SATURN
Final Publishable Summary Report

1. Executive Summary

Following the initial remarkable success of antibiotics, the emergence and spread of human pathogenic bacteria resistant to antibiotics has become a major phenomenon in the past fifty years. Antimicrobial Resistance (AMR) is rampant among bacteria that cause healthcare- and community-acquired infections, driving up costs and increasing the difficulty of therapeutic management. To gain a handle on the factors that are propelling the problem of AMR, molecular and patient-level investigations are necessary to better elucidate the time-varying and heterogeneous role of antibiotic selection pressure on emergence and selection of AMR.

Many results drawn from previous studies of the effect of antibiotic use on emergence, selection and spread of AMR have lacked a holistic view combining all aspects into one study. The SATURN project had as aim to study the impact of antibiotic exposure on AMR with a multidisciplinary approach that bridges molecular, epidemiological, clinical and pharmacological research.

Two types of clinical studies were conducted :

- A randomised trial was performed to resolve an issue of high controversy (antibiotic cycling vs. mixing).
- Three observational studies were conducted to rigorously study issues surrounding the effect of antibiotic use on AMR that are not easily assessable through randomised trials.

These clinical studies served as a platform to two complementary work packages (Microbiology and Pharmacology) that performed futher investigations:

- The work package focusing on molecular studies generated new evidence about the changes effected by antibiotic therapy on commensal organisms or opportunistic pathogens in the oropharyngeal, nasal and gastro-intestinal flora and study AMR mechanisms and the dissemination of successful clones of fluoroquinolone-resistant, carbapenem-resistant or extended-spectrum beta-lactamase harboring Gram-negative bacteria, MRSA and fluoroquinolone-resistant viridans streptococci.
- The pharmacodynamic study modelled the relationships between antibiotic exposure and AMR emergence over time for various classes of agents.

In summary, the overarching rationale of SATURN was to improve methodological standards and conduct research that will help to better understand the impact of antibiotic use on acquisition, selection and transmission of AMR in different environments, by combining analyses of molecular, individual patient-level and ecologic data. The anticipated results may guide clinical and policy decisions to ultimately reduce the burden of AMR in Europe.

With a total budget of 7.8 M€ and a funding support of 6 M€ from the EC's 7th Framework Programme for 5 years, SATURN was coordinated by Stephan Harbarth (University of Geneva) and brought together leading European researchers in antimicrobial resistance. The SATURN consortium comprised 14 partners from 11 countries which included Switzerland, Italy, Israel, the Netherlands, Belgium, Poland, France, Spain, Germany, Serbia and Romania.

2. Project context and objectives

The emergence and spread of human pathogenic bacteria resistant to antibiotics has become a major problem in the past fifty years. Antimicrobial resistance (AMR) is rampant among bacteria that cause healthcare- and community-acquired infections, driving up costs and increasing the difficulty of therapeutic management.

SATURN aimed to respond to an urgent need, as stated in the EU Council conclusions (June 2008) on AMR, in which the EU "*stresses the need of research in the area of AMR, e.g. to increase the understanding of the mechanisms and underlying risk factors that advance the development of AMR and to increase the knowledge of the effectiveness of current and future control measures*."

Thus, the main global objectives of SATURN were to **study the impact of antibiotic exposure on antimicrobial resistance** (AMR) and to **define strategies to improve knowledge on antibiotic selection pressure and judicious antibiotic use**.

Also, SATURN had as mission to improve methodological standards and conduct research to better understand the impact of antibiotic use on acquisition, selection and transmission of antibiotic-resistant bacteria (ARB) in different environments, by combining state-of-the-art analyses of molecular, ecologic and individual patient-level data. The proposed program and anticipated results would help reduce the burden of AMR in Europe and guide both clinical decision making and policy decisions in this area.

In this perspective, SATURN decided to follow a multidisciplinary approach that bridges microbiological, clinical, epidemiological and pharmacological research.

One intervention study (WP2) and three observational clinical studies (WPs 3-5) were conducted that had as objective to produce demonstrable improvements over previously generated evidence regarding the effect of antibiotic exposure and selection pressure on acquisition, selection and transmission of ARB within hospital and community settings. Molecular (WP1) and pharmacologic (WP6) issues generated by the four clinical studies were addressed by two additional studies. The latter two project components are essential elements of the research project and interrelated synergistically with the clinical studies.

The specific objectives of each study and the workplan structure showing the interaction between the studies is shown hereafter.

WP2 – Antibiotic rotation strategies to reduce antibiotic resistance in the Intensive Care Unit: A cluster randomized, crossover study

In SATURN, special focus was brought on ICUs as they are clinical environments where patient care may be severely hampered by AMR problems. The primary objective of this ICU-trial was to assess the impact of antibiotic mixing to a strategy of antibiotic cycling on the mean unit-wide prevalence of antibiotic-resistant Gram-negative bacteria (ARGNB). The primary hypothesis was superiority of one intervention arm over the other. In this perspective, a study of AMR in Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* species in 10 European ICUs was undertaken.

WP3 – Observational community study

A prospective, observational multi-centre cohort study of antimicrobial resistance in *E. coli* and other clinically relevant enteric bacteria in a well-defined community population in three European countries was undertaken with the following primary objectives:

- To determine the impact of antibiotic class and duration use on the carriage of resistant Enterobacteriaceae among individuals consuming antibiotics for urinary tract infections (UTIs) and their household members;
- To assess other epidemiologic factors associated with carriage of resistant bacteria or changes in the resistance pattern of the commensal flora;
- To determine the impact of antimicrobial use on the carriage of susceptible Enterobacteriaceae among individuals consuming antibiotics

WP4 – Observational nosocomial acquisition study

The main goal of this study was to compare nosocomial acquisition rates of MRSA and ESBL-producing gram-negative bacteria (*E.coli*, *Klebsiella* spp. and *Proteus* spp.) among hospitalized patients treated with antibiotics and to define temporal relationship between the start of antibiotic therapy, the acquisition of new colonisation in patients previously not colonized, and the development of a bacterial infection caused by the same strain isolated in a screening sample. This goal was achieved by completing the following primary objectives:

- To determine the rate of acquisition of target antibiotic-resistant bacteria by 1,000 antibiotic-days according to different classes of antibiotics, duration of therapy, and antibiotic combination (monotherapy versus combination therapy);
- To determine genotypic relation between colonising and infecting strain in the same patient and patients' and hospital staff colonising strains;
- To study the virulence and fitness of the isolates (i.e. new colonising strains) causing subsequent nosocomial infections;
- To predict the risk for nosocomial acquiring of a target bacteria after a single treatment therapy adjusted by length of hospitalisation and ward colonisation pressure.

