshold effect was also observed with Co-amoxiclav use above 235-241 DDDs/1,000 OBDs being followed by similar increases in CC22 and CC5/other prevalence density, but a positive association with CC30 was only seen at lower levels of consumption (up to 160 DDDs/1,000 OBDs).

Other strain-specific associations reflected the resistance phenotype of the strain. Clindamycin consumption above 25 DDDs/1,000 OBDs was positively associated with rates of CC30, but was not significantly related to CC22 or CC5/other strains at any level of use. Increases in CC30 prevalence density were seen at levels of fluoroquinolone use up to 68 DDDs/1,000 OBDs (lag 4). Consumption above this level was inversely associated with CC30 but positively associated with CC22, suggesting selective advantage of CC22 under higher antibiotic pressure.

Where antibiotic consumption was positively associated with strain prevalence density, the median (range) % isolates within strains with related resistances was 98.1% (40% to 100%), compared to 3.7% (3.5% to 32%) where no association was identified (Mann-Whitney U test, P = 0.004). Consumption of other antibiotics in hospital or community were not significantly related to strain dynamics.

DISCUSSION

This 16-year retrospective study represents the first ever application of non-linear time-series analysis to investigate ecological determinants of MRSA strain dynamics. Following recent declines in hospital-associated epidemic strains such as CC22 and CC30, clonal complexes arising from the community, including CC5, became increasingly important in the region. Large shifts in strain distribution were underpinned by more subtle changes in sub-lineages and antibiotic phenotypes associated with changes in selective pressure from antibiotic use. Even after accounting for interactions between clinical populations, and natural progression within time series, we demonstrated that changes in infection control and antibiotic use were important predictors of this evolving MRSA population structure. Improved hand-hygiene, and reductions in bed-occupancy or length-of-stay, were followed by declining inpatient burdens from hospital-associated epidemic strains but had little or opposite effects on community strains. The hospital-associated prevalence density of all clonal complexes declined with increasing intensity of admission screening, but thresholds for association were strain-specific. Responses to consumption of antibiotics reflected the resistance phenotype of the strain and were subject to total use thresholds.

This study had several limitations. An observational and ecological design meant that associations may not be causal, may be explained by unidentified confounding variables, and may not reflect variation in molecular epidemiology due to individual-level exposures. However, although retrospective in nature, use of routinely collected data from electronic databases and standardised microbiological [34] and clinical definitions minimised risks of information bias. Change in criteria for sending isolates for typing (March 2011) was not likely to introduce bias since: major changes in antibiotic use and infection control occurred before this time, and covariates and direction of associations in baseline models for months before were unchanged; time-series for strain-distribution derived from isolate types sent throughout the study period were strongly correlated with time-series derived from wider range of isolates typed before the change in criteria (R^2 for 5-month moving averages =0.85 to 0.96). Use of a long time series (N = 192) and restriction of candidate explanatory variables through two-step GAM and MARS procedures helped to reduce the potential for spurious (chance) associations. Nevertheless measures of uncertainty around associations may be underestimated where data are used for model estimation and hypothesis testing. Further applications of our approach to other, similar, datasets is required to validate parameters reported here. Between 6% and 42% of variation in strain prevalence densities was not explained by multivariate models, suggesting unidentified determinants. We were unable to obtain consistent data on: staffing-levels;[35] transfer-rates;[23] isolation and decolonisation performance;[36] and compliance with hand-hygiene and environmental cleaning before initiation of national strategies.[37] External validity was strengthened by exploring strain-dynamics in a geographically-defined population covered by a universal health system, and in various levels of care. However, our findings also highlight the importance of healthcare environments and local ecological exposures in shaping strain-dynamics, which may limit generalisability of specific associations.

Previous evidence on associations between infection control measures or antibiotic use and MRSA strain-dynamics has largely been from in-vitro or animal experiments,[21,38] and mathematical models.[39] While such studies have demonstrated important concepts of stain competition and strain-specific impacts of manipulating selective pressures, examining the evolution of MRSA in real-life contexts provides greater ecological and population validity. Wyllie and colleagues have highlighted the importance of considering internal strain-dynamics when evaluating the contribution of national infection control strategies to

recent declines in MRSA within the UK.[16] In a large observational study, these authors explored the evolution of MRSA and two epidemic strains (CC30 and CC22) in Oxfordshire hospitals alongside infection control strategies.[17] They concluded that recent falls in MRSA rates were more likely attributable to spontaneous strain dynamics than interventions since: declines were seen before intensification of infection control; and decline in CC30 was much steeper than that in CC22. Elsewhere, in a 10 year study of an MRSA population in a London hospital, Knight *et al.* noted a similar shift in dominant strain from CC30 to CC22, and attributed it to fitness advantage in CC22 after acquisition of additional resistances.[22] This evolution was independent of ecological pressures, but fluoroquinolone resistance was a key feature of successful hospital strains and overall MRSA declined after restriction of these antibiotics. These investigations made limited attempts to model impacts of interventions and changing antibiotic use, adjust for expected progression of time-series, or consider population interactions. In overcoming these methodological weaknesses, our study helps reconcile conflicting evidence.

Firstly, results of multivariate models suggest that even those infection control measures expected to have general effects can have strain-specific impacts due to differences in the temporal and spatial distribution of clonal complexes. Threshold effects of hand-hygiene have been identified previously.[40] Our findings also suggest that impacts of a national initiative to improve hand-hygiene were dependent upon background prevalence densities of CC22 and CC30 during the campaign.[40] Greater impact during period of high prevalence density is consistent with the role of hand-hygiene in reducing transmission, and of diminishing returns at lower prevalence density.[41] Several time-series analyses have demonstrated the importance of bed-occupancy in determining rates of MRSA,[42] with both guidelines[43] and research[35] suggesting safety thresholds between 82 and 90%. We found highly consistent associations between bed-occupancy and rates of CC22 and CC30 above thresholds of 74-78%: much lower than average bed-occupancies of 82-88% across the UK.[44] The association with bed-occupancy was not explained by variation in mean inpatient age and seasonality, but may reflect changes in case-mix during winter rather than increased transmission. Congruent with hospital burdens from CC5/other strains being driven by importation from the community, no associations were seen with hand-hygiene or bed-occupancy. Similarly while lower average length-of-stay anticipated declines in CC22 and CC30, it was associated with increases in hospital burdens from CC5/other strains. Given that antibiotic-resistant infections lead to longer length-of-stay a complex bidirectional relationship is likely.[45] We noted the threshold of hospital-wide MRSA admission screening at which declines were seen varied considerably between strains, probably reflecting the roll-out among different clinical populations, and background rates of strains.[46] Population interaction models suggested that ICU was a key environmental niche for CC30, consistent with a highly drug resistance phenotype. Early introduction of admission screening in this unit (May 2001) resulted in an abrupt and permanent decline in total MRSA rates,[47] which this study suggests was attributable to control of CC30. Responsiveness may also reflect much more frequent carriage of qacA, encoding for chlorhexidine resistance, in CC22 compared to CC30.[48] However, we have not identified increasing chlorhexidine resistance in the ICU. Declines in CC22 and CC5/other strains were limited to months when hospital-wide screening exceeded 70 and 110 admissions screens/1,000 OBDs: a level only seen during expansion to HDU/surgical and universal admission screening, respectively. On the basis of cost-effectiveness,[49] risk-factor based (targeted) screening is advocated in Scotland. However, since community strains can appear in patients without traditional risk-factors for MRSA, this approach may be insufficient to prevent invasion into hospitals.[50]

