Antibiotic strategies in critical care: back to square one?

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Lancet Infect Dis 2018

Published Online
January 24, 2018
http://dx.doi.org/10.1016/S1473-3099(18)30057-4

See Online/Articles
http://dx.doi.org/10.1016/S1473-3099(18)30056-2

The worth of antibiotics to human health is without bound, but antimicrobial resistance poses a serious threat to this treasured resource.[1] Reduction of the selection pressure on pathogens through the rational use of antimicrobials, and discernment of which antibiotic treatment strategies achieve this goal, are now international priorities. Antibiotic stewardship encompasses different evidence-based measures to improve the appropriate use of antibiotics by promoting selection of optimal drug regimens, including dosing, duration of therapy, and route of administration.[2,3] There have been important recent advances in our understanding of these approaches, but we are still some way from truly knowing how to best use antibiotics in the setting of patients with sepsis in critical care. Although there is a strong consensus that, at the patient level, prescribers need to ensure the right antibiotic, at the right dose, at the right time is used in cases where antibiotics are truly warranted,[4] treatment strategies at the unit level remain unclear. Among manifold others, two such strategies are cycling and mixing of antibiotics.[5] Antibiotic cycling refers to prescription of a specific antibiotic drug preferentially as first-line therapy during a prespecified period, with subsequent rotation to another antibiotic drug with different selective properties later on. Antibiotic mixing refers to the changing of antibiotics each time a new patient in need of this drug arrives.

Which antibiotic prescription strategy results in the lowest risk of resistance and is therefore preferable is an important, but open, clinical and evolutionary question. Evidence from laboratory studies suggests that drug changes can negatively impact bacterial growth and adaptation.[6] Theorists have wrestled with this question for decades, and initial enthusiasm for mixing[7] has waned in light of analyses showing that mixing and cycling are probably hard to distinguish in the clinic,[5] although cycling has received some theoretical support at times too.[8]

To better understand whether antibiotic mixing has more favourable outcomes than cycling for the risk of antibiotic-resistant, Gram-negative bacteria, Pleun Joppe van Duijn and colleagues[9] report in *The Lancet Infectious Diseases* the results of a cluster-randomised crossover trial in eight European intensive care units (ICUs). The primary endpoint was the change in unit-wide prevalence of antibiotic-resistant, Gram-negative bacteria, defined as carriage of Enterobacteriaceae harbouring extended-spectrum β-lactamase genes, or phenotypical resistance of Enterobacteriacae to piperacillin–tazobactam or of *Acinetobacter* spp and *Pseudomonas aeruginosa* to piperacillin–tazobactam or carbapenems. This endpoint was measured in 745 of 4069 patients present in the ICUs during cycling and 853 of 4707 patients during mixing through monthly point-prevalence screening cultures. Because randomisation was not implemented at a patient level, but rather at an institutional level, the investigators adjusted their analyses for potential patient-related and ICU-related confounders. The trial was pragmatic, whereby treating physicians could deviate from the protocol in case of safety concerns and for the use of combination therapy, including with non-study antibiotics.

The investigators noted no differences in antibiotic use between randomisation groups, and study antibiotics accounted for 42% and 43% of all antibiotics used in the cycling and mixing groups, respectively. For the primary endpoint, investigators did not find a significant difference in the mean prevalence of antibiotic-resistant, Gram-negative bacteria (168 [23%] patients with carriage during cycling *vs* 184 [22%] during mixing) even when considering the incidence rate ratio derived from a mixed effects analysis adjusted for potential confounders (1·039, 95% CI 0·837–1·291, p=0·73). No difference was observed in several subgroup analyses. These results thus strongly suggest that cycling of antibiotics has no beneficial effect over antibiotic mixing against the emergence of antibiotic resistance for Gram-negative bacteria.

Where does this trial leave us? This study had a negative outcome, but the findings are consistent with theoretical arguments.[5] However, a negative study can still be highly informative if the study quality was high, meaning sufficient statistical power to avoid a type II error, high protocol adherence, and a thorough statistical analysis accounting for potential biases. The trial reported by van Duijn and colleagues fulfils these criteria. In their study protocol, the investigators predefined the statistical approach using different

sensitivity analyses with sufficient power to detect effects if any were present. High adherence to the study protocol was observed despite two major deviations (one in which data were excluded due to missing point-prevalence information, and another in which the washout period was prolonged due to an outbreak of carbapenem-resistant *Klebsiella pneumoniae*). There is, therefore, no obvious reason that another trial would contradict these findings. However, reduction of the prevalence of antibiotic-resistant, Gram-negative bacteria in ICUs remains a priority; there is an urgent need to find new institutional strategies that prove beneficial in clinical trials. Until then, we need to reinforce the patient-level tools that are available, including (among others) improved hand hygiene and better selection of patients in need of antibiotics by host-response markers such as procalcitonin and other pathogenic markers.[10,11]

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PS is supported by the Swiss National Science Foundation (SNSF Professorship, PP00P3_150531) and the Forschungsrat of the Kantonsspital Aarau (1410.000.058 and 1410.000.044). PS has received research support from BRAHMS/Thermofisher, BioMerieux, Roche, Abbott, Nestle, and Novo Nordisk. RB is supported by a UK EPSRC Healthcare Technology Impact Fellowship (EP/N033671/1) and has received previous research support from AstraZeneca.

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The effects of antibiotic cycling and mixing on antibiotic resistance in intensive care units: a cluster-randomised crossover trial

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Summary

Background Whether antibiotic rotation strategies reduce prevalence of antibiotic-resistant, Gram-negative bacteria in intensive care units (ICUs) has not been accurately established. We aimed to assess whether cycling of antibiotics compared with a mixing strategy (changing antibiotic to an alternative class for each consecutive patient) would reduce the prevalence of antibiotic-resistant, Gram-negative bacteria in European intensive care units (ICUs).