WP5 – Observational AMR carrier study

A prospective, observational longitudinal trial designed to examine the effect of various agents on selection and amplification of AMR organisms and resistance genes among carriers of these strains was conducted in 4 countries (Italy, Romania, Serbia and Israel) in which AMR is a prevalent problem with the following objectives:

- To determine the effect of various antibiotics on amplification of AMR Enterobacteriaceae pathogens, i.e. ESBL producing Enterobacteriaceae and Carbapenem resistant Enterobacteriaceae (CRE), and on dissemination of resistance genes.
- To determine the effect of duration of antibiotic therapy on amplification of AMR pathogens and on dissemination of resistance genes
- To determine the PK/PD indices that influence the amplification of AMR pathogens and on dissemination of resistance genes
- To correlate the effects of antibiotics on phenotypic and genotypic expansion of AMR
- To determine the effects of various antibiotics on epidemicity and fitness of various ARB
- To determine the correlation between amplification of AMR pathogens in the GI tract and environmental contamination

- To study the impact of antimicrobial treatment.

WP1 – Bacterial Genetics and Functional Studies

The major objective of the microbiology study was to carry out microbiologic analysis of samples/strains collected during the community- or hospital-based clinical trials (WPs 2-5). These studies aimed to quantify the resistance burden of ESBL- or carbapenemase-harbouring or fluoroquinlone-resistant Enterobacteriaceae, and of viridans streptococci in stool samples/rectal swabs and oropharyngeal samples prior to antibiotic treatments and follow their dynamics under and after antibiotic treament. These studies utilized both phenotypic and genotypic methods to realise this aim, and these results are presented in the clinical WP summaries.

WP1 also aimed to study known and novel/emerging mechanisms of resistance in the bacterial pathogens enumerated above. Except for basic screening on culture, these studies utilized state-of-the-art molecular techniques.

Finally the third major aim of WP1 was to study clonality, fitness costs of resistance gene carriage and virulence using comparative genomics, genome-wide arrays, high-throughput sequencing, in vitro competitive growth experiments, and mice models of infection.

WP6 – Pharmacodynamics

The efficacy of antimicrobials has been shown to be dependent on both exposure of antimicrobials *in vivo* as well as susceptibility of the micro-organism, the latter usually expressed as MIC. In particularly over the last decade, the increased sophistication and insight in pharmacokinetics and pharmacodynamics has allowed for exposure-response relationships that have elucidated exposure response relationships for several antimicrobials. However, few data existed with respect to colonization by resistant micro-organisms, emergence of resistance, and exposure and susceptibility, respectively. Thus, the main purpose of SATURN pharmacodynamics study was to explore relationships between exposure and emergence of resistance respectively, both qualitatively as well as quantitatively. The objectives to reach that goal were fourfold : (1) to ensure that databases were build and datasets were available that would allow such analyses (2) inventorize population pharmacokinetic models and where appropriate build these models of specified antimicrobials (3) build software and validate software that allowed the calculation of individual exposures of large numbers of patients (4) explore exposure response relationships, response being defined as either efficacy, becoming colonized [with resistant strains] or emergence of resistance. The data that could collected in each of the clinical studies differed because the extent and quality of data collection differed by work package, depending on the set-up of each study.

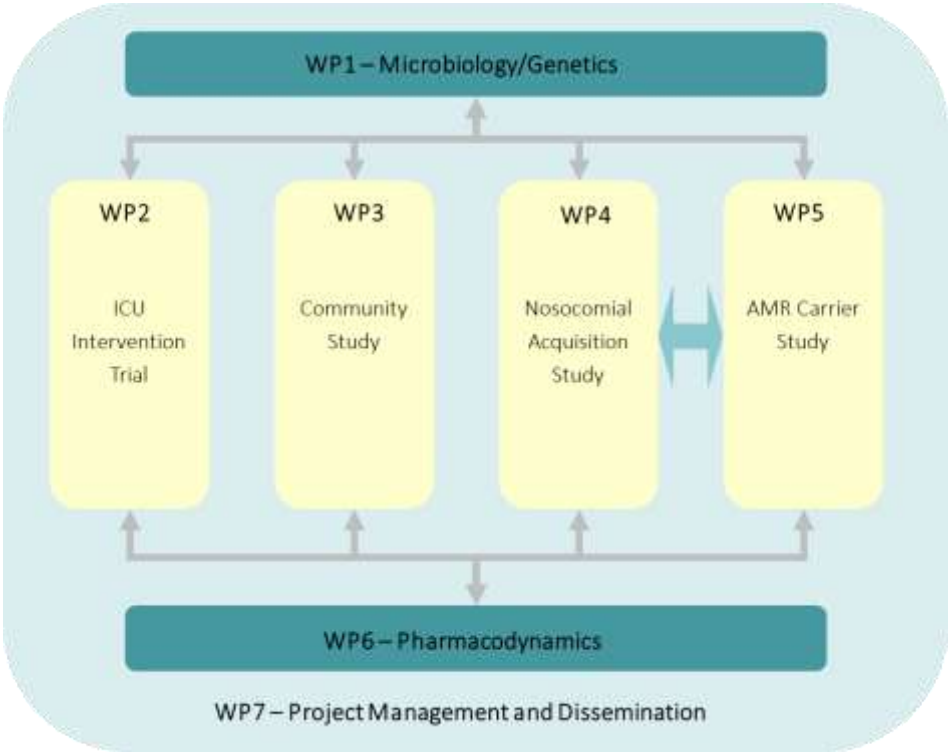


Figure 1 SATURN work plan structure showing the interaction between the studies

3. Main S&T results/foregrounds

3.1 WP2 – Antibiotic rotation strategies to reduce antibiotic resistance in the Intensive Care Unit: A cluster randomized, crossover study

Scientific approach of the study / the procedures, methods used

After a standard care period of 4 months, ICUs were randomized in a cluster-randomized, crossover design. The two intervention periods (mixing and cycling) that follow the baseline are both 9 months. In between the intervention periods is a wash-out period of 1 month. The patient inclusion period ranged from January 2011 until February 2014.