We further demonstrated the importance of selective pressures from population antibiotic use in determining the molecular epidemiology of MRSA. Alongside non-linear associations strongly related to the typical resistance profiles of strains, declining use of '4C' and macrolide antibiotics coincided with changes in antibiotic resistance phenotypes and shift towards more susceptible sub-lineages within all clonal complexes. Total antibiotic use thresholds may represent 'tipping points' at which ability to adapt to different selective pressure determines strain success within environmental niches. The rapidity of change within strains during hospital antibiotic stewardship is in keeping with mathematical models demonstrating declines in resistance within weeks to months, even in the absence of high fitness costs.[31, 51] Studies in France have described secular trends towards strains and resistance phenotypes with susceptibility to macrolides and gentamicin despite a lack of change in antibiotic consumption.[15,19] However, use of macrolides in these areas was around 40 DDDs/1,000 OBDs, and well below the thresholds for association with strain prevalence in our study. The studies also highlighted the selective advantage of strains carrying SCC type IV, associated with high genetic plasticity mediated by the frequent transfer of[42] mobile genetic elements.[15] Consistent with Knight *et al.* we noted that dominance of CC22-IV in hospital coincided with acquisition of multiple antibiotic resistances.[22] We have previously noted increasing trimethoprim resistance in major epidemic strains associated with regional use in MRSA throat decolonisation.[52] Our finding that CC22 outcompeted CC30 at higher intensity of fluoroquinolone (FQ) use is congruent with lower fitness costs of FQ resistance in CC22,[21] and its critical role in the dissemination of CC22 through the UK health system.[23]

Our findings suggest that implementation and evaluation of interventions to control MRSA can be improved by consideration of non-linear and strain-specific impacts. Recognising critical thresholds in modifiable ecological pressures may enhance cost-effectiveness by determining optimal levels of intervention and identifying areas where impacts are unlikely.[53] Limiting population antibiotic use to below critical levels may provide a powerful means to balance immediate clinical need with avoidance of resistance and sustainability of use.[30] Further applications of our approach in other populations and clinical contexts is required to elucidate factors modifying thresholds for association with ecological variables, and to adapt antibiotic stewardship or infection control policies to local scenarios. These factors may include: age and comorbidities in the clinical population; baseline rates of MRSA; existing strain distributions; importation pressures;[50] and interactions with other populations.[23] Previous investigations have demonstrated complex within-host strain dynamics. Multi-level analyses could quantify the relative contribution of individual and population level exposures to acquisition or infection with specific strains.[54] The relative weakness of existing hospital-based infection control measures in controlling CC5/other strains seen in this study suggests a pressing need for strategies to control burdens from clonal complexes arising in the community.[55]

In conclusion, this study found evidence that changes in infection control and population antibiotic use have contributed to MRSA strain dynamics in Scotland over the past 16 years. Declines in overall clinical burdens from MRSA were convergent with intensified hospital infection control and antibiotic stewardship strategies removing selective pressures favouring hospital epidemic strains. Future efforts to control MRSA, and in particular evolving community strains, should consider thresholds for effects and strain-specific impacts.

ACKNOWLEDGEMENTS The authors wish to thank the Scottish MRSA Reference Laboratory, and health intelligence, medical microbiology and pharmacy departments of NHS Grampian, for their help in obtaining data. TL, IMG, JMML and CN designed the study, strain-typing was coordinated by GE, data-collection was by TL, GE, GM and RSS, and analysis by TL, JMML and CN. All authors reviewed and approved the final manuscript.

COMPETING INTERESTS TL, CRJD, GM, RSS, CN, JMML and GE have no conflicts of interest to declare. IMG consults and lectures for various pharmaceutical and diagnostic companies specialising in control of MRSA.

FUNDING This study was part of the TSARINAS (Time Series Analysis of Resistance, Infection control, and Antibiotic Stewardship) project, supported by grants from the NHS Grampian Clinical Microbiology Endowment Fund. No external funding was attached to this project.

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FIGURE LEGENDS

Figure 1: (a) Epidemiological typing of clinical MRSA isolates, and distribution of clonal complexes† as (b) cumulative % typed isolates or (c) prevalence density by population. † 'Other' clonal complexes included CC7, CC15, CC59, CC88, CC93 and C239.