Methods In a cluster-randomised crossover study, we randomly assigned ICUs to use one of three antibiotic groups (third-generation or fourth-generation cephalosporins, piperacillin–tazobactam, and carbapenems) as preferred empirical treatment during 6-week periods (cycling) or to change preference after every consecutively treated patient (mixing). Computer-based randomisation of intervention and rotated antibiotic sequence was done centrally. Cycling or mixing was applied for 9 months; then, following a washout period, the alternative strategy was implemented. We defined antibiotic-resistant, Gram-negative bacteria as Enterobacteriaceae with extended-spectrum β-lactamase production or piperacillin–tazobactam resistance, and *Acinetobacter* spp and *Pseudomonas aeruginosa* with piperacillin–tazobactam or carbapenem resistance. Data were collected for all admissions during the study. The primary endpoint was average, unit-wide, monthly point prevalence of antibiotic-resistant, Gram-negative bacteria in respiratory and perineal swabs with adjustment for potential confounders. This trial is registered with ClinicalTrials.gov, number NCT01293071.

Findings Eight ICUs (from Belgium, France, Germany, Portugal, and Slovenia) were randomly assigned and patients enrolled from June 27, 2011, to Feb 16, 2014. 4069 patients were admitted during the cycling periods in total and 4707 were admitted during the mixing periods. Of these, 745 patients during cycling and 853 patients during mixing were present during the monthly point-prevalence surveys, and were included in the main analysis. Mean prevalence of the composite primary endpoint was 23% (168/745) during cycling and 22% (184/853) during mixing (p=0·64), yielding an adjusted incidence rate ratio during mixing of 1·039 (95% CI 0·837–1·291; p=0·73). There was no difference in all-cause in-ICU mortality between intervention periods.

Interpretation Antibiotic cycling does not reduce the prevalence of carriage of antibiotic-resistant, Gram-negative bacteria in patients admitted to the ICU.

Funding European Union Seventh Framework Programme.

Lancet Infect Dis 2018

Published Online January 24, 2018 http://dx.doi.org/10.1016/S1473-3099(18)30056-2

See Online/Comment http://dx.doi.org/10.1016/S1473-3099(18)30057-4

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Introduction

Antibiotic resistance poses a risk to patient safety, because it is associated with increased morbidity and mortality, and prolonged length of stay in health-care settings.[1,2] Within hospitals, antibiotic resistance is usually most prevalent in intensive care units (ICUs). Here, selective antibiotic pressure is high, opportunities for cross- transmission are frequent, and patients are susceptible to the acquisition of carriage and subsequent infections with antibiotic-resistant bacteria. Across different ICU settings, study findings support the clinical effectiveness of improved hand hygiene, universal chlorhexidine bathing, universal use of mupirocin nasal ointment, and universal gowning to minimise the acquisition of carriage and infections of Gram-positive bacteria, such as meticillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[3–5] However, none of these interventions seem effective to control the emergence of antibiotic-resistant, Gram-negative bacteria, such as Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL) or carbapenemases.

Antibiotics accelerate selection of antibiotic resistance, but they are also indispensable for the treatment and protection of critically ill patients. Intravenous antibiotics have been associated with selection for antibiotic resistance within individual patients.[6,7] It has been hypothesised that alternation of ecological selective antibiotic pressure at the ward level, through structured modifications in antibiotic policies, will reduce antibiotic resistance.[8,9] Mathematical models predict that lengthy periods of homogeneous selective pressure create a

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Research in context

Evidence before this study

The study was designed in 2010 on the basis of findings from a systematic review done in 2006. We had searched PubMed with the terms (first as individual terms and subsequently in a combined approach and snowballing from references): ("antibiotic" or "antimicrobial"), "resistance", ("ICU" or "intensive care"), ("colonization" or "infection"). Selection criteria included antibiotic-resistant bacteria of any kind, interventions targeting antibiotic use to reduce prevalence or incidence of antibiotic-resistant bacteria (restriction of specific classes or individual antibiotics, rotation, or cycling), and non-outbreak settings. Reports in languages other than English and reviews were excluded. This search yielded 1017 articles, which were first screened by title and abstract to establish whether selection criteria were indeed met. Studies published only as abstracts or that were published without abstracts were excluded. Ultimately, nine studies, done between 1984 and 2006, met these criteria and were reviewed. This search was repeated on Oct 6, 2017, yielding eight additional studies. We also searched meta-analyses from the Cochrane Collaboration, American and later European and German guidelines on antibiotic stewardship (including antibiotic rotation), and reviews of antibiotic rotation focusing on methodological, clinical, and in-silico studies, as well as mathematical studies. The overall conclusion emerging from these sources is that the theoretical basis for antibiotic rotation is complex and that there is insufficient clinical evidence for a recommendation of a particular antibiotic rotation strategy in intensive care units (ICUs).

Added value of this study

Compared with previously published studies on antibiotic cycling and mixing in ICUs, this cluster-randomised, crossover study is to our knowledge the largest prospective evaluation of the effects of mixing and cycling on the prevalence of carriage with antibiotic-resistant, Gram-negative bacteria in the ICU, with measurement of the primary endpoint based on point-prevalence surveys. Special emphasis was made to control for important potential confounders, such as colonisation pressure, use of non-study antibiotics, and basic infection control measures.