The primary objects of study are the clusters or ICUs. The secondary endpoints are the individual patient endpoints. ICUs have been selected through a tendering procedure according to EU regulations. IRB approval with a waiver for individual patient written informed consent was obtained. The SATURN ICU trial was led by University Medical Centre Utrecht and was registered in the ClinicalTrials.gov (NCT01293071) register. All admitted patients were included in the study. Those subject to empiric treatment of Gram-negative bacteria were eligible for treatment according to the intervention protocol. Individual treatment and treatment indication were not registered, adherence to the intervention was measured at the cluster-(e.g. ICU-)level.

Summary of the main steps/phases of the study

In the participating ICUs, empiric treatment for Gram-negative therapy was rotated systematically. The two main strategies of antibiotic rotation are mixing and cycling. They differ in the speed at which antibiotics are rotated and can be seen as two extremes of one spectrum with regard to heterogeneity.

Mixing is rotation per patient antibiotic course (with possibly more than one course within a patient). This leads to a high heterogeneity in antibiotic consumption on a ward or ICU unit at any point in time. Cycling has a slower rotation, in periods of 6 weeks. Cycling leads to “blocs” in time with monotonic selective pressure, and a large contrast in selective pressure when a new antibiotic becomes the first-line treatment.

The three rotated antibiotic (group)s are 1) third- or fourth- generation cephalosporins, 2) piperacillin-tazobactam and 3) carbapenems.

The order of 9 month interventions and the order of rotated antibiotics were randomized for each ICU at the beginning of the trial by a person not involved in the design or execution of the study.

The treating physician has ultimate decision over antibiotic treatment. Combination therapy (such as addition of an aminoglycoside or coverage of Gram-positive bacteria) is allowed. ICU-specific procedures related to standard hygienic measures, monitoring practices, outbreak management and any other infection prevention measures will not be dictated by study protocol.

With optimal adherence to the interventions, the three preferred antibiotics should be in equal proportions during mixing, whereas the preferred antibiotic should be dominant during cycling. For this the heterogeneity, or balance, of proportions is measured between the three rotation antibiotics.

The primary endpoint is the mean point-prevalence of ARGNB, and is obtained from the monthly point-prevalence screening results of each study period. These measurements were performed by obtaining swabs from oropharynx and perineum from all patients present in the ICU. Swabs are frozen directly and cultured in a single centralized microbiological laboratory. The resistance endpoint is an aggregated outcome, and is defined as 1) extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. 2) Piperacillin-Tazobactam resistance in: Enterobacteriaceae, *Pseudomonas aeruginosa* and Acinetobacter species, and 3) Carbapenem resistance in *P. aeruginosa* and Acinetobacter species (Table 1).

Table 1 Resistance endpoints

Species	Extended-spectrum beta-lactamase (ESBL)-producing	Piperacillin-Tazobactam resistance	Carbapenem resistance
Enterobacteriaceae	+	+	
Pseudomonas aeruginosa		+	+
Acinetobacter species		+	+

The study focused on the primary unadjusted analysis of ICU-level resistance. Potential confounding by case-mix, and the effects of the interventions on antibiotic consumption will be assessed in the secondary, adjusted analyses.
Baseline characteristics and covariates will be tested, with appropriate bivariate tests. For the primary endpoint, a Pearson Chi-square test will be used for estimation of the crude effect of the interventions on resistance prevalence.

During the implementation of the trial, part of the microbiological protocol was changed with regard to the screening swabs. This followed criteria posed by the IRB of one of the ICUs. It required that the screening swabs were not sent directly to the central laboratory, but were first cultured and isolated on location and then shipped. Media plates were provided by the central laboratory. Shipment, culturing, isolation and typing at the central laboratory was all done according to protocol.

Main scientific results

During the standard care and intervention study periods all admitted patients (n=11.158) were included for data collection on endpoints and potential confounders.
For the intervention periods; standard care (baseline), *cycling* and *mixing*, 2.210, 4.237 and 4.711 patients were included (Table 2). All variables values between the standard care and intervention periods and between *cycling* and *mixing* were comparable, except for differences between illness severity scores. There was no statistically significant difference in mortality (10.9 versus 11.5%, p=0.369).
Of note, not all centers documented the same illness severity scores. Therefore, these were represented as mean per type of score per study period. The amount of patients contributing to these averages, and thus their weight with regard to the impact on overall mortality, is also noted. The totals can exceed the total number of admissions, as some ICUs deliver more than one illness-severity score.

Table 2 Main demographics

	Standard care	Cycling intervention	Mixing intervention	Mixing versus Cycling p-value
Admissions (n)	2.210	4.237	4.711	
Patients (n)	2.074	3.913	4.433	
Age (mean)	61,6	61,2	61,9	.234*
Gender female/male %	40,0	39,1	40,3	.244#
LOS mean	6,99	6,82	7,06	.289*
median	3	3	3	
Admissions (mean/week/ICU)	16,0	14,8	15,2	
Short stay %	35,0	30,9	31,6	.643#
Ilness severity scores (mean)				
APACHE II (n)	19,4 (323)	19,8 (645)	20,6 (733)	.176*
SAPS II (n)	33,6 (975)	34,5 (1904)	37,4 (1933)	<0.001*
SAPS III (n)	47,9 (1019)	48,5 (1882)	46,7 (2246)	<0.001*
TIS-28 (n)	22,0 (433)	21,0 (780)	22,7 (877)	<0.004*
Mortality (%)	11,0	10,9	11,5	.369#

Chi-square test
* T-test

The primary outcome is the incidence of resistant bacteria in the two intervention groups, cycling and mixing. For the aggregated endpoint, there were 206 positive screenings of 750 total screenings (24,4%) in the *cycling* period, and 183 in 869 screenings (23,7%)in the *mixing* period (Table 3). *Mixing* and *cycling* have almost exactly the same incidences (p=.744). The ESBL-producing Enterobacteriaceae and Carbapenem resistant Enterobacteriaceae had a relative reduction of 11,7% and 13,7% respectively, during *mixing* but this was not statistically significant (p-values 0.267 and 0.664). For all resistance categories non-fermenters (*P. aeruginosa* and Acinetobacter species), incidence of resistance was increased, though non-significantly, during *mixing*.

The antibiotic results show that ICUs vary considerably in baseline antibiotic prescription. The antibiotic consumption of ICUs over the entire study ranged from 0,09 - 3,74 DDD per admission day in the standard care and intervention periods.