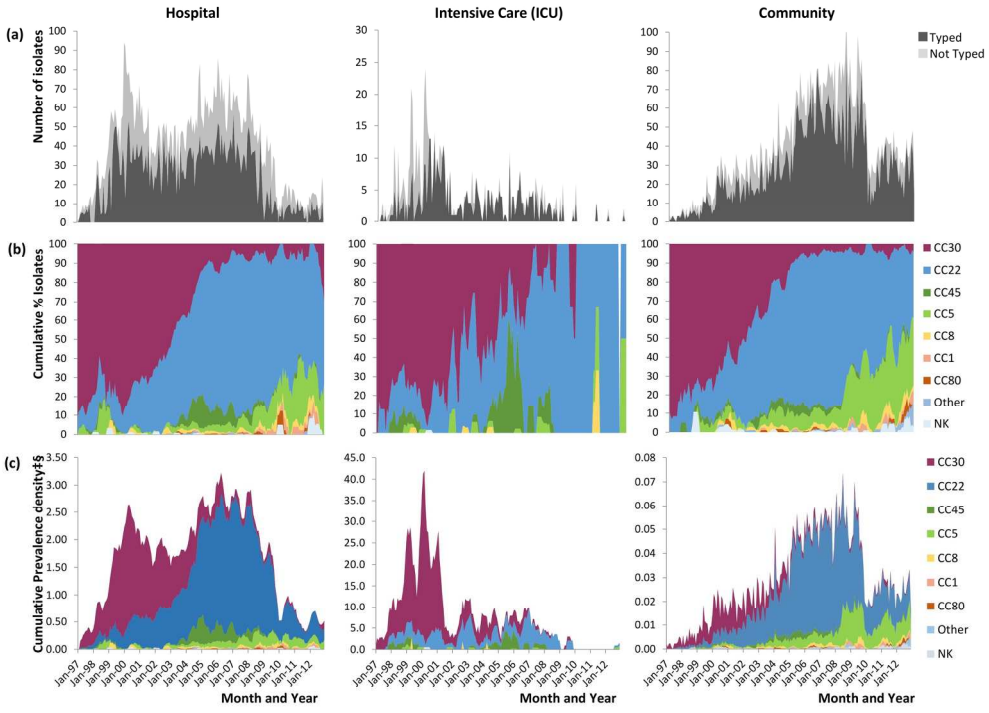
Figure 2: Heat map of antibiotic resistance phenotypes including total number in study period, % of isolates in each strain, and % of all isolates per quarter of year

Figure 3: Percentage of isolates within strains resistant to Erythromycin, Ciprofloxacin or Clindamycin and consumption of related antibiotics from univariate ARIMA time-series models (3m moving averages)

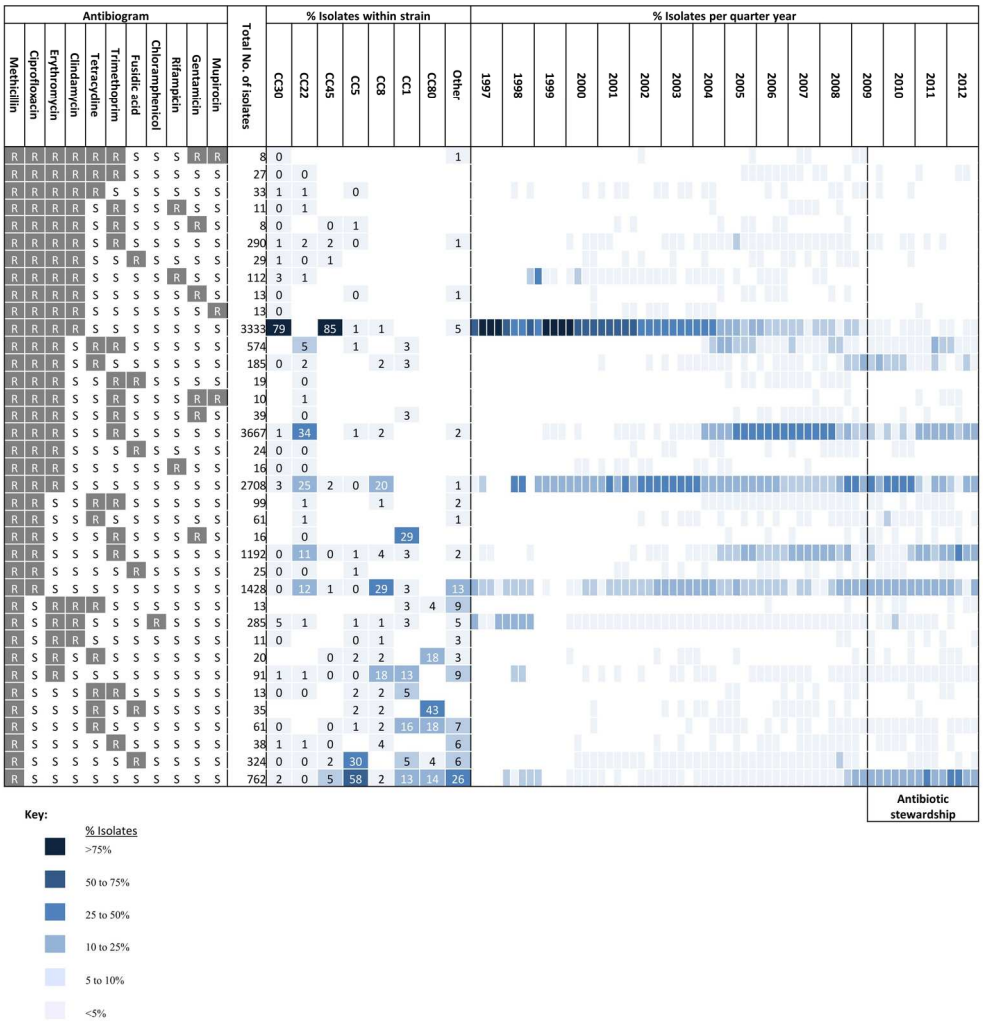
Figure 4: Heat map describing relative frequency (% total isolates in strain per quarter) of sub-lineages of the five most prevalent clonal complexes

Figure 5: Flow charts of temporal associations between prevalence density of MRSA strains in different clinical populations, as derived from Vector Error Correction (VEC) models. Boxes represent patient populations, arrows the direction of temporal association, and numbers (months) the delay in associated changes. Arrow width is proportional to the % of total variation in response time-series (population prevalence density) explained by input time-series.