Implications of all the available evidence

Findings from our study do not provide evidence that antibiotic mixing, compared with antibiotic cycling with 6-week cycling periods, with third-generation or fourth-generation cephalosporins, piperacillin–tazobactam, and carbapenems reduces the prevalence of carriage with antibiotic-resistant, Gram-negative bacteria in the ICU. Therefore, one strategy cannot be recommended over another, and neither strategy can be recommended in ICUs.

higher selective pressure than a strategy in which antibiotics with different selective properties are rotated.[10] Translated to real-life settings, such strategies would include the scheduled alternation of first-line empirical treatment choices to increase diversity in antibiotic use. Different approaches have been used, such as antibiotic cycling and mixing. The most frequently researched strategy, dating back to the 1980s, is antibiotic cycling. In this approach, a specific antibiotic is preferentially used as first-line therapy in all patients that need treatment during a prespecified period, after which another antibiotic—presumed to have different selective properties—becomes the preferred therapy for all patients needing treatment.[11] This strategy increases homogeneity of selective pressure within each cycling period, and heterogeneity between periods. In antibiotic mixing, such antibiotics would alternate after each patient in which treatment has been started, thereby continuously maximising heterogeneity in antibiotic selective pressure.

In clinical studies, antibiotic cycling and mixing have yielded inconclusive results.[12,13] Studies we found in the literature were mostly single centre (n=15), yet sometimes in multiple wards (n=5), and more frequently testing cycling interventions (n=15) than mixing strategies (n=3). In most studies (n=12), a quasi-experimental, before-and-after design was used. Potential confounders (such as patient characteristics and infection prevention measures) and clustering of outcomes were poorly considered. These methodological shortcomings and the small number of studies investigating mixing strategies, preclude definite conclusions on the benefits of these strategies, as has been expressed in international guidelines.[14–17] We therefore aimed to compare the effects of both strategies on the prevalence of antibiotic-resistant, Gram-negative bacteria in the ICU.

See Online for appendix

Methods

Study design and participants

We did an international, multicentre, cluster-randomised, crossover study. The protocol for this study was previously published.[13] Eligibility criteria for ICUs are listed in the appendix (p 1); we included medical, surgical, or mixed ICUs with at least eight ventilator beds, the ability to implement the study, and the presence of a digital infrastructure to deliver study data. ICUs were approached and selected according to a tender defined by the European Union, including an assessment with questionnaires and on-site visits.

The study protocol was approved by each local institutional review board (IRB) and all centres obtained a waiver for written informed consent from individual patients.

Randomisation and masking

After a baseline period of 4 months, in which ICUs applied standard care treatment practices, ICUs were randomly assigned to two 9-month intervention periods,

separated by a 1-month washout period (figure 1). During the intervention periods, the preferred empirical treatment choices for ICU-acquired infections in which Gram-negative bacteria were covered were third-generation or fourth-generation cephalosporins (eg, cefotaxime, ceftriaxone, ceftazidime, and cefepime), piperacillin–tazobactam, and carbapenems (eg, imipenem, meropenem); the antibiotic class was determined by the study protocol, but the choice of specific antibiotic was determined by factors such as physician discretion, hospital availability, and local guidelines. The order of the tested strategies (cycling or mixing) and the order of rotated antibiotics within each strategy were randomised before the start of the trial by a person not part of the study team. ICUs were randomised separately for which intervention (cycling or mixing) was implemented first. The order of antibiotics was randomised per ICU. The intervention did not allow concealment of allocation.

During mixing, the preferred empirical treatment choice changed with every consecutive empirical treatment course. During cycling, preferred empirical treatment changed every 6 weeks, creating six cycling periods of 6 weeks each. Treating physicians could only deviate from the study-preferred antibiotic for reasons of patient safety (eg, previous antibiotic use, colonisation with resistant bacteria, or allergies). De-escalation and the use of combination therapy that included study and non-study antibiotics were allowed. Infection control procedures were not dictated by the study protocol and practices were monitored during the study period.

Outcomes

The primary endpoint of this study was the change in the unit-wide prevalence of carriage with antibiotic-resistant, Gram-negative bacteria. This composite endpoint included carriage with Enterobacteriaceae harbouring ESBL genes, or with phenotypical resistance to piperacillin–tazobactam (for Enterobacteriaceae, *Acinetobacter* spp and *Pseudomonas aeruginosa*) or carbapenems (for *Acinetobacter* spp and *P aeruginosa*). Unit-wide prevalence of carriage was measured through monthly point-prevalence screening cultures of the oropharynx and perineum of all patients present in the ICU on a single day. This subset of patients was used for the primary analysis. Carriage with one of the indicator bacteria in either the oropharynx or the perineum was considered a primary endpoint. Patients with extended ICU stay could be included in multiple monthly measurements. Secondary endpoints included length of stay and mortality in ICU; additional secondary outcomes not addressed here are described in the protocol.

Procedures

Data were collected for individual patient-level outcomes (eg, age, sex, admission diagnosis, length of stay, validated illness severity scores, and in-ICU mortality) for all patients admitted, or aggregated at ICU level with monthly point-prevalence measurement (eg, ICU bed size, bed occupancy, isolation precautions, and staffing ratios).[18] Adherence to hand hygiene protocol was measured monthly by direct observations by trained research nurses following standardised methods.[19]

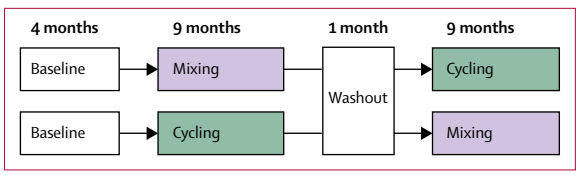

Figure 1: **Study timeline**

Full adherence to study protocol during cycling should result in dominance of the preferred antibiotic during the 6-week cycling periods and high variance between these periods. Mixing should yield equal use of antibiotics during the mixing period. We therefore quantified the use of study antibiotics in defined daily dose (DDD) per patient-day for 6-week periods during cycling and mixing. To optimise protocol adherence, we registered the point prevalence of antibiotic use for all patients in the ICU on a single day each week, and communicated the calculated proportions of the different study antibiotics to the ICUs. For the analysis on antibiotic consumption, overall unit-wide data were used, based either on individual courses (five ICUs), or on ward-level administrative orders (three ICUs).