Comparing the standard care period against the two intervention periods combined, overall consumption increased from 1,53 to 1,69 DDDs per admission day. Between the two intervention periods, mixing had a slightly higher use of antibiotics per admission day than cycling, 1,70 versus 1,67 (Figure 2).
This small difference between intervention periods was attributable mainly to an overall higher use of meropenem (+1.6%), ampicillin-sulbactam (+1,1%) and linezolid (+1.0%). The increase compared to standard care was associated with a concomitant reduction of daily consumption of flucloxacillin (-1,1%), Ciprofloxacin (-1,0%) and cefepime (-1,0%). With regard to the study antibiotics therefore, meropenem use increased with a reduction in cefepime. All other rotated study antibiotics had <1% change. Overall consumption of the three study antibiotics increased from 36,0% of total consumption in DDDs during standard care, to 41,9 and 42,5% for cycling and mixing respectively (Graph 2).
Over the course of the study, these proportions of study versus non-study antibiotics remained comparable (Figure 3).

Table 3 Screening swab endpoint results

	Baseline	Cycling	Mixing	p-value (Chisq)*
Screening events N	458	750	869	
CRE phenotype N	8	17	17	.664
	1,75%	*2,27%*	*1,96%*	
ESBL-E phenotype N	95	131	134	.267
	20,74%	*17,47%*	*15,42%*	
Enteroacteriaceae piperacillin-tazobactam resistance	102	93	108	.986
	22,27%	*12,40%*	*12,43%*	
Acinetobacter spp. resistance	5	6	8	.794
	1,09%	*0,80%*	*0,92%*	
Acinetobacter spp. Piperacillin-tazobactam resistance	6	7	8	.979
	1,31%	*0,93%*	*0,92%*	
***Pseuasomonas aeruginosa* carbapenem resistance**	32	42	53	.670
	6,99%	*5,60%*	*6,10%*	
***Pseuasomonas aeruginosa* piperacillin-tazobactam resistance**	16	31	23	.097
	3,49%	*4,13%*	*2,65%*	
All non-fermenters any resistance endpoint**	39	56	66	.922
	8,52%	*7,47%*	*7,59%*	
Aggregated all endpoints	132	183	206	.744
	28,82%	*24,40%*	*23,71%*	

* *Mixing versus cycling interventions*
** Pseudomonas aeruginosa *and Acinetobacter spp.*

Figure 2 DDD per admission day per intervention period

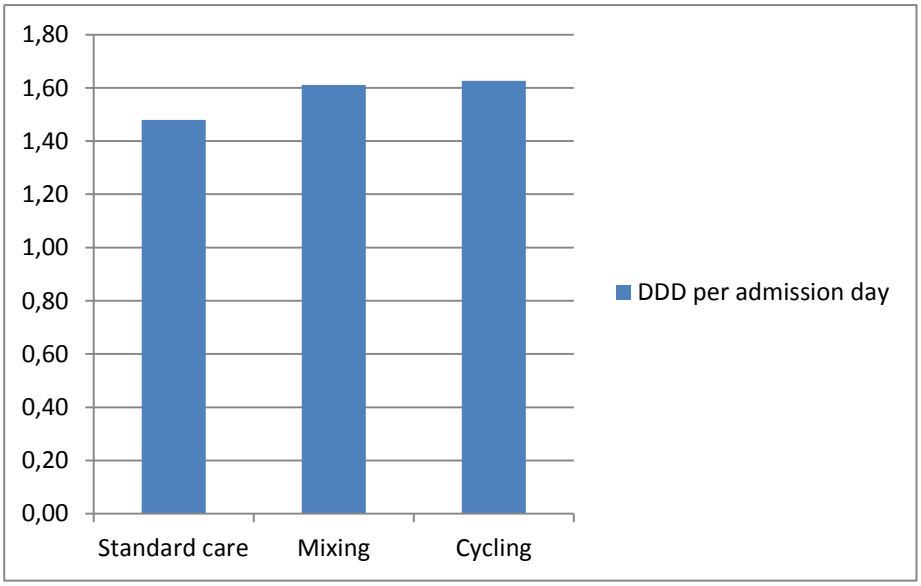
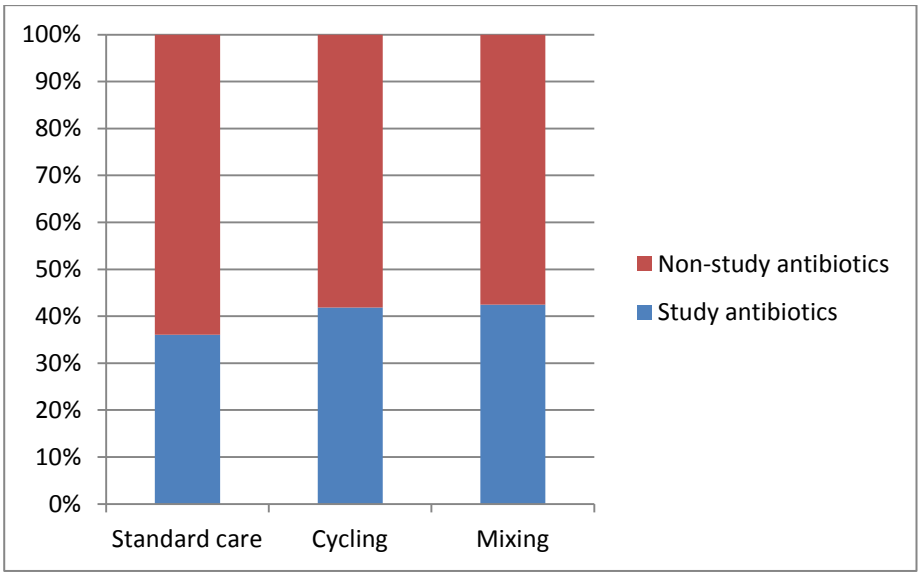


Figure 3 Study and non-study antibiotics, proportions per intervention



The mean 1-weekly proportions of the 3 study antibiotics were not different across intervention periods (Figure 4). For 33 out of 46 cycling periods, the study protocol-driven preferred antibiotic was prescribed the most. This resulted in an adherence of 72%. The non-compliant periods were reasonably distributed over the ICUs, All but one hospital had at least 1 non-adherent cycling period. Two hospitals had only one, and two successful periods respectively, out of six cycling periods. When pooling the periods by their preferred antibiotic the most prescribed antibiotics were concordant with the preferred study antibiotics (Figure 5). The largest dominance of a cycling antibiotic is seen for meropenem during the meropenem period, then cephalosporins and piperacillin-tazobactem.

