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Supplementary information

A nonlinear time-series analysis approach to identify thresholds in associations between population antibiotic use and rates of resistance

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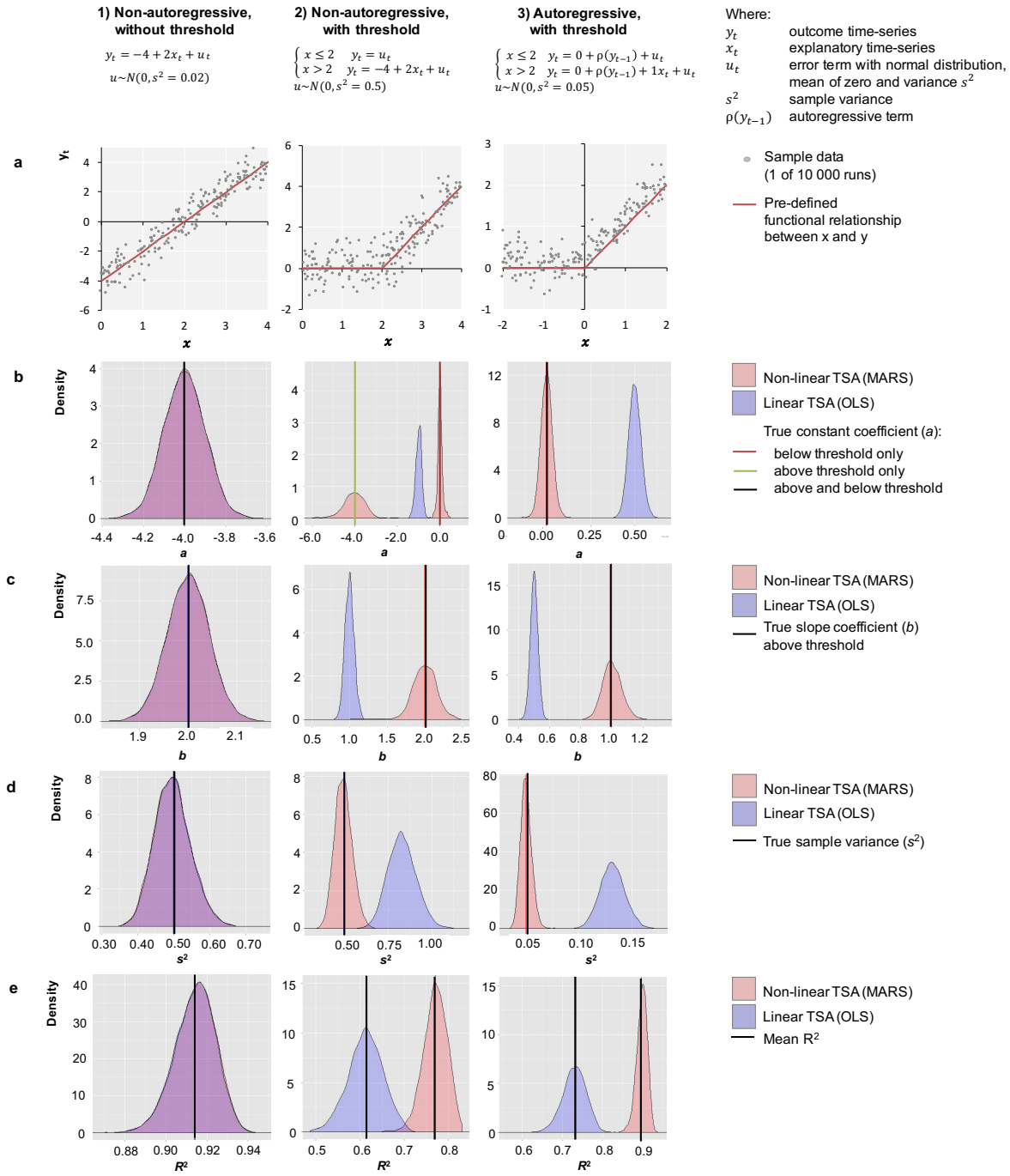
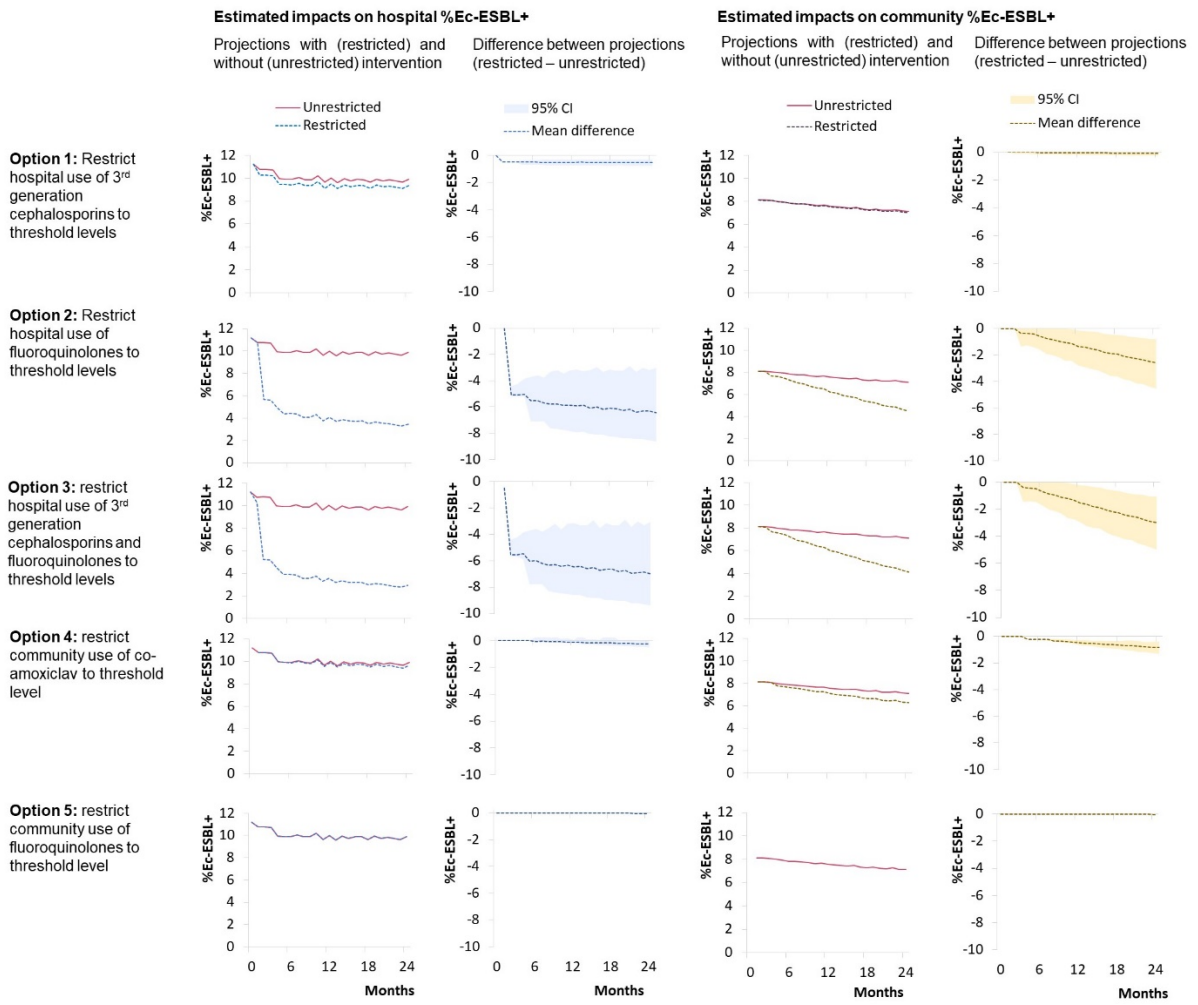
A non-linear time-series analysis approach to identify thresholds in associations between population antibiotic use and rates of resistance