Swabs obtained as part of the monthly point-prevalence studies were inoculated in brain–heart infusion glycerol medium and stored at –70°C. From seven ICUs, swabs were processed at a central laboratory by inoculation on five different (selective) media plates: MacConkey agar without antibiotics, MacConkey agar with ceftriaxone (0·5 mg/L), piperacillin–tazobactam (4 mg/L), or meropenem (0·125 mg/L), and an ESBL chromogenic agar plate (Oxoid Brilliance ESBL Agar, ThermoScientific, Hampshire, UK). In one centre, the local institutional review board required that screening swabs were processed locally. Isolates were sent to the central laboratory for further analysis. Presence of ESBL genes was assessed by PCR (for genes encoding CTX-M, SHV, and TEM, including subtyping) in all Enterobacteriaceae with phenotypic resistance to ceftazidime or ceftriaxone. For details of the microbiology protocol and breakpoints for non-susceptibility see appendix p 2.

Statistical analysis

We did sample size calculations using a parallel group comparison of two proportions, adjusted for clustering within ICUs, and based on an intraclass correlation coefficient of 0·01 and a design effect of 2·35; we calculated the sample size to be 921 patients per intervention type (total 1842) with 95% confidence (α) and 80% power (1–β), to show an absolute unadjusted difference in carriage prevalence of 10% between cycling and mixing. The minimum number of clusters required was seven. For each participating ICU, 135 measurements

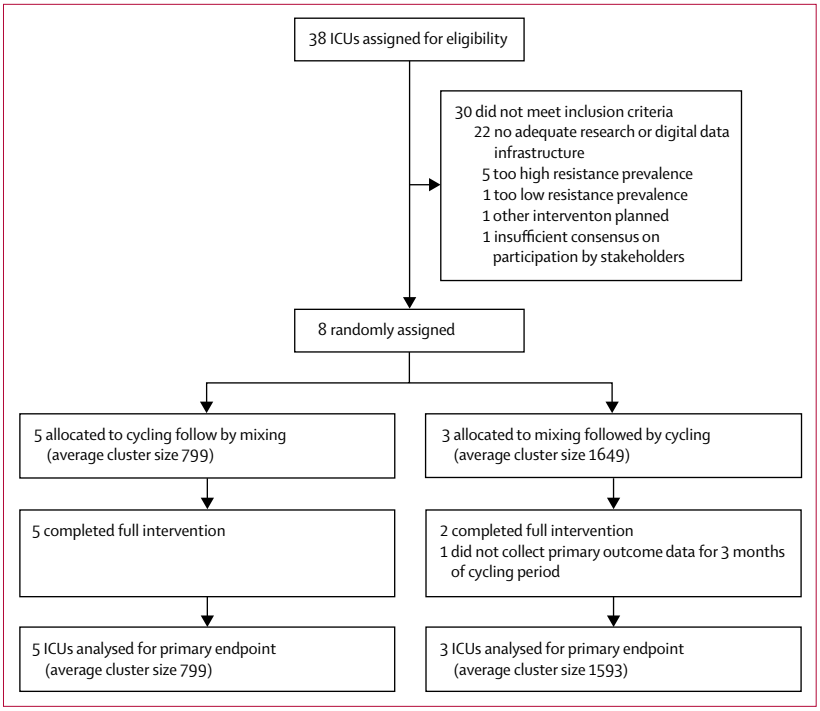

Figure 2: **Study design**
ICU=intensive care unit.

were assumed to be done per intervention period (nine monthly point-prevalence measurements multiplied by 15 estimated beds per ICU), yielding 1080 screened patients per intervention period.

Unadjusted analysis of the prevalence of antibiotic-resistant, Gram-negative bacteria in the monthly prevalence surveys during mixing and cycling was based on a univariable χ^2 test, and adjusted analysis was done with a generalised linear mixed model, accounting for clustering of endpoints (proportion of patients carrying these bacteria in point-prevalence screening) within hospitals, time trends, and patient-level and ICU-level confounders. The adjusted analysis uses a Poisson distribution and a logarithmic link with a random intercept per ICU and random slope for intervention weeks. The resulting time-trend thus describes the change in prevalence of antibiotic-resistant, Gram-negative bacteria over time under the reference intervention. Link and variance functions were chosen on the basis of expert opinion by a senior statistician not involved in study design, data collection, or result inference. Confounder variable selection before forward stepwise selection was on the basis of expert opinion and visual assessment of collinearity. Preselected confounders were age and sex, and point-prevalence percentages of short-stay patients (with admission <48 h),

	Baseline	Cycling	Mixing
Patient characteristics			
Number of admissions	2204	4069	4707
Sex			
Men	1323 (60%)	2484 (61%)	2813 (60%)
Women	881 (40%)	1585 (39%)	1894 (40%)
Age, years	61·6 (19·2)	61·1 (19·1)	61·5 (18·7)
Length of stay in ICU, days	6·9 (3·0; 2·0–7·0)	6·9 (3·0; 2·0–7·0)	7·1 (3·0; 2·0–7·0)
Patients discharged before day 3	846 (38%)	1570 (39%)	1834 (39%)
Mean SAPSII score (six ICUs)	33·5	33·8	37·4
Mean SAPSIII score (two ICUs)	47·9	48·5	46·7
Mean APACHE score (three ICUs)	19·4	19·8	20·3
Mean TISS-28 score (three ICUs)	22·0	21·0	22·7
Mortality (%)	243 (11%)	430 (11%)	544 (12%)
Number of patients included in point-prevalence measurements	467	773	927
ICU characteristics*			
Bed occupancy, occupied/available (%)	467/568 (82%)	773/999 (77%)	927/1157 (80%)
Patients in contact isolation	101 (22%)	184 (24%)	226 (24%)
Patients in droplet isolation	11 (2%)	12 (2%)	19 (2%)
Patients in respiratory isolation	8 (2%)	7 (1%)	15 (2%)
Number of nurses per patient†	0·64	0·65	0·65
Number of student nurses per patient	0·19	0·12	0·13
Hand hygiene compliance (observed health-care worker hand hygiene opportunities)	70% (1085)	69% (2824)	72% (2810)
Patients colonised with antibiotic-resistant, Gram-negative bacteria on admission‡	14/117 (12%)	25/201 (12%)	18/221 (8%)