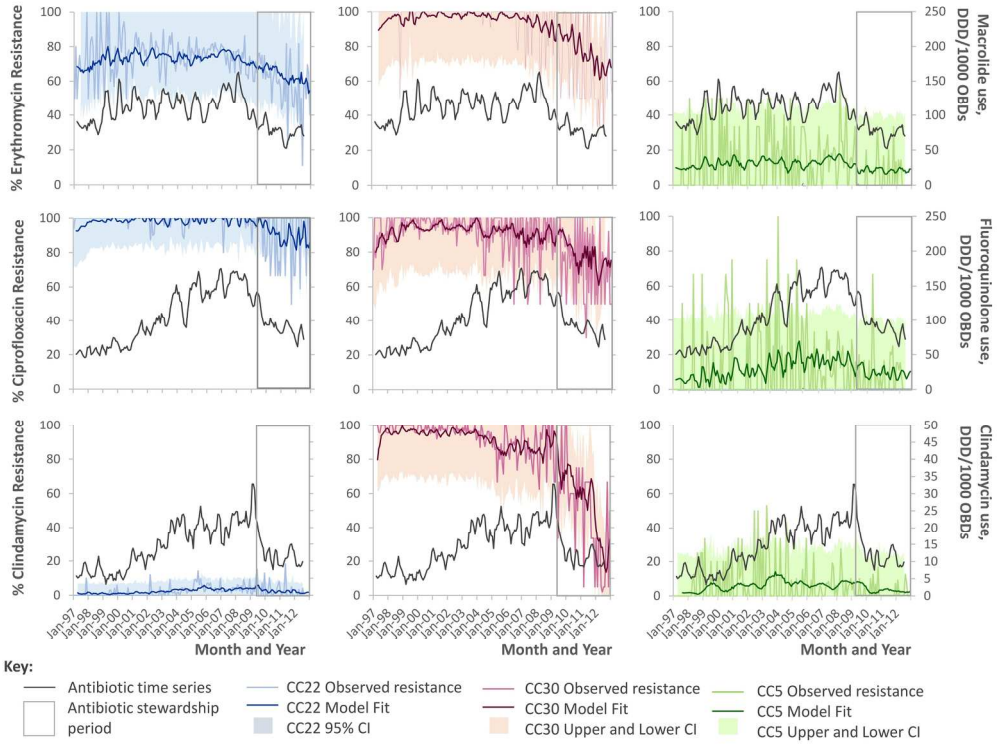
Figure 6: Contribution charts illustrating non-linear associations between explanatory variables and prevalence density of CC22, CC30, CC5/other strains. Lines represent the change in (Δ) prevalence density (y-axis) associated with changes in explanatory variables over their observed range (see boxplots). Thresholds ('knots') are represented by a change in direction in the line. Where y = 0 there is no association with the explanatory variable. A dotted line represents an area of uncertainty within which the actual threshold is likely to be located.



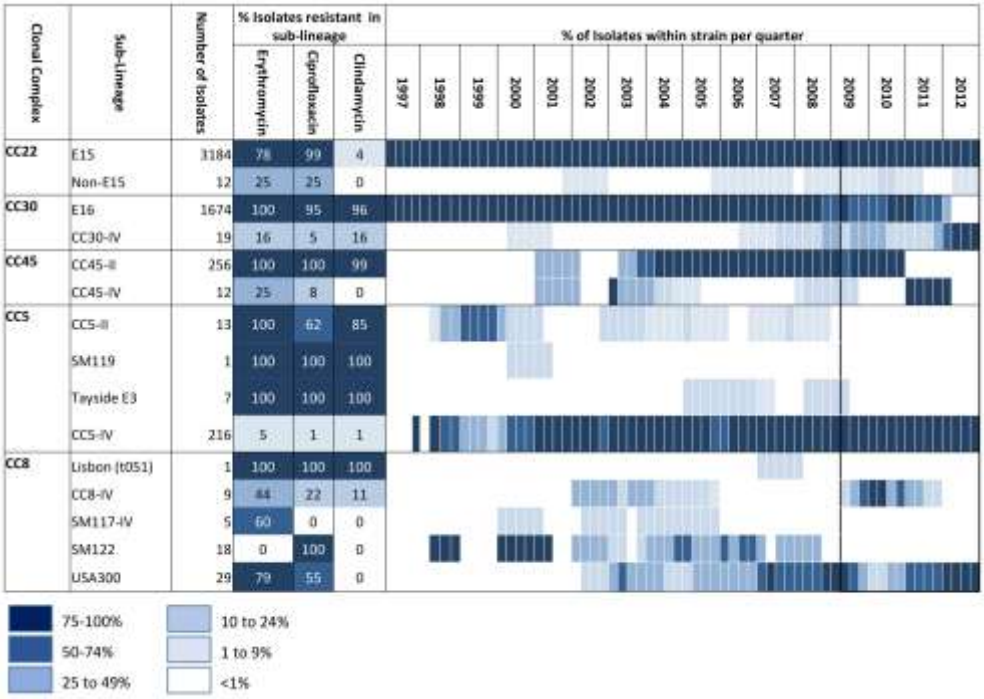
(a) Epidemiological typing of clinical MRSA isolates, and distribution of clonal complexes† as (b) cumulative % typed isolates or (c) prevalence density by population. † 'Other' clonal complexes included CC7, CC15, CC59, CC88, CC93 and C239.
190x134mm (300 x 300 DPI)



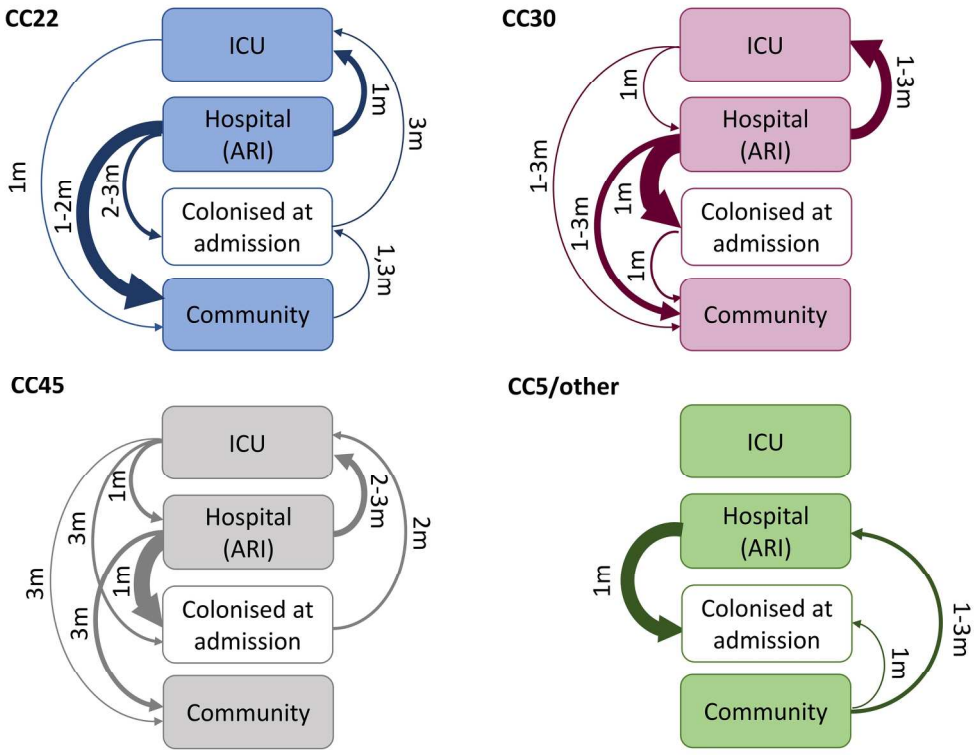
Heat map of antibiotic resistance phenotypes including total number in study period, % of isolates in each strain, and % of all isolates per quarter of year
171x177mm (300 x 300 DPI)