Supplementary Figures

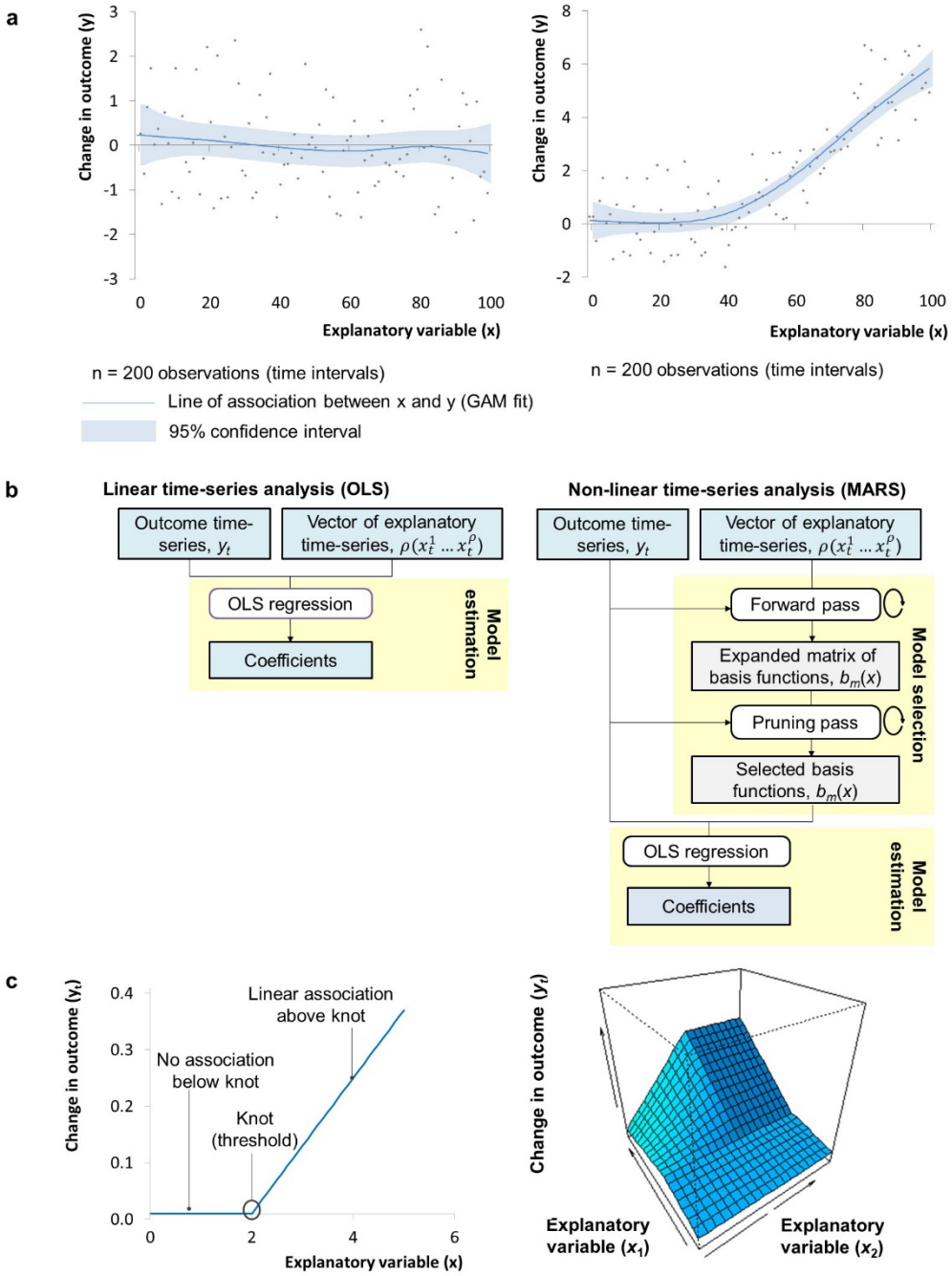
Supplementary Tables



Supplemental Figure 1 | Monte-Carlo experiments comparing linear and non-linear time-series analyses The ability of linear (ordinary least squares, OLS) and non-linear (Multivariate Adaptive Regression Splines, MARS) time-series analyses (TSA) to identify known relationships between explanatory (x) and outcome (y_t) time-series was assessed by applying both model types to 10,000 datasets generated from three simple pre-defined stochastic processes (1,2 and 3). **(a)** sample simulated dataset and pre-defined functional relationship. **(b-d)** frequency distributions for model estimates of coefficients (constant *a,* slope *b*), and variance (s^2), along with true (pre-defined) values displayed as vertical lines. **(e)** frequency distribution and means for overall model fit statistic (R^2) for linear and non-linear models applied to simulated data.



Supplemental Figure 2 | Projections for percentage of *Escherichia coli* producing extended-spectrum β-lactamase (%Ec-ESBL+) in hospital and community populations of Orihuela, Spain, under five different antibiotic stewardship options. 24-month projections represent medians of 1000 simulated future time-series generated using best-fit MARS models from observed data, steady-state values of %Ec-ESBL, and levels of antibiotic use reflecting one of five antibiotic stewardship interventions (restriction of one or more antibiotics to previously identified threshold values) or no intervention (unrestricted, 'business as usual' usage patterns). Expected impacts of antibiotic stewardship options 1 to 5 are summarised as mean differences in %Ec-ESBL+ with 95% confidence intervals, from comparisons of projections with and without intervention. Note that due to population interactions restriction of antibiotic use in hospital was projected to affect %Ec-ESBL+ in the community, and vice-versa.