Data are n, n (%), mean (SD), or mean (median; IQR), unless otherwise stated. SAPS=Simplified Acute Physiology Score. APACHE=Acute Physiologic Assessment and Chronic Health Evaluation. TISS-28=Therapeutic Intervention Scoring System-28. *Based on monthly point-prevalence surveys. †Registered nurses on duty per number of patients on the ward during point prevalence. ‡Calculated within patients from point-prevalence measurements: number of patients testing positive for antibiotic-resistant bacteria (study endpoint) during the first 2 days of admission, divided by total patients screened during the first 2 days of admission.

Table 1: **Patient and intensive care unit characteristics**

bed occupancy, proportion of patients requiring ventilation, and the staffing ratio (number of patients per qualified nurse). All variables were means over the 4 weeks preceding outcome point-prevalence measurement.

A crossover design can induce carry-over effects, occurring when effects from a preceding intervention period affect outcome in a following intervention period. Before and after building the model, we assessed carry-over effects by comparing the intervention type in the first versus the second intervention period, using a statistical test for the interaction of intervention and period. We analysed mortality using a Cox proportional hazard model. Analyses and sample size calculations were done with R software version 3.2.0.

The study is registered with ClinicalTrials.gov, number NCT01293071.

Role of the funding source

The funders had no access to the data, nor had influence on the design, execution, analysis, or writing of the report of this study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 38 assessed ICUs, eight fulfilled all eligibility criteria (one in Belgium, two in France, two in Germany, one in Portugal, and two in Slovenia; figure 2) and were randomly assigned from June 27, 2011, to Feb 16, 2014. Three ICUs were assigned to mixing followed by cycling (mean cluster size 1649, range 446–3824) and five were assigned to cycling followed by mixing (mean cluster size 799, range 428–1535). The randomised sequences of rotated antibiotics in the eight ICUs are provided in the appendix (p 2).

10 980 patients were admitted during the study period: 2204 during baseline, 4069 during cycling, and 4707 during mixing. 1598 (18·2%) patients were present during the monthly point-prevalence surveys (745 during cycling and 853 during mixing), and were therefore included in the main analysis (table 1). Patient-variable and ICU-variable values, as well as hand hygiene adherence, prevalence of isolation precautions, and nurse-per-patient staffing ratios, were similar during the 23-month course of the study (table 1).

There were two major protocol deviations. One ICU failed to collect point-prevalence screening swabs during the last 3 months of the study. We therefore decided to exclude all data for these months for this ICU, reducing the cycling intervention period from 9 to 6 months and changing the mean cluster size to 1593 (range 446–3824).

In another ICU, an outbreak with a carbapenem-resistant *Klebsiella pneumoniae* occurred during the washout period. Due to reduced treatment options, full adherence to study protocol became impossible and outbreak management measures would introduce confounding. Therefore, the washout period was extended until the outbreak had ended, outbreak management measures had been terminated, and antibiotic policy had returned to that before the outbreak. The duration of interruption was 5 months.

The average volume of antibiotic use was 1·51 DDD/patient-day during baseline, 1·59 DDD/patient-day during cycling, and 1·53 DDD/patient-day during mixing (difference between mixing and cycling 0·053 DDD/patient-day, 95% CI –0·16 to 0·15, p=0·93; table 2). However, we noted substantial variation in antibiotic use between ICUs (range 0·5–2·8 total DDD/patient-day during baseline; appendix p 3). Study antibiotics accounted for 39% of all antibiotics during baseline, 42% of all antibiotics during cycling, and 43% of all antibiotics during mixing. Overall use of study antibiotics was similar between the intervention periods (table 2). Carbapenems were used most frequently (0·33 DDD/patient-day during cycling *vs* 0·31 DDD/patient-day during mixing; difference 0·02, 95% CI –0·02 to 0·08), followed by third-generation and fourth-generation cephalosporins (0·21 DDD/patient-day *vs* 0·22 DDD/patient-day; difference –0·01, 95% CI –0·07 to 0·014) and piperacillin–tazobactam (0·13 DDD/patient-day in both study periods; difference –0·005, 95% CI –0·018 to 0·020).