Figure 4 Study antibiotic proportions, intervention periods

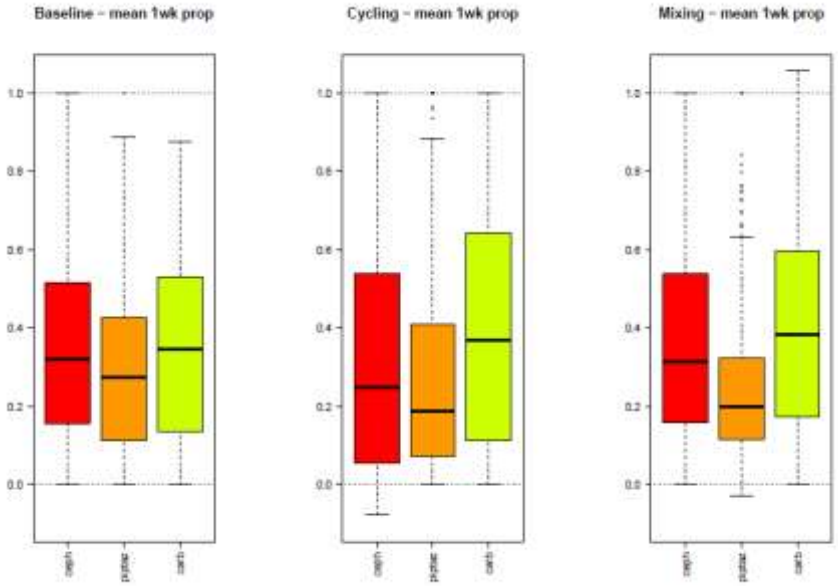
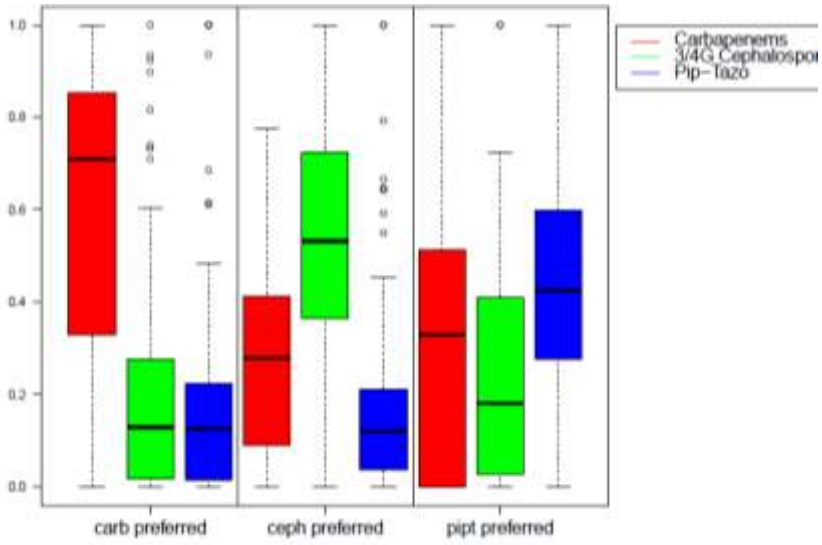


Figure 5 Antibiotic weekly proportions, cycling periods pooled per preferred antibiotics



Variance of the proportions for the 3 study antibiotics was used as a measure for heterogeneity in antibiotic prescription. The 6-week proportions stratified to hospital, showed individual differences in ICU variance, with increased variance for cycling (Figure, 6, 7, 8).

There was a decrease in mean variance (of 6-weekly proportions) for mixing compared to cycling for all 8 hospitals, for the proportions of 3rd and 4th generation cephalosporins, piperacillin-tazobactam and meropenem.

Figure 6-7-8 Distribution of 6-week proportions for 3rd and 4th generation cephalosporin, piperacillin-tazobactam and carbapenems, per hospital. Dotted line is the proportion mean

Figure 6

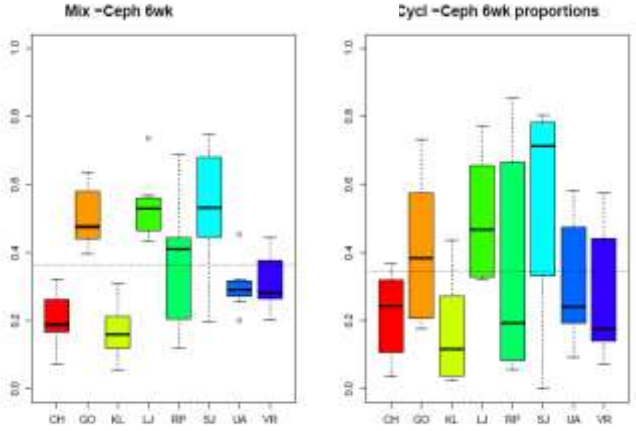


Figure 7

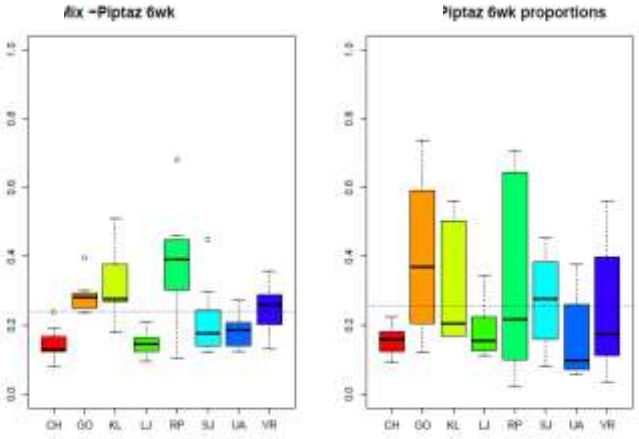
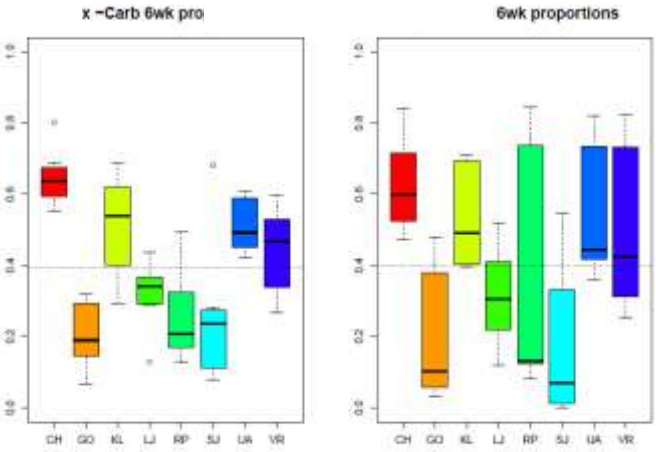


Figure 8



In addition to non-clustered missing values, there were clusters with missing data owing to systematic hiatus in data collection.