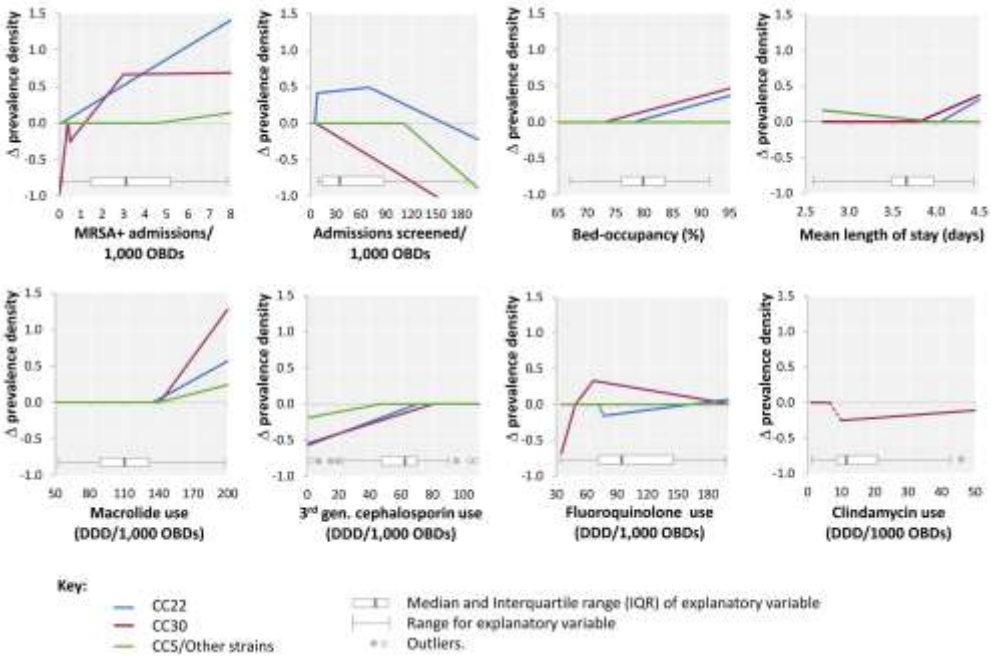
Percentage of isolates within strains resistant to Erythromycin, Ciprofloxacin or Clindamycin and consumption of related antibiotics from univariate ARIMA time-series models (3m moving averages)
190x142mm (300 x 300 DPI)



Heat map describing relative frequency (% total isolates in strain per quarter) of sub-lineages of the five most prevalent clonal complexes
177x124mm (300 x 300 DPI)



Flow charts of temporal associations between prevalence density of MRSA strains in different clinical populations, as derived from Vector Error Correction (VEC) models. Boxes represent patient populations, arrows the direction of temporal association, and numbers (months) the delay in associated changes. Arrow width is proportional to the % of total variation in response time-series (population prevalence density) explained by input time-series.
190x149mm (300 x 300 DPI)



Contribution charts illustrating non-linear associations between explanatory variables and prevalence density of CC22, CC30, CC5/other strains. Lines represent the change in (Δ) prevalence density (y-axis) associated with changes in explanatory variables over their observed range (see boxplots). Thresholds ('knots') are represented by a change in direction in the line. Where y = 0 there is no association with the explanatory variable. A dotted line represents an area of uncertainty within which the actual threshold is likely to be located.
168x113mm (300 x 300 DPI)

Supplemental File 1: Statistical Appendix detailing non-linear time-series analysis method

In the present article, we applied a novel time-series analysis (TSA) method to detect non-linear relationships between methicillin resistant *Staphylococcus aureus* (MRSA) and ecological exposures, including antibiotic use and infection control measures. We intend to publish a more detailed review of this methodology elsewhere, but present here a summary for those wishing to replicate our approach.

Non-linear TSA provides a more general form of the linear transfer-function (TF) models based on the Autoregressive Integrated Moving Average (ARIMA) approach. In linear TF models an outcome time-series (e.g. rate of resistant infection) is predicted as a linear function of contemporaneous or recent (lagged) ecological exposures and terms defining stochastic elements of natural time-series, including autoregression (response to prior values of the outcome time-series), moving average (response to prior 'shocks' (deviation from trend) in the outcome time-series) and integration of long-term trends (differencing of outcome time-series).

Mirroring the approach suggested by Box and Jenkins (1976) for ARIMA analysis,[1] we conducted non-linear TSA by a '3-step' process:

1. Identification

Firstly, we identified potentially significant (non-linear) associations between ecological exposures and resistance prevalence densities via inspection of the output from a General Additive Model (GAM) procedure.[2,3]. The GAM procedure is an extension of linear regression where we suspect the relationships between predictor variables ($x_{1\text{-}k}$) and dependent variable or outcome time-series (y) are nonlinear. A model of the form $y = f(x_1, x_2, ..., x_k)$ in GAM can be written as a sum of smooth functions:

$$E(y \mid x_1, x_2, ..., x_k) = \alpha_0 + \sum_{j=1}^{k} \alpha_j(x_j) + e$$

where smooth functions $\alpha_j(.)$ are standardised such that $E[\alpha_j(x_j)] = 0 \quad \forall j = 1, ..., k$

Functions $\alpha_j(.)$ are estimated one at a time, in a forward stepwise manner, using a *scatterplot smoother*. Each time-series is centered to zero (x_i - $\bar{x}_i$) and a spline series (s_i) added to form a smoothed series x_i^*:

$$x_i^* = (x_i - \bar{x}_i) + s_i$$

The new function with splines can be estimated by the Ordinary Least Square approach:

$y^* = \beta_0^{gam} + \sum_{i=1}^{k} \beta_i^{gam} x_i + e_i$ where we have removed the nonlinearities from y ($y^* = y - \sum_{i=1}^{k} s_i$).