Supplemental Figure 3 | Illustrations of GAM and MARS procedures for non-linear time-series analysis (a) Examples of contribution charts from GAM procedure in (left) the absence and (right) the presence of significant relationships between (x) and the outcome (y) variables. **(b)** Schematic summary of (left) linear (OLS) and (right) non-linear (MARS) regression analyses. **(c)** Contribution charts illustrating basis (spline) functions of relationships between the outcome (y_t) and: (left) a single explanatory variable with a minimum threshold; and (right) interacting explanatory variables (x_1, x_2) in which the association with outcome of one variable is dependent upon the level of the other variable.

Supplementary Table 1: Results of multivariable non-linear time-series analyses●

Predicting variable	Lag[a]	Threshold (95% CI)[b]	Relation to threshold[c]	Coefficient (95% CI)[d]	T-ratio	p value
1: Carbapenem-resistant *Acinetobacter baumannii* (CRAb)‡ Debrecen, Hungary [n=143 months; R^2 0.859; MAPE 45.3%; MGCV 2.254]						
Constant	-	-	-	6.962 (6.157 to 7.767)	16.9	<0.0001
Previous CRAb incidence density	1	6.2 (4.7 to 8.0)	Below	0.563 (0.326 to 0.801)	4.64	<0.0001
Previous CRAb incidence density	1	9.4 (7.0 to 11.2)	Above	0.932 (0.478 to 1.386)	4.02	0.0001
Previous CRAb incidence density	2	9.4 (7.8 to 12.3)	Below	0.397 (0.235 to 0.559)	4.79	<0.0001
Carbapenem use	3	14.9 (8.1 to 23.9)	Above	0.067 (0.028 to 0.106)	3.39	0.0007
Piperacillin-tazobactam use	3	7.0 (5.0 to 14.0)	Above	0.296 (0.070 to 0.523)	2.56	0.0102
Fluoroquinolone use	1	85.2 (65.6 to 103.0)	Above	0.060 (0.019 to 0.100)	2.89	0.0038
2: Extended-spectrum β-lactamase producing *E. coli* (%Ec-ESBL+)^ Orihuela, Spain						
***a) Hospital* [n=304 months; R^2 0.622; MAPE 57.1%; MGCV 19.97]**						
Constant	-	-	-	5.858 (3.288 to 8.428)	4.46	<0.0001
Previous %Ec-ESBL+ (hospital)	3	9.9 (6.3 to 13.3)	Below	0.266 (0.488 to 0.045)	2.51	0.0129
Previous %Ec-ESBL+ (hospital)	3	9.9 (6.3 to 13.3)	Above	-0.254 (-0.45 to -0.058)	2.77	0.0061
%Ec-ESBL+ (community)	2	10.1 (5.9 to 20.0)	Below	0.359 (0.579 to 0.138)	3.08	0.0024
%Ec-ESBL+ (community)	2	10.1 (5.9 to 20.0)	Above	-1.291 (-2.066 to -0.516)	3.26	0.0013
Third-gen. ceph. use (hospital)	1	65.15 (36.5 to 95.5)	Above	0.061 (0.029 to 0.093)	3.28	0.0012
Fluoroquinolone use (hospital)	2	82.28 (70.5 to 101.1)[e]	Above	0.122 (0.085 to 0.159)	6.07	<0.0001
Fluoroquinolone use (hospital)	2	150.83 (145.5 to 155.2)[e]	Above	-0.269 (-0.495 to -0.044)[f]	-2.34	0.0203
Fluoroquinolone use (hospital)	2	160.32 (153.3 to 168.0)[e]	Above	0.010 (-0.300 to 0.319)[f]	0.06	0.9522
***b) Community* [n = 304 months; R^2 0.768; MAPE 46.2%; MGCV 4.715]**						
Constant	-	-	-	4.828 (3.774 to 5.883)	8.97	<0.0001
Previous %Ec-ESBL+	3	7.3 (5.9 to 10.3)	Below	0.423 (0.266 to 0.597)	5.11	<0.0001
Previous %Ec-ESBL+	4	4.1 (2.1 to 5.8)	Above	0.473 (0.302 to 0.645)	5.40	<0.0001
Previous %Ec-ESBL+	5	1.1 (0.9 to 4.7)	Above	0.167 (0.062 to 0.273)	3.10	0.0021
%Ec-ESBL+ (hospital)	1	5.5 (1.61 to 12.50)	Below	0.286 (0.106 to 0.466)	3.11	0.0020
Co-amoxiclav use (community)	4	6.7 (5.0 to 10.0)	Above	0.549 (0.223 to 0.875)	3.30	0.0010
Fluoroquinolone use (community)	6	2.8 (1.4 to 5.2)	Above	0.635 (0.174 to 1.100)	2.70	0.0070
3: Cefepime-resistant *E. coli* (%EcFepR)* Seville, Spain. [n = 108 months; R^2 0.301; MAPE 41.6%; MGCV 12.11]						
Constant	-	-	-	6.163 (5.164 to 7.161)	12.1	<0.0001
Previous %EcFepR	12	11.6 (1.2 to 21.9)	Above	0.580 (0.254 to 0.907)	3.48	0.0005
Audit 2*%EcFepR	1	5.8 (4.6 to 7.0)	Above	-2.801 (-4.669 to -0.994)	-2.93	0.0033
Audit 2*%EcFepR	1	7.1 (5.8 to 8.4)	Above	3.301 (0.923 to 5.680)	2.72	0.0065
Fluoroquinolone use	3	138.2 (79.5 to150.4)	Above	0.105 (0.012 to 0.198)	2.2	0.0272
3rd/4th gen cephalosporin use	4	45.8 (29.1 to 55.6)	Above	0.197 (0.103 to 0.291)	4.1	<0.0001
4: Gentamicin-resistant *Pseudomonas aeruginosa* (GRPa)§ Besançon, France [n=192 months; R^2 0.857; MAPE 27.3%; MGCV 2.66]						
Constant	-	-	-	3.236 (2.894 to 3.577)	18.5	<0.0001
Previous GRPa incidence density	1	4.8 (3.4 to 5.8)	Above	0.670 (0.563 to 0.773)	12.4	<0.0001
Previous GRPa incidence density	12	2.7 (1.6 to 5.1)	Above	0.170 (0.073 to 0.268)	3.43	<0.0001
Gentamicin and tobramycin use	1	8.0 (3.9 to 10.6)	Above	0.221 (0.077 to 0.365)	4.18	<0.0001
Fluoroquinolone use	1	81.0 (78.0 to 84.0)	Above	0.045 (0.029 to 0.061)	3.01	0.003
5: Methicillin-resistant *Staphylococcus aureus* (MRSA)§ Antrim, Northern Ireland [n=105 months; R^2 0.561; MAPE 15.1%; MGCV 0.331]						
Constant	-	-	-	1.982 (1.762 to 2.202)	17.6	<0.0001
Previous MRSA incidence density	3	1.9 (1.1 to 2.5)	Above	0.273 (0.086 to 0.460)	2.86	0.0042
Fluoroquinolone use	3	16.8 (14.2 to 20.7)	Above	0.003 (0.000 to 0.006)	2.13	0.0299
Third-gen. cephalosporin use	3	4.9 (4.3 to 5.7)	Above	0.026 (0.015 to 0.037)	4.51	0.0005
Co-amoxiclav use	1	224.9 (132.8 to 296.6)	Above	0.003 (0.002 to 0.005)	4.39	<0.0001
Alcohol-based hand rub use	2	6.9 (3.9 to 16.1)	Below	-0.133 (0.047 to 0.218)	3.04	0.0024