	Baseline, DDD/patient-day	Cycling, DDD/patient-day*	Mixing, DDD/patient-day	p value†
Total	1·51 (0·62–2·66)	1·59 (0·45–2·56)	1·53 (0·4–3·32)	0·93
Cephalosporins‡	0·17 (0·03–0·27)	0·21 (0·03–0·53)	0·22 (0·05–0·72)	0·21
Cephalosporin cycle	..	0·36 (0·05–0·74)	..	..
Piperacillin–tazobactam cycle	..	0·14 (0·01–0·46)§	..	..
Carbapenem cycle	..	0·14 (0·01–0·38)§	..	..
Piperacillin–tazobactam	0·17 (0·05–0·25)	0·13 (0·04–0·18)	0·13 (0·04–0·20)	0·91
Cephalosporin cycle	..	0·08 (0·00–0·12)§	..	..
Piperacillin–tazobactam cycle	..	0·21 (0·02–0·31)	..	..
Carbapenem cycle	..	0·08 (0·01–0·19)§	..	..
Carbapenems	0·25 (0·02–0·50)	0·33 (0·04–0·61)	0·31 (0·04–0·55)	0·26
Cephalosporin cycle	..	0·24 (0·01–0·39)§	..	..
Piperacillin–tazobactam cycle	..	0·26 (0·00–0·59)§	..	..
Carbapenem cycle	..	0·49 (0·01–0·85)	..	..
Cephalosporins‡				
Ceftriaxone	0·05 (0·02–0·14)	0·04 (0·01–0·17)	0·05 (0·02–0·11)	0·87
Cefotaxime	0·02 (0·00–0·13)	0·02 (0·00–0·21)	0·02 (0·00–0·18)	0·61
Ceftazidime	0·04 (0·00–0·22)	0·05 (0·00–0·09)	0·04 (0·01–0·07)	0·33
Cefepime	0·06 (0·00–0·12)	0·10 (0·00–0·24)	0·11 (0·00–0·48)	0·08
Fluoroquinolones	0·14 (0·04–0·40)	0·13 (0·02–0·29)	0·14 (0·04–0·33)	0·37
Aminoglycosides	0·07 (0·00–0·16)	0·06 (0·00–0·10)	0·05 (0·00–0·08)	0·09
Co-trimoxazole	0·02 (0·00–0·12)	0·02 (0·00–0·08)	0·03 (0·00–0·21)	0·35
Macrolides	0·11 (0·03–0·25)	0·10 (0·03–0·22)	0·08 (0·03–0·15)	0·19
Amoxicillin–clavulanic acid	0·17 (0·06–0·41)	0·17 (0·06–0·41)	0·15 (0·05–0·34)	0·27

Values represent means per study period (ranges of individual intensive care units). DDD=defined daily dose.
*t test p value comparison of preferred antibiotic consumption rates with other two study antibiotics. †t test p value comparison of cycling and mixing periods. ‡Refers to third-generation or fourth-generation cephalosporins. §p<0·01.

Table 2: **Antibiotic use in DDD per patient-day**

	Baseline	Cycling	Mixing	Mixing *vs* cycling*	Difference (95% CI)
Point-prevalence surveys	32	59†	70	..	..
Screened patients	462	745	853	..	..
Patients with antibiotic-resistant, Gram-negative bacteria‡	129 (28%)	168 (23%)	184 (22%)	0·64	1·0 (–3·1 to 5·1)
Enterobacteriaceae					
ESBL phenotype	97 (21%)	128 (17%)	127 (15%)	0·21	2·3 (–1·3 to 5·9)
ESBL genotype	58 (13%)	72 (10%)	68 (8%)	0·23	1·69 (–1·1 to 4·5)
CRE genotype	4 (1%)	7 (1%)	10 (1%)	0·65	–0·2 (–1·2 to 0·8)
Non-fermenters§					
Resistant to piperacillin–tazobactam or carbapenems	40 (9%)	61 (8%)	66 (8%)	0·74	0·5 (–2·2 to 3·1)
Pseudomonas aeruginosa					
Resistant to ceftazidime	5 (1%)	5 (1%)	2 (<1%)	0·19	0·4 (–0·2 to 1·1)
Resistant to piperacillin–tazobactam	20 (4%)	37 (5%)	25 (3%)	0·04	2·0 (0·1 to 4·0)
Resistant to carbapenems	29 (6%)	43 (6%)	53 (6%)	0·71	–0·4 (–2·8 to 1·9)
Acinetobacter spp					
Resistant to piperacillin–tazobactam	4 (1%)	7 (1%)	6 (1%)	0·60	0·2 (–0·7 to 1·1)
Resistant to carbapenems	1 (<1%)	6 (1%)	6 (1%)	0·81	0·1 (–0·8 to 1·0)

Data are n (%) unless otherwise stated. ESBL=extended-spectrum β-lactamase. CRE=carbapenem-resistant Enterobacteriaceae. ICU=intensive care unit. *χ^2 test for mixing *vs* cycling. †Number of point-prevalence surveys during cycling was lower than in mixing due to three missed surveys in one ICU and overall shorter total time-period of cycling compared with mixing. ‡Defined as carriage with Enterobacteriaceae bacteria harbouring ESBL genes, or with phenotypical resistance to piperacillin–tazobactam (Enterobacteriaceae, *Acinetobacter* spp or *P aeruginosa*) or carbapenems (*Acinetobacter* spp or *P aeruginosa*). §*P aeruginosa* and *Acinetobacter* spp.