First, screening swabs have not been collected for the last 3 months of the study in one hospital. These missing values are due to an unforeseen shortage in personnel. All data

from this period will be excluded from the study. This means the concerning ICU will have an intervention period of 6-months instead of 9.
Second, for the duration of the baseline period of the same ICU, Hand Hygiene observations are missing due to incomplete protocol implementation. For the primary analysis between the two intervention periods, data from the standard care period are not essential. Sensitivity-analyses will be performed for other analyses where they are used (such as time-trend analyses).
One hospital deviated from the original timeline due to an outbreak of multi-resistant Gram-negative bacteria.. This lead to a reduction in treatment options for empiric treatment, to the point that there were no alternative options for physicians to rotate between. As a result, the intervention was halted automatically. This outbreak and cessation of the study occurred during the wash-out period. The wash-out month was therefore extended till the end of the outbreak, 4 months later, after which the normal protocol was restarted. As a result, despite a delayed start of the second intervention, the protocol could be restarted without any additional changes.

Conclusion

Antibiotic rotation is a intervention that is safe, easy- and low-cost to implement. In this preliminary, unadjusted analysis of antibiotic prevalence under *mixing* or *cycling* interventions, no statistical differences were found. There were differences between standard care and the interventions periods, but the study was not initially powered to show such an effect. Adherence was high, but especially for *mixing*, measuring adherence is challenging and not yet formalized.
Given the current results there is no preference for either *mixing* or *cycling* interventions but antibiotic rotation still has potential benefits over standard care. If current results hold, the choice between *cycling* and *mixing* will not depend on higher effectiveness but more on feasibility.
Concluding, this study uses a level of methodology and inclusion size not performed before, showing equivocal results. Final, more sophisticated analyses will assess the features that underlie this result and their potential effects they have on resistance. It has also made a first step to compare antibiotic rotation with normal care. It will provide a large mass of data valuable for additional studies, such as, mathematical modeling and pharmacological population models.

3.2 WP3 – Observational community study

Scientific approach of the study / the procedures, methods used

University of Geneva performed a prospective cohort study from January 2010 to August 2013 (trial registration, ISRCTN26797709). The human research ethics committee at each site provided approval. All subjects provided written informed consent.

Ambulatory patients were recruited from three European sites: general practice networks in Antwerp (Belgium) and Łódź (Poland), and ambulatory clinics at the University of Geneva Hospitals (Switzerland).

We recruited—as the ‘exposed’ group—a convenience sample of patients with suspected UTIs who required antibiotic treatment. Simultaneously, we recruited an unmatched group of ‘non-exposed’ patients presenting to the same clinics for an indication not requiring antibiotic therapy. Inclusion criteria applied to both groups were age ≥18 years and current

residence with at least one other person. Exclusion criteria were treatment with systemic antibiotics or hospitalisation within the previous 30 days; residence in a long-term care facility; current urinary catheter; and renal transplant or renal replacement therapy. We recruited 2–3 household contacts for each index patient.

The exposure of interest was antibiotic therapy, stratified by agent and duration.

The main outcomes were intestinal colonisation by extended-spectrum beta-lactamase (ESBL)-producing and ciprofloxacin-resistant Enterobacteriaceae, defined as detection of such organisms in faecal samples taken at the end of antibiotic therapy and 28 days later. Intestinal colonisation with nitrofurantoin-resistant Enterobacteriaceae was a secondary outcome for those subjects receiving nitrofurantoin.

The investigator completed a case report form at the time of index subject recruitment. Each subject completed a self-administered baseline questionnaire. Finally a prospective questionnaire was completed with each sample collection to record antibiotic exposure during the study period.

Participants provided three faecal samples: baseline (time point [TP] 1); completion of antibiotic therapy (TP2); and 28 days after the second sample, without further antibiotic exposure (TP3). For non-exposed households, TP2 was 7–10 days after TP1. Subjects collected their own stool samples using a disposable Protocult^{TM} kit (Ability Building Center, Rochester, USA), and were kept on ice for a maximum of 24 hours before being frozen at –80° Celsius until further microbiologic analysis.

Faecal samples were analysed by quantitative plating on non-selective (blood agar & CHROMagar Orientation) and selective (CHROMagar ESBL, KPC, CIP0.12 (0.12 µg/ml ciprofloxacin), CIP2 (2 µg/ml ciprofloxacin) media. Samples from households of patients receiving nitrofurantoin or fosfomycin were additionally cultured on chromogenic media with 64 µg/ml nitrofurantoin or 8 µg/ml fosfomycin, respectively.

Ten colonies of each morphology type were sampled from selective plates. Antibiotic susceptibility was determined by the disc diffusion method according to CLSI guidelines. Strains not identified as *E. coli* by coloration on the chromogenic agar underwent species identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. ESBL-producing strains of same species from a single household were selected for MLST on basis of similar antibiogram.

Microbiologic analyses from all sites were centralized and performed by WP1 at the UA.

Baseline covariates were presented for each participant group. Categorical variables were presented as counts and proportions. Continuous variables were presented using mean and standard deviation if normally distributed, and otherwise with median (or geometric mean) and interquartile range.

We accounted for repeated measurements and the clustered nature of collected data by using mixed-effects generalised linear regression models, with subject, household and study site included as random effects.