Predicted values for y can be recovered as $\hat{y} = \hat{y}^* + \sum_{i=1}^{k} s_i$

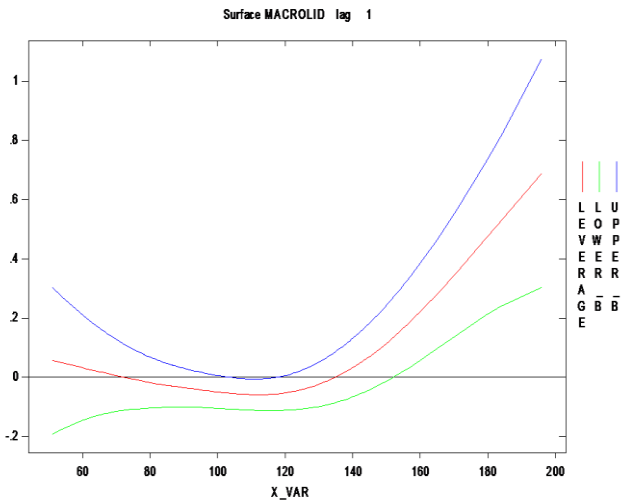
The forward stepwise estimation procedure uses a diagnostic test based on the residual sum square (RSS) differences between enhanced and restricted estimation.

$\frac{(RSS_R - RSS_E)}{RSS_E / (n-p)}$. Where; n = number of observations and p = parameters

Parameters (p) are added until level (k) where no significant improvements can be made to the estimate. Graphical illustration of the model estimate and confidence limits provides a means to identify independent variables (and lags) demonstrating potentially significant non-linear associations with the outcome (dependent) variable – figure i.

Further explanation of the General Additive Model (GAM) procedure can be found in Simon Wood's book "*General Additive Models: An Introduction with R*".[4]

Figure i: Example of output from a GAM. A significant non-linear relationship is found between population macrolide consumption (x-axis) and resistance (y-axis represents change in prevalence density) 1 month later (lag 1). The central red graph line (labelled 'leverage') represents the model estimate for the change in rate of antibiotic resistant infection across all observed values of macrolide use (c. 50 to 200 DDDs/1,000 OBDs). Lines above and below represent upper and lower 95% confidence limits, respectively. Where a line at y = 0 (no change in prevalence density) falls within the 95% confidence limits no association between macrolide use and prevalence density is likely. Where the model estimate and 95% confidence limits deviate substantially from this line (the y=0 line falls outside the 95% CI) a significant association is likely. Based on visual inspection we expect a 'threshold' for association between the 95% Confidence limits (c. 120 to 150 DDDs/1,000 OBDs here). Below this threshold no association exists between macrolides and resistance. Above this threshold a positive association is seen, with increasing use of macrolide associated with increasing rate of resistance.



Intervention variables and Intervention analysis

The above holds for continuous time-series variables (e.g. antibiotic use in DDDs/1,000 OBDs) but in some instances only the date of the intervention +/- some idea of the shape of effect may be known. In intervention analysis (IA) it may be the explicit aim of the researcher to identify the total effect of the introduction of a new strategy. In both instances it is necessary to construct transfer functions for intervention variables describing (i) change in level (ii) change in slope (or trend).

To estimate a transfer function including intervention variables and other covariates we can proceed as follows:

Let us consider a transfer function model of the general form: $y_t = \alpha_0 + \sum_{j=1}^{p} \phi_j y_{t-j} + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$

Where; $\sum_{j=1}^{p} \phi_j y_{t-j}$ = sum of p-order autoregression terms ($y_{t\text{-}j}$ = (y) in previous time-periods)

$\sum_{i=1}^{k} \rho_i x_{t-i}$ = the sum of transfer functions between explanatory variables (x_t) and (y_t).

Now, we can add dummy variables related to an intervention started at period τ such that:

$$d_t, \begin{cases} d_t = 0 \text{ for } t < \tau \\ d_t = 1 \text{ for } t \geq \tau \end{cases}$$

Our transfer function model, incorporating an intervention then consists of:

$$y_t = \alpha_0 + \alpha_0^I d_t + \sum_{j=1}^{p} (\phi_j + \phi_j^I d_{t-j}) y_{t-j} + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$$

Where; α_0^I is the parameter for the immediate effect on y_t (level effect)

ϕ_j^I is the parameter for the effect on the j^{th} autoregression term (slope effect)

The model can be rewritten as: $y_t = \alpha_0 + \alpha_0^I d_t + \sum_{j=1}^{p} \phi_j y_{t-j} + \sum_{j=1}^{p} \phi_j^I (d_{t-j} y_{t-j}) + \sum_{i=1}^{k} \rho_i x_{t-i} + e_i$

Where; $d_{t-j} y_{t-j}$ = interaction between the intervention dummy ($d_{t\text{-}j}$) and an autoregressive ($y_{t\text{-}j}$).

The total impact of an intervention is the sum of:

i. The level effect ($a_0^I d_t$)
ii. The slope effect, reflected in changes in autoregressive terms $\sum_{j=1}^{p} \emptyset_j^I (d_{t-j} y_{t-j})$

2. Estimation

After identifying significant non-linear associations by the GAM procedure, we then enter candidate variables (and lags) into a Multivariate Adaptive Regression Spline (MARS) model which is able to define thresholds in the relationships between independent and dependent variables. This procedure provides a systematic nonlinear estimation strategy that fit splines according to the seminal work of Friedman (1991).[5] It can detect and fit models in situations where there are distinct break points in associations, such as a result of a change in the underlying probability density function of the coefficients, i.e. a change in the slope.

As in GAM, we assume a nonlinear model $y = f(x_1, \cdots$ involving *N* observations for variables $x_1, \cdots$.
The MARS procedure attempts to approximate the nonlinear function with the addition of a weighted basis function: $\hat{f}(X) = \sum_{j=1}^{s} c_j K_j(X)$

Where; each $\{K_j(X)\}_{j=1}^{s}$ is associated with *s* sub-regions $\{R_j\}_{j=1}^{s}$ in the range of values of the independent variable.
and c_j is the coefficient for the j^{th} product basis function.

OLS is a particular case of a MARS procedure in which a single function defines the relationship between explanatory and outcome variables across all sub-regions from the total range of an independent variable.