DDDs Defined Daily Doses; MGCV Modified Generalised Cross Validation criteria (a lower value indicates a more efficient model; MAPE Mean Average Percentage Error (observed/predicted × 100); OBDs Occupied Bed Days; R^2 indicates the percentage of total variation in outcome explained.

● lags, thresholds, coefficients, T-ratios and p-values were derived from multivariate adaptive regression splines (MARS) models, starting with a restricted set of candidate explanatory variables (and lags). P-values are 2-sided.

a average delay (in months) between change in predictive time-series and associated change in outcome time-series

b value of the predicting variable at which the association with the outcome changes, with 95% conditional conservative inverted likelihood ratio (CCILR) confidence intervals (see Methodology section for details).

c Indicator of whether the association is seen below or above the threshold.

d Coefficient refers to the change in the outcome for a 1 unit (e.g. DDD) increase in the explanatory variable

e CCILR confidence intervals around thresholds may be slightly wider than estimated due to multiple closely occurring thresholds in association between fluoroquinolone and %Ec-ESBL+ in hospital.

f contribution above this threshold derived from sum of coefficients operating above current and any other lower thresholds for same variable and lag combination.

‡ Cases per 10 000 OBDs. § Cases per 1000 OBDs. ^ Percentage of all non-duplicate *E. coli* isolates positive for ESBL. * Percentage of all non-duplicate *E. coli* isolates resistant to cefepime.