***Table 3*: Prevalence of antibiotic resistance at the patient level**

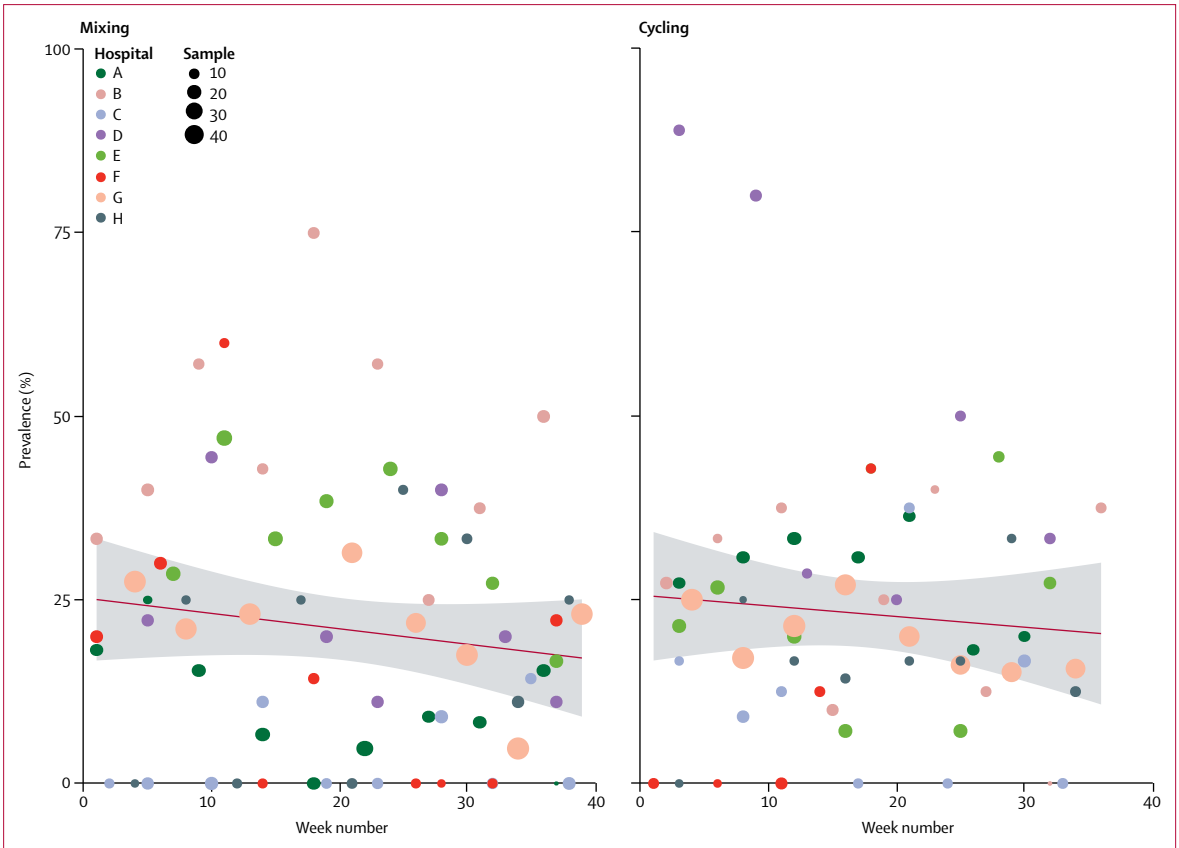

***Figure 3*: Primary endpoint time trend per intervention, using linear regression for time trend**
Dot size depicts number of patients in the point prevalence. Y-axis depicts the percentage of patients with (primary endpoint) antibiotic-resistant bacteria. The grey shaded area is the 95% CI for the regression coefficient of the time-trend of the primary outcome.

During cycling, the volume of study antibiotics varied per 6-week period. Carbapenem use was 0·49 DDD/patient-day during the carbapenem cycles and half that (0·24–0·26 DDD/patient-day) in the other cycle periods. Third-generation and fourth-generation cephalosporin use was 0·36 during the cephalosporin cycle and almost two-thirds lower (0·14 DDD/patient-day) in both other cycles. Piperacillin–tazobactam use was 0·21 DDD/patient-day during the piperacillin–tazobactam period and almost two-thirds lower (0·08 DDD/patient-day) in the other cycle periods. During mixing, the volume of study antibiotics (analysed in 6-week periods to mimic the duration of cycling periods), was stable (appendix p 1).

We used microbiological screening results and demographic data for patients present during the monthly point-prevalence surveys (745 during cycling and 853 during mixing) for the primary analysis. The mean prevalence of antibiotic-resistant, Gram-negative bacteria (composite primary endpoint) was 23% (168/745) during cycling and 22% (184/853) during mixing (p=0·64; table 3). There were no relevant differences in prevalence for subgroups or specific species (table 3, appendix p 2). The incidence rate ratio between mixing and cycling of the mixed effects analysis was 1·039 (95% CI 0·837–1·291; p=0·73), adjusted for hand hygiene compliance, patient sex, and proportion of short-stay patients. The model was built using forward stepwise parameter selection, based on a decrease of the Akaike information criterion (AIC), an indicator of how well the new parameter relates to the other data of the model, including the resistance prevalence. If the AIC decreases, this new model better describes the other study confounders and the primary outcome. In the model with the lowest AIC, compliance with hand hygiene, patient sex, and the percentage of short-stay admissions best described the ICU-level prevalence of the primary endpoint. All variables in the final model improved model fit, thereby increasing overall model validity. However, none of the correlations between confounders and primary outcome (eg, proportion of short-stay patients and prevalence reduction) were statistically significant (data not shown).

Assessment of carry-over effects did not change the model fit significantly, with a trend towards reduced fit (AIC increase of 0·04 and 1·31 with and without adjustment for confounders). By comparison, the AIC decreased by 23·7 after adding hand hygiene compliance to the model. Straightforward weighted linear regression of point-prevalence measurements did not demonstrate significant trends of resistance prevalence decrease during both intervention periods (figure 3). In crude analysis there was evidence of autocorrelation of prevalence that extended up until 5–6 weeks, which disappeared after adjustment for potential confounders (appendix p 4).

ICU mortality was 11% during baseline, 11% during cycling, and 12% during mixing (p=0·38 in unadjusted Cox proportional hazard analysis). We noted no statistically significant (p<0·01) differences in subgroup endpoints (species and resistance type; appendix p 2).

Discussion

In this cluster-randomised crossover study in eight ICUs, 9-month periods of antibiotic cycling and mixing did not change the unit-wide prevalence of antibiotic-resistant, Gram-negative bacteria. Therefore, structured rotation of antibiotic prescription policies for possible Gram-negative bacteria cannot be considered as a measure to reduce antibiotic resistance in ICUs.

The epidemiology of antibiotic resistance in ICUs is complex. Acquisition and prevalence of carriage is affected by the number of patients with colonisation in the unit.[20] This colonisation pressure might reduce the validity of a study when individual patients are randomly assigned to interventions. Prevention of colonisation due to an intervention might then also reduce the risk of colonisation in a patient present in the unit but receiving another intervention, and vice versa. This patient dependency could affect accurate quantification of the effects of an intervention. We avoided this issue by using a cluster-randomised design. Furthermore, changes in the proportion of admitted patients who carry resistant bacteria, infection control practices, patient casemix, adherence to hand hygiene, and the use of non-study antibiotics could also affect acquisition of resistant bacteria; these variables therefore were carefully monitored during the study periods. On the basis of the observed absence of changes in these potential confounders during the study periods and the small effects on outcome after adjustment in the statistical analysis, we conclude that it is unlikely that they affected the findings and interpretation of this study.