MARS procedure can identify the sub-regions in which the coefficients are stable (approximately linear) and other regions when they are zero. For a function with two sub-regions defined by different slopes,

$$\begin{cases} y = \alpha + \beta_1 x + e & \text{for } x > 100 \\ y = \alpha + \beta_2 x + e & \text{for } x < 100 \end{cases}$$

MARS specification can be written as $y = \alpha' + c_1 \max(x - \tau^*, 0) - c_2 \max(\tau^* - x, 0) + e$

Where; the knot value (τ^*) = 100 and each max( ) is a truncated spline function (isolated to the area above (x-τ^*, 0) or below (τ^* - x, 0) the knot) , so $c_1 \equiv \beta_1$ and $c_2 \equiv \beta_2$.

It is worth to notice that it correspond a OLS estimation with a transformed independent variable (z):

$$y = \alpha' + c_1 z_1 - c_2 z_2 + e$$
$$z_1 = \max(x - \tau^*, 0)$$
$$z_2 = \max(\tau^* - x, 0)$$

To reach convergence in the MARS procedure Friedman (1991) suggested using a modified form of the generalized cross validation criterion (MGCV): $MGCV = [(1/N)\sum_{i=1}^{N}(y_i - \hat{f}(X))^2] / [1 - [C(M)^* / N]^2]$

Where; N is the number of observations,
$\hat{f}(X_i) \equiv \hat{y}_i$ (so $(y_i - \hat{f}(X))$ is the error for observation number i); and
$C(M)^*$ is a complexity penalty.

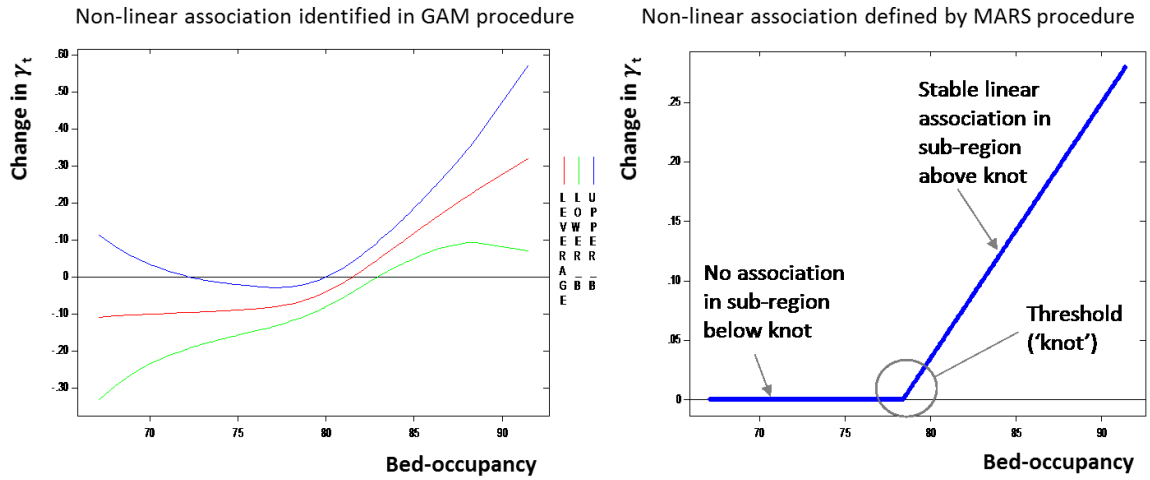
The default is to set $C(M)^*$ equal to a function of the effective no. of parameters: $C(M)^* = C(M) + \delta M$

Where; δ can be set by the user (Friedman suggests a value of 3).(Friedman 1991).
$C(M)$ is the number of parameters being fit; and
M is the number of non-constant basis functions in the model.

Minimizing the MGCV value controls how many parameters will finally remain in the model and can be used to form an estimate of the relative importance of each x_i variable. Once we include in MARS all those relevant variables detected by GAM convergence works in an approximation of the econometric general to specific approach, removing non-significant variables.

For each model, contribution charts show the nonlinear relationship of independent and dependent variables. Slopes are estimated c_i in MARS specification, and changes in slopes are knots τ^* (figure ii.)

Figure ii. Example of contribution chart from MARS output (right) with associated non-linear association identified in GAM.



3. Diagnosis:

A number of checks are made to ensure adequacy of model fit, as follows:

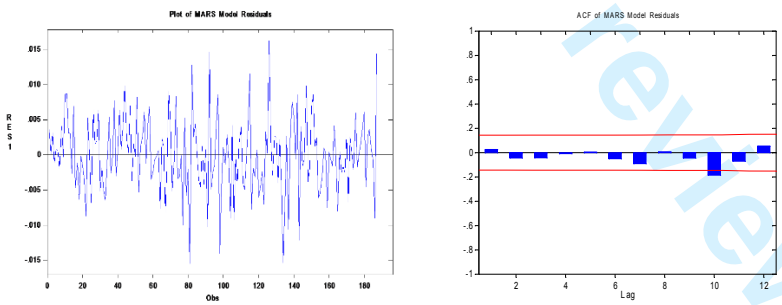
i. Residuals should correspond to 'white noise' (be normally distributed, with homogeneous variance, and mean = 0)

ii. A Hinich test is used to identify any non-linearities not detected by the model.[6]

iii. Autocorrelation functions (ACF) display values not significantly different from zero.

Figure iii. Diagnostic checks of MARS model (a) residuals by observation (b) ACF of residuals

(a) **(b)**



Model performance is compared by:

i. R^2, representing the % of total variance in the outcome variable predicted by the model.

ii. Mean Absolute Percentage Error (MAPE) and Root Mean Squared Error (RMSE), provide measures of forecasting error. Improvement in fit is identified by smaller MAPE and RMSE.

Software:

GAM and MARS procedures can be run in a number of free or commercially available software packages. In the current paper we used SCA 8.1 (Scientific Computing Associates Corp. Illinois, US).

References

[1] Box G, Jenkins G. Time series analysis: Forecasting and control, San Francisco, CA. Holden-Day 1970.

[2] Hastie T, and Tibshirani R. *Generalized Additive Models*. Chapman & Hall 1990.

[3] Faraway J. *Extending the Linear Model with R*. New York: Chapman & Hall/CRC 2006.

[4] Wood, S. *Generalized Additive Models: An introduction with R*. Chapman & Hall/CRC 2006.

[5] Friedman J. Multivariate Adaptive Regression Splines. *Annals of Statistics* 1991;19 (1):1-67

[6] Hinich M. Testing for Gaussianity and Linearity of a Stationary Time Series. *Journal of Time Series Analysis* 1982;3:169-176.