Supplementary Table 2: Comparison of rates of non-susceptibility to potentially selecting antibiotics in carbapenem-resistant and carbapenem-susceptible *Acinetobacter baumannii* isolates from Debrecen, Hungary[a]

		CRAb isolates[a] (n=1776)		CSAb isolates[a] (n=883)			
ATC antibiotic sub-group	**Antibiotic**	**Tested**	**I/R, N (%)**	**Tested**	**I/R, N (%)**	**Difference in %I/R**	**p-value[b]**
3rd gen cephalosporin (J01DD)	Cefotaxime	1154	1134 (98%)	758	548 (72%)	26%	<0.0001[c]
	Ceftazidime	1164	1085 (93%)	762	430 (56%)	37%	<0.0001[c]
Fluoroquinolones (J01MA)	Ciprofloxacin	1776	1692 (97%)	852	571 (67%)	30%	<0.0001[c]
Combined penicillin and β-lactamase inhibitor (J01CR)	Piperacillin-tazobactam	1114	1046 (94%)	748	161 (22%)	72%	<0.0001[c]
Aminoglycoside (J01GB)	Gentamicin	1762	1600 (91%)	863	473 (55%)	36%	<0.0001[c]
	Amikacin	1763	1571 (89%)	869	369 (43%)	47%	<0.0001[c]
Polymyxins (J01XB)	Colistin	1619	14 (1%)	381	8 (2%)	-1%	0.0376

a. non-duplicate clinical isolates from single tertiary level hospital, October 2004 to August 2016 (n=143 months).
b. 2-sided p-value from Chi-squared test of independence
c. significant at adjusted critical significance level with Bonferroni correction (α_{adj} =0.00714 = α / n = 0.05 (5%) probability of type I error / 7 comparisons.
ATC, Anatomical Therapeutic Chemical classification. CRAb, carbapenem-resistant *Acinetobacter Baumannii.* CSAb, carbapenem-susceptible *Acinetobacter Baumannii.* I/R, intermediate/resistant (non-susceptible) to antibiotic as defined by zone diameter <23mm on disc-diffusion testing according to CLSI (Oct 2004 to Aug 2013) or EUCAST (Sep 2013 to Aug 2016) interpretive criteria.

Supplementary Table 3: Comparison of rates of non-susceptibility to potentially selecting antibiotics in ESBL-producing and non-ESBL producing *Escherichia coli* isolates from Orihuela, Spain.[a]

		ESBL-producing *E.coli* isolates (n=3641)		Non-ESBL *E.coli* isolates (n=3641)			
ATC antibiotic group	**Antibiotic**	**Tested**	**I/R, N (%)**	**Tested**	**I/R, N (%)**	**Difference in %I/R**	**P-value**
3rd gen cephalosporin (J01DD)	Ceftazidime	735	735 (100%)	11108	778 (7%)	93%	<0.0001[c]
	Cefotaxime	735	735 (100%)	11108	666 (6%)	94%	<0.0001[c]
Monobactams (J01DF)	Aztreonam	735	728 (99%)	11108	220 (2%)	97%	<0.0001[c]
Fluoroquinolones (J01MA)	Ciprofloxacin	735	596 (81%)	11108	3442 (31%)	50%	<0.0001[c]
4th gen cephalosporin (J01DE)	Cefepime	735	574 (78%)	11108	335 (3%)	75%	<0.0001[c]
Aminoglycoside (J01GB)	Tobramycin	735	287 (39%)	11108	1002 (9%)	30%	<0.0001[c]
	Gentamicin	735	213 (29%)	11108	1111 (10%)	19%	<0.0001[c]
Combined penicillin and β-lactamase inhibitor (J01CR)	Co-amoxiclav	735	228 (31%)	11108	1005 (9%)	22%	<0.0001[c]
	Piperacillin-tazobactam	735	37 (5%)	11108	226 (2%)	3%	0.0001[c]
Sulfonamide and trimethoprim combination (J01EE)	Co-trimoxazole	735	390 (53%)	11108	3555 (32%)	21%	0.0001[c]

a. non-duplicate clinical isolates from single tertiary level hospital, January 1999 to December 2014 (n=192 months)
b. 2-sided p-value from Chi-squared test of independence
c. significant at adjusted critical significance level with Bonferroni correction (α_{adj} =0.005 = α / n = 0.05 (5%) probability of type I error / 10 comparisons.
ATC, Anatomical Therapeutic Chemical classification. ESBL, extended-spectrum β-lactamase. I/R, intermediate/resistant (non-susceptible) to antibiotic as defined by microdilution testing according to CLSI (Jul 1991 to Dec 2015) or EUCAST (Jan to Oct 2016) interpretive criteria.