Summary of the main steps/phases of the study

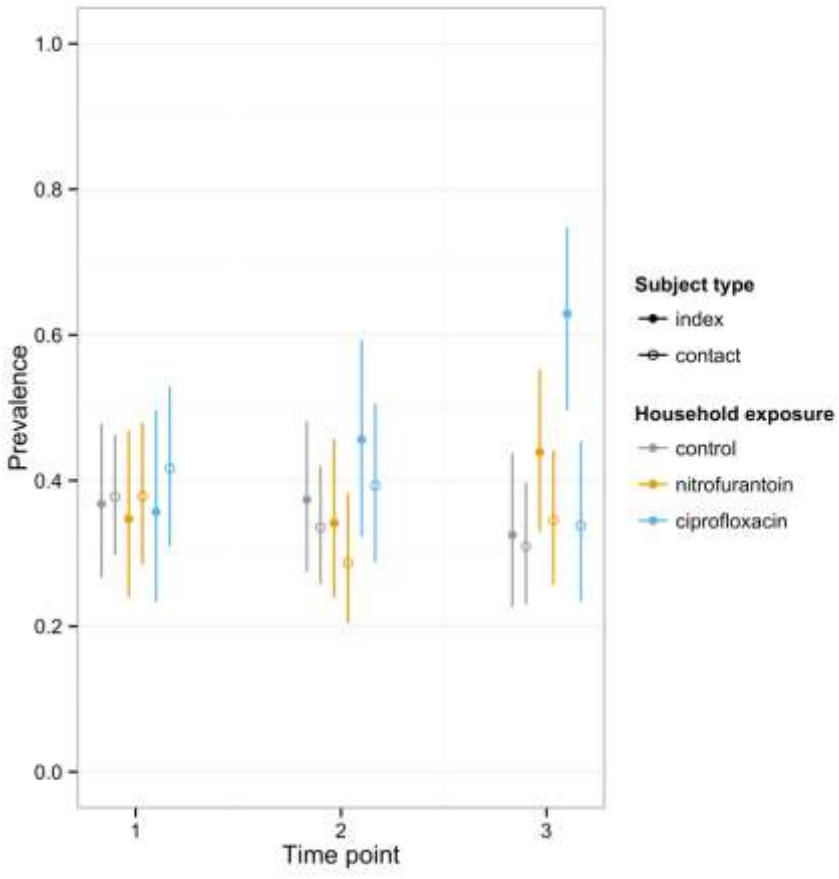
Study preparation occurred during 2010. Recruitment was performed from 2011 to 2013. Microbiologic analysis was completed by mid-2014. Primary analyses were concluded in early 2015.

Main scientific results

A total of 309 households (98 control and 211 antibiotic-exposed) completed the study and provided samples at three time points. Extended-spectrum beta-lactamase producing Enterobacteriaceae were isolated from 6.8% (95% confidence interval [95% CI], 5.8-8.0%) of samples and ciprofloxacin-resistant Enterobacteriaceae were isolated from a surprisingly high proportion of participants in 36.4% (95% CI, 34.3-38.9%) of samples. The most common antibiotics prescribed to index patients with UTIs were ciprofloxacin and nitrofurantoin – other antibiotics were relatively uncommon. The key results are presented below.

Impact of antibiotic use on the carriage of resistant Enterobacteriaceae among individuals consuming antibiotics for suspected or confirmed urinary tract infections and their household members

Interestingly, antibiotic exposure of any class or duration was not associated with emergence of ESBL-producing Enterobacteriaceae amongst commensal flora. Less surprisingly, antibiotic exposure was also not associated with emergence of resistance to nitrofurantoin. The most notable effect of antibiotics on the emergence of resistance was seen amongst patients treated with ciprofloxacin. The prevalence of ciprofloxacin resistant Enterobacteriaceae amongst such patients increased from 34% (prevalence ratio, 0.88 [95% CI 0.52-1.49] compared to household contacts in control households at TP1) at baseline to 61% 28 days after the completion of antibiotic treatment (prevalence ratio, 1.68 [95% CI 1.09- 2.57]). Nitrofurantoin was not associated with a significant increase in the prevalence of ciprofloxacin resistance. The plot below illustrates the prevalence of colonisation by ciprofloxacin-resistant Enterobacteriaceae amongst index patients and household contracts in control households and UTI households where the index patient was treated with ciprofloxacin or nitrofurantoin.



Epidemiologic factors associated with carriage of resistant bacteria or changes in the resistance pattern of the commensal flora

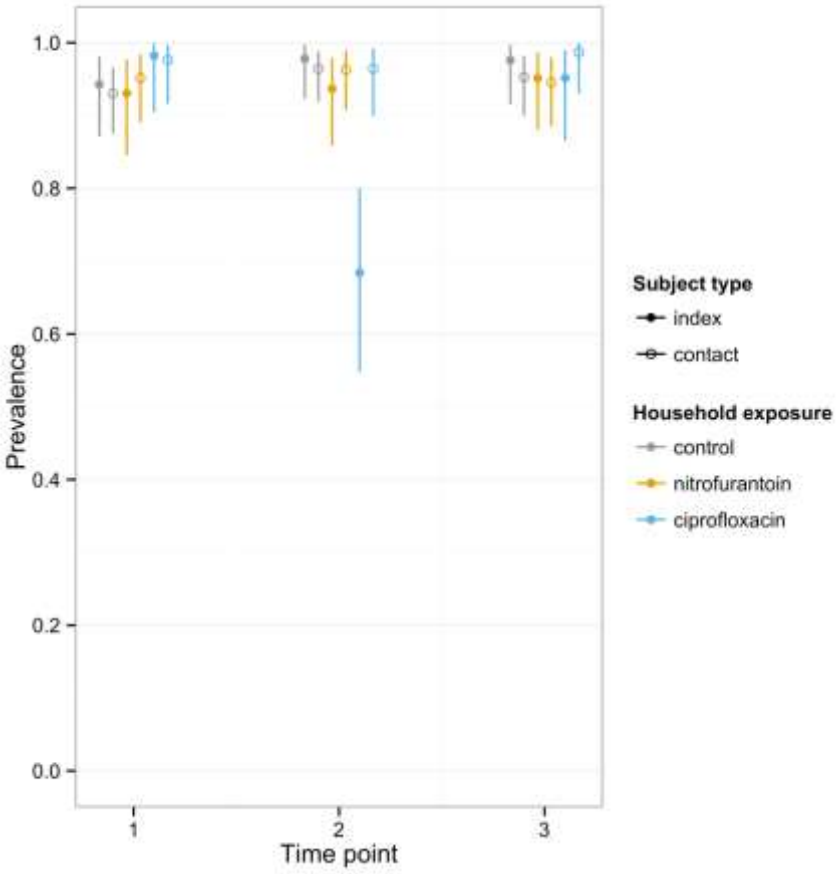
Importantly, clustering within households was noted for ESBL-production, ciprofloxacin-resistance and nitrofurantoin-resistance. At baseline, colonisation of one or more household contacts by Enterobacteriaceae with these forms of resistance was associated with a significant increase in risk of colonisation: prevalence ratio, 2.69 (95% CI, 1.39-5.20), 1.48 (1.14-1.93) and 9.84 (1.48-65.55), respectively.