ORION Checklist for Intervention Studies or Outbreak Reports of Nosocomial Infection. (Stone et al. 2007)

Item	Descriptor (*If possible; ** If relevant)	Author's evaluation	Comment
1. Title & Abstract	Is paper described as an outbreak report (OR) or intervention study (IS)?	Yes	See "Objectives" in abstract.
	Is design of IS described?	Yes	Retrospective ecological study and time-series analysis
	Is Intervention & main outcomes described?	Yes	See "Interventions" and "Outcome measures" in abstract
INTRODUCTION			
2. Background	Are background and rationale for IS/OR explained?	Yes	See background
	Is organism described as epidemic, endemic?	Yes	"In the UK, national initiatives of infection control and antibiotic stewardship have been linked to a declining MRSA epidemic"
3. Type of paper	Is paper described as IS or OR?	Yes	See last paragraph of background.
	If OR, is number of outbreaks given?	N/A	N/A
4. Dates	Are start & finish dates of IS or OR given?	Yes	See last paragraph of background.
5. Objectives	Are objectives stated for OR? Are hypotheses stated for IS?	Yes	See last paragraph of background.
METHODS			
6. Design	Is study design described? How?	Yes	Retrospective ecological study and time-series analysis
	Is study described as retrospective, prospective or ambidirectional?	Yes	Retrospective.
	Does it state if decision to report or intervene prompted by any outcome data or not?	Yes	...national infection control and antibiotic stewardship strategies, prompted by detection of high-rates of nosocomial infection in mandatory surveillance
	Is it stated if study formally implemented or not, with protocol & endpoints?	Yes	Retrospective observational study (Not formally implemented).
7. Participants	Is number patients admitted given?	Yes	See table 1.
	Is age & length of stay given?	Yes	See table 1.
	Are eligibility criteria for IS or case definitions for OR given?	Yes	See table 1.
	Is % inter/intra-hospital transfers or admissions from care homes given?*	Yes	< 5% of admissions are transferred from other hospitals or regions
	Are potential risk factors for acquisition organism given?**	Yes	See "Outcomes and exposures"
8. Setting	Is unit, ward or hospital (and its units) described?.	Yes	See table 1 and "population and setting"
	Are number of beds, presence and staffing of infection control team given?	Yes	See table 1 and "population and setting"
9. Interventions	Are phases defined by major change in specific infection control practice?	Yes	See table 1.

	Is a summary table given, with details of interventions, their delivery and timing given?	Yes	See table 1.
10. Culturing & Typing	Are details of culture media, antibiograms and/or typing given?	Yes	See Laboratory methods
	Are details environmental sampling given?*	N/A	N/A
11. Infection-related outcomes	Are there clearly defined primary& secondary outcomes?	Yes	See table 1. and "Outcomes and exposures"
	Are they given at regular time intervals ?	Yes	Monthly over 16 years.
	Are there sufficient time points per phase? (see ORION author's checklist)?	Yes	192 time-points, including 148 before antibiotic stewardship
	Are denominators given (eg admissions, discharges, bed days)?	Yes	Occupied Bed Days (hospital); Inhabitant-days (community)
	Is all cause mortality given?	N/A	Not a study objective or outcome.
	Is prevalence organism, or incidence of colonisation on admission at same time intervals*?	Yes	Importation pressure and overall MRSA prevalence density measured at same time-intervals.
	In a short IS or OR is a chart used with duration patient stay & dates detection of organism ? (see author checklist)	N/A	N/A
12. Economic outcomes	Is this a formal economic study?	N/A	N/A
	If so, are outcomes defined? Are resources (for interventions) described? Are costs in basic units? Are assumptions stated?.	N/A	N/A
13. Potential Threats to internal validity	Which potential confounders were considered, recorded or adjusted for?(eg: length of stay, case mix, occupancy, staffing levels, hand-hygiene,, antibiotic use, strain type, processing of isolates, seasonality).	Yes	See "Outcomes and exposures"
	Are measures to avoid bias described? (eg blinding; standardisation outcome assessment/provision of care).	Yes	Informational bias reduced by electronic records and microbiologically defined outcome measures.
14. Sample size	Are power calculations given? (if appropriate)	N/A	Justifiation given in "Study Design" for time-period.
15. Statistical methods	Are statistical methods to compare groups or phases described?	Yes	"Statistical methods" and supplemental file 1.
	Do these account for dependencies in outcome data?	Yes	Explicitly measured in autoregression.
	Do they adjust where necessary for confounders?	Yes	Multivariate time-series analysis adjusted for ecological variables
	Are methods for subgroup or adjusted analyses described? Are they planned or not (exploratory)?	Yes	Variables used in adjusted analysis (multivariate time-series analysis) determined *a priori.*
	Is statistical analysis of an OR appropriate/necessary?	N/A	N/A
RESULTS			
16. Recruitment	Are the dates defining periods of recruitment & follow up given**?	N/A	N/A
	Is there a flow diagram**?	N/A	N/A

17. Outcomes and estimation	Is the estimated effect size & its precision given for main outcomes?	Yes	Coefficients (+ 95% CI) given in table 3.
	Is there a graphical summary of outcomes (for dependent data and most time series)?	Yes	Figure 6 provides summary of relationships between antibiotic use, infection control measures and strain prevalence densities
18. Ancillary analysis	Are subgroup analyses reported?	N/A	
	Are possible confounders adjusted for?	N/A	
19. Harms	Are these pre-specified in each group or phase?	N/A	Not a study objective or outcome.
DISCUSSION			
20. Interpretation	IS: is evidence for/against hypotheses assessed?	Yes	Hypothesis that antibiotic stewardship and infection control measures affect MRSA strain-dynamics discussed.
	Are plausible alternative explanations considered, including regression to mean &reporting bias?	Yes	Study limitations and threats to internal validity discusssed.
	OR: Is clinical significance of observations considered?	N/A	N/A
	Are explanatory hypotheses generated?	Yes	Concept of 'critical thresholds' in total antibiotic use.
21. Generalisability	Is there discussion of how results may generalise to different target populations or settings?	Yes	Comment made on external validity, and likely dependence of non-linear associations on clinical context
	Is feasibility of interventions considered?	Yes	Need to balance clinical priority with control of resistance noted as key to antibiotic stewardship. Limits to current infection control noted.
22. Overall evidence	Are results interpreted in context of current evidence?	Yes	Evaluated in light of previous evidence on MRSA strain dynamics