Supplementary Table 4: Comparison of rates of non-susceptibility to potentially selecting antibiotics in gentamicin-resistant and gentamicin-susceptible *Pseudomonas aeruginosa* isolates from Besançon, France[a]

		GRPa isolates[a] (n=3641)		GSPa isolates[a] (n=6507)		Difference	
ATC antibiotic sub-group	Antibiotic	Tested	I/R, N (%)	Tested	I/R, N (%)	in %I/R	p-value[b]
Fluoroquinolones (J01MA)	Ciprofloxacin	3641	2338 (64%)	6507	1557 (24%)	40%	<0.0001[c]
Combined penicillin and β-lactamase inhibitor (J01CR)	Ticarcillin-clavulanate	3641	2056 (56%)	6507	1337 (21%)	35%	<0.0001[c]
	Piperacillin-tazobactam	3641	1185 (33%)	6507	414 (6%)	27%	<0.0001[c]
Carbapenems (J01DH)	Imipenem	3641	1719 (47%)	6507	912 (14%)	33%	<0.0001[c]
Monobactams (J01DF)	Aztreonam	3641	1645 (45%)	6507	1272 (20%)	25%	<0.0001[c]
4th gen cephalosporin (J01DE)	Cefepime	3641	1456 (40%)	6507	386 (6%)	34%	<0.0001[c]
3rd gen cephalosporin (J01DD)	Ceftazidime	3641	911 (25%)	6507	375 (6%)	19%	<0.0001[c]

a. non-duplicate clinical isolates from single tertiary level hospital, January 1999 to December 2014 (n=192 months)
b. 2-sided p-value from Chi-squared test of independence
c. significant at adjusted critical significance level with Bonferroni correction (α_{adj} =0.00714 = α / n = 0.05 (5%) probability of type I error / 7 comparisons.
ATC, Anatomical Therapeutic Chemical classification. (GRPa, gentamicin-resistant *Pseudomonas aeruginosa* and GSPa, gentamicin-susceptible *Pseudomonas aeruginosa*) I/R, intermediate/resistant (non-susceptible) to antibiotic as defined by zone diameter <16mm on disc-diffusion testing according to EUCAST interpretive criteria.

Supplementary Table 5: Summary of study populations, outcomes and exposures

Centre	1. Debrecen (Hungary)	2. Orihuela (Spain)	3. Seville (Spain)	4. Besançon (France)	5. Antrim (N.Ireland)
Months of study (No.)	Oct 2004 to Aug 2016 (N=143)	July 1991 to Oct 2016 (N=304)	Feb 2008 to Dec 2016 (N=108)	Jan 1999 to Dec 2014 (N=192)	Jan 2005 to Sep 2013 (N=105)
Setting	1559-bed tertiary hospital. Serving population of 1 500 000	a) 350-bed district hospital b) Primary care services for 199 000.	778 bed hospital Serving population of 481 000	1200-bed tertiary hospital. Serving population of 1 200 000.	340-bed teaching hospital. Serving population of 147 103.
Population	General adult and paediatric inpatients, 75 ICU beds, 100 chronic-care beds, haematology, oncology, transplantation services 39 300 OBDs per month	General adult inpatients, 10-bed ICU. Oncology services. 6 670 OBDs per month	General adult and paediatric inpatients, 6-bed PICU, 6-bed NICU, 50-bed ICU. Renal, oncology services. 21 300 OBDs per month	General adult and paediatric inpatients, including 40-bed ICU and 12-bed NICU. 27 710 OBDs per month	General adult inpatients including 7-bed ICU and oncology services, renal replacement therapy. 9 750 OBDs per month
Primary Outcome	Carbapenem-resistant *Acinetobacter baumannii* isolates per 10 000 OBDs (CRAb)	% *Escherichia coli* producing extended-spectrum ß-lactamases (%Ec-ESBL+)	% *Escherichia coli* resistant to cefepime (%Ec-FepR)	Gentamicin resistant *Pseudomonas aeruginosa* cases per 1000 OBDs (GRPa)	Methicillin-resistant *Staphylococcus aureus* per 1000 OBDs (MRSA)
Laboratory methods /susceptibility testing	Disc-diffusion Breakpoint: - CLSI (to Aug 2013) - EUCAST (from Sep 2013) - Non-susceptible if zone diameter <23mm (for imipenem).	Microdilution Breakpoint - CLSI (to Dec 2015) - EUCAst (from Jan 2016). - ESBL+ if: (i) MIC >1mg/L for cefotaxime or ceftazidime. and; (ii) ≥3 two-fold decrease in MIC when combined with clavulanic acid.	Microdilution Breakpoint: - CLSI (to Dec 2015) - EUCAST (from Jan 2016) - Non-susceptible if MIC ≤8mg/L (to Sep 2014) ≤2mg/L (from Oct 2014) ≤1mg/L (from Jan 2016)	Disc-diffusion Breakpoint: - EUCAST (all months) - Non-susceptible if zone diameter <16mm.	Disc-diffusion (Oxicillin to Nov 2007, Cefoxitin from Nov 2007). Breakpoint: -NCCLS from (Jan 2005-Mar 2007) -CLSI (Mar 2007 - Nov 2011) -EUCAST (from Nov 2011) - Non-susceptible if zone diameter <22mm (Cefoxitin)
Isolate types	All body sites (excluding infection control isolates)	All body sites (excluding infection control isolates)	All body sites (excluding non-clinical isolates)	All body sites	All body sites
Hypothesised explanatory variables	Carbapenems Fluoroquinolones Piperacillin-tazobactam Cephalosporins Aminoglycosides Glycopeptides	$2^{nd}/3^{rd}$ gen cephalosporins Co-amoxiclav Fluoroquinolones Co-trimoxazole Aminoglycosides %EcESBL+ in other population	Fluoroquinolones $3^{rd}/4^{th}$ gen cephalosporins Clindamycin Co-amoxiclav Carbapenems	Aminoglycosides Fluoroquinolones Carbapenems $3^{rd}/4^{th}$ gen cephalosporins Piperacillin-tazobactam	Fluoroquinolones $3^{rd}/4^{th}$ gen cephalosporins Clindamycin Co-amoxiclav Macrolides Alcohol-Based Hand Rub