Some aspects of the study design deserve explanation. First, the rationale of the study was based on the observed emergence of antibiotic-resistant, Gram-negative bacteria and decline in invasive infections caused by MRSA in Europe, which warranted an intervention targeting antibiotics that influence the epidemiology of these bacteria. The choice of antibiotics eligible for rotation at the time of study design (2010) already excluded the use of amoxicillin–clavulanic acid, second-generation cephalosporins, or fluoroquinolones as suitable options for empirical treatment of presumed Gram-negative infections in many European ICUs. In the absence of endemic carbapenem-resistant Enterobacteriaceae, we considered third-generation or fourth-generation cephalosporins, piperacillin–tazobactam, and carbapenems as equally acceptable for empirical treatment. There was (and is) no evidence base to define the optimal duration of cycling periods. Previous studies have used cycling periods ranging from 1 to 8 months. Our decision for two 9-month study periods was, at least partly, guided by the available funding. Within the 9-month cycling period, we decided to use two 6-week periods for each

preferred antibiotic, instead of one 3-month period per antibiotic without reintroduction. This decision was guided by the available theoretical evidence and discussions with experts in mathematical modelling.[10,21,22] Determination of the primary outcome was based on monthly point-prevalence studies. For feasibility reasons these surveys were fixed on a standard day each month, and the point-prevalence days, therefore, did not coincide with the end of the 6-week cycling periods. We aimed to establish the effects of changing antibiotic exposures during a total of 9 months, and not to record immediate effects per 6 weeks.

The study was underpowered for subgroup analyses of individual resistance and species types. This aspect should be taken into account when interpreting results. Additionally, we did not achieve the sample size initially calculated for the main outcome, although by not taking into account the crossover design we probably overestimated the needed sample size. Patient follow-up was restricted to the ICU period, and mortality was not a primary study outcome because we did not expect it to be seriously affected by the intervention with the planned population size. Eight ICUs in five European countries might not be fully representative for all European ICUs. Although participating ICUs did not have extraordinary features with regard to patient population and prevalence of carriage with antibiotic-resistant Gram-negative bacteria, their characteristics should be taken into account when extrapolating results to individual ICUs. Additionally, there were two major protocol deviations in two ICUs, one of which missed three point-prevalence measurements. However, sensitivity analyses excluding these ICUs did not change interpretation of results (data not shown). Finally, as individual-level prescription data were not available from three ICUs, our analyses of antibiotic use were restricted to aggregated data.

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On the basis of observed antibiotic use during baseline, implementation of the study protocol significantly changed neither overall antibiotic use nor the overall use of study antibiotics. The three study antibiotics accounted for about 40% of all antibiotics used and the total volume of antibiotics and the amounts of the study antibiotics were very similar in both study periods. The study intervention therefore represented—as intended—variance in the use of the three study antibiotics, without change in the volume of these antibiotics used over time. Substantial differences in exposure to the three study antibiotics during the study periods were achieved. During the cycling periods, antibiotic use for the non-preferred drugs fell by half to two-thirds, whereas use of study antibiotics was remarkably stable during mixing. However, the achieved differences in antibiotic exposure did not affect the unit-wide prevalence of antibiotic resistance, lending support to findings from a recent theoretical study[11] that cycling and mixing in real-life circumstances are unlikely to achieve large effects on antibiotic resistance.

Controlling the emergence of antibiotic-resistant, Gram-negative bacteria in ICUs is important, but universally useful and successful strategies remain to be identified. Previously, the combined intervention of improved adherence to hand hygiene, universal chlorhexidine body washing, and screening on admission for carriage and isolation of carriers did not reduce the acquisition of antibiotic-resistant, Gram-negative bacteria carriage in 13 European ICUs.[3] In settings with low levels of antibiotic resistance, the use of non-absorbable, prophylactic antibiotics in the gastrointestinal and upper-respiratory tract has been successful in preventing infections and improving patient outcomes, while maintaining low prevalence of antibiotic-resistant, Gram-negative bacteria.[23,24] Yet, whether that approach is equally successful and safe in settings with higher levels of antibiotic-resistant, Gram-negative bacteria remains to be established. Reductions of the total volume of antibiotics, however, will probably contribute to control of the emergence of antibiotic-resistant, Gram-negative bacteria through a reduction in antibiotic selective pressure. This goal can be achieved by improved diagnostics, distinguishing which patients do and do not need antibiotics, as was demonstrated with invasive diagnostics for patients with a clinical suspicion of ventilator-associated pneumonia.[25] Furthermore, selective pressure can be reduced by biomarker-guided reductions in the duration of antibiotic treatment.[26,27]

Contributors

PJvD and MB designed the study and were responsible for study management, data interpretation, and manuscript preparation. MB was co-applicant of the SATURN consortium, funded by the European Union's Seventh Framework Programme. WV, PGJ, FSp, DS, MD, AR, DA, CL, J-CNV, BM, MJ, KS, FSi, VT, FE, and JC were responsible for the on-site managerial and executive part of implementing the study. MJCE provided consultation for the statistical analysis. SH was the academic coordinator of the SATURN consortium and provided input in the study design, data interpretation, and final revision of the manuscript. All authors read and approved the final manuscript.

Declaration of interests

SH has received consulting fees from GSK, Bayer, Janssen, and Novartis. All other authors declare no competing interests.

Acknowledgments

We are grateful to the many investigators and staff who were involved in enrolling and screening patients over the course of this clinical trial. We also thank Martin Bootsma for valuable discussions on the study design. This work was supported financially by the European Commission under the Life Science Health Priority of the Seventh Framework Programme, under grant agreement number 241796 (SATURN project).

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