Supplementary Table 6: Summary of antibiotic stewardship and infection prevention and control resources and interventions during study period by study population

Centre	1. Debrecen (Hungary)	2. Orihuela (Spain)	3. Seville (Spain)	4. Besançon (France)	5. Antrim (N.Ireland)
Antibiotic stewardship					
Resources	None specific.	Local AMT 0.5 WTE antibiotic pharmacist	0.5 WTE antibiotic pharmacist, 0.5 WTE stewardship doctor	Regional and local AMT 1.0 WTE antibiotic pharmacist.	Regional AMT. 1.5 WTE antibiotic pharmacists
Restrictive interventions	None.	Permissions for use required for fluoroquinolones, linezolid, vancomycin, 3rd gen cephalosporins (from Jan 1995)	None	Permissions for use required for carbapenems, daptomycin (from Jan 2012)	Removal of stock and permissions for use (from Jan 2008) for: clindamycin fluoroquinolones, cephalosporins, vancomycin, linezolid, colistin, amikacin, chloramphenicol, co-trimoxazole, meropenem, Limited disclosure of antibiograms (all years)
Persuasive interventions	Voluntary consultation with clinical microbiologist available throughout study period	Empirical guidelines	Empirical guidelines Education & reminders (from Jan 2012-) Ward-based audit (from Jan 2013-) Root cause analysis for HCAIs (all months)	Empirical Guidelines Ward-based audit of antibiotic use	Empirical guidelines Ward-based audit Root-cause analysis for HCAIs (all study months)
Infection prevention & control					
Resources	3.0 WTE ICN, 1.0 WTE ICD	1.0 WTE ICN, 1.0 WTE ICD	4 WTE ICN, 0.5 WTE ICD	4 WTE ICNs, 2 WTE ICDs	10 WTE ICNs, 2 WTE ICDs
Hand-hygiene	ABHR throughout study period	ABHR (Jan 2000 ongoing) Regional / National Hand Hygiene campaign (Jan 2000 ongoing)	ABHR (2001-) Auditing of compliance (2006-) Regional hand-hygiene campaign (2008-)	ABHR (Jan 2000 ongoing) Auditing of compliance.	ABHR throughout study period, monthly hospital-wide consumption measured.
Isolation practices	MRSA cases isolated. Other multiresistant pathogens depend on isolation room availability	As per CDC recommendations. Nasal mupirocin / chlorhexidine washes for MRSA (Jan 1991 -)	Of all CRE,VRE cases Nasal mupirocin for MRSA	Isolation of MRSA, ESBLE, VRE, CRE, CDI and CRAb cases.` Nasal mupirocin for MRSA	Isolation or cohorting of MRSA and CDI cases
Admission screening for outcome pathogen	No	ICU (Jan 2013 -)	No	*P. aeruginosa* screening in both adult ICUs (Jan 2000-)	Risk-factor based screening for MRSA in all wards (all study months)
Environmental interventions	None	Audit of hospital cleanliness (Jan 1995 -) Hospital Environment Inspections – continuous (Jan 1995 -)	Audit of hospital cleanliness Hospital environment inspections	None	None