Otherwise, few risk factors were identified. Consistent with other recent studies, travel to the Indian subcontinent was associated with an increased risk of colonisation by ESBL-producing Enterobacteriaceae (prevalence ratio 8.37 [95% CI, 2.61-26.91]). No other association between travel and colonisation by any form of resistant Enterobacteriaceae was identified. Likewise, other potential risk factors were not significantly associated with colonisation by resistant strains. These include socio-economic factors such as level of education, dietary factors such as consumption of or contact with meat, contact with animals, living on a farm, and the presence of children in the household.

Impact of antimicrobial use on the carriage of susceptible Enterobacteriaceae among individuals consuming antibiotics

One means by which antibiotic consumption can facilitate the amplification and spread of antibiotic-resistant bacteria is by supressing the amount of antibiotic susceptible bacteria. Suppression of antibiotic-susceptible bacteria may increase the probability of being colonised if an individual is then exposure to antibiotic resistant bacteria (loss of so-called ‘colonisation resistance’). Furthermore, suppression of susceptible bacteria may allow amplification of resistant strains already present amongst commensal flora in small amounts by removal of competition.

To explore this effect, we examined the impact of different antibiotics on the presence of Enterobacteriacaea in stool samples. The plot below illustrates the proportion of subjects – stratified by subject type (index or contact) and household (control, nitrofurantoin and ciprofloxacin) – with any Enterobactericeae present in stools samples at the three time points. As would be expected for a group of bacteria generally considered ubiquitous gut commensals, Enterobacteriaceae are cultured from almost all stool samples. The notable exception was that Enterbacteriaceae were not cultured from the time point 2 stool samples (end of antibiotic treatment) of one third of patients treated with ciprofloxacin.



This finding that ciprofloxacin has more substantial impact on commensal flora than nitrofurantoin was further developed in a SATURN sub-study performed on a small cohort of WP3 samples using culture-free methods to analyse the entire gut microbiota rather than only Enterobacteriaceae. Using this approach we found that ciprofloxacin had a significant global impact on the gut microbiota whereas nitrofurantoin did not. The end of ciprofloxacin treatment correlated with a reduced proportion of *Bifidobacterium* (Actinobacteria), *Alistipes* (Bacteroidetes) and four genera from the phylum Firmicutes (*Faecalibacterium*, *Oscillospira*, *Ruminococcus* and *Dialister*) and an increased relative abundance of *Bacteroides* (Bacteroidetes) and the Firmicutes genera *Blautia*, *Eubacterium* and *Roseburia*. Similarly to the plot above, substantial recovery had occurred four weeks later. In contrast, nitrofurantoin treatment correlated with a reduced relative proportion of the genus *Clostridium* and an increased proportion of the genus *Faecalibacterium*.

Conclusion

These data are novel in presenting an alarmingly high prevalence of colonisation by ciprofloxacin-resistant Enterobacteriaceae in the European community. Exposure to ciprofloxacin clearly increased the risk of colonisation by such strains, and this effect is likely to by mediated – at least in part – by the impact of this antibiotic on the gastrointestinal microbiota. By contrast, nitrofurantoin had minimal impact on commensal microbiota and was not associated with emergence of resistance. Interestingly, these data did not demonstrate and association between exposure to antibiotics and colonisation by ESBL-producing Enterobacteriaceae. Household clustering was noted for both ciprofloxacin and nitrofurantoin resistance, as well as ESBL-producing Enterobacteriaceae. Interruption of household transmission therefore represents a hitherto largely neglected opportunity for

interventions to reduce the community-based spread of antibiotic resistant Enterobacteriaceae.

3.3 WP4 – Observational nosocomial acquisition study

Scientific approach of the study / the procedures, methods used

A 24-month multicenter prospective cohort study and two nested case-control studies were conducted in 6 medical and 6 surgical wards in three centers (UCSC-Rome, CCS-Belgrade, IDMB- Bucharest).

All consenting adult inpatients (> 16 y) starting antibiotic therapy orally and/or intravenously were included. Exclusion criteria were surgical antibiotic prophylaxis and pregnancy.

Nasal samples for the detection of MRSA and rectal sample for ESBL-producing gram-negative bacteria were obtained at hospital admission and discharge. Patients starting antibiotic therapy *per os* and/or intravenously were sampled at antibiotic start (t0, within one hour) and at the following intervals: day 3 (t1), 7 (t2), 15 (t3), 30 (t4). Patients colonised with MRSA and/or ESBL-producing gram-negative bacteria before starting antibiotic therapy were excluded from follow-up cultures and analysis in this WP and included in a different study protocol (WP5). Screenings were performed in outpatient clinics after patients' discharge from the hospital. Nasal and rectal cultures were also obtained from the ward staff at the beginning and at the end of the study. This group includes nurses and all staff and research-dedicated doctors having contact with patients. These cultures were handled in the same manner as the patients' cultures. All patients included in the study were followed to determine whether they had developed clinical infection with the target antibiotic-resistant bacteria. Patients were followed during the hospitalisation and afterwards for a total of 30-day from the inclusion in the study.

The assessable population for each target organism has included all treated patients from whom baseline screening and at least one follow-up screening was available. The incidence of colonisation has been defined as the number of new cases of colonisation for 1,000 days of antibiotic therapy. Only patients with a negative baseline (t0) have been included in the longitudinal analysis. The length of exposure to antibiotic therapy for patients newly colonised by antibiotic-resistant bacteria has been defined as the number of days between the inclusion in the study and the date of the first positive sample. The length of exposure to antibiotic therapy for patients not colonised has been defined as the number of days between the inclusion in the study and the date of antibiotic suspension. The Mongo-DB (Python) was used to find the most effective machine-learning model to deal with a multi-factorial and heterogeneous dataset. Complex models were chosen since they have a significant advantage over traditional statistical methods in reasoning different features at the same time.

The number of patients required to test the hypothesis that antibiotics may result in a significant increase in colonisation by antibiotic resistant strains within 30 days of antibiotic exposure was calculated as 1,500 patients starting antibiotics (500 per center). According to this study design and considering a percentage of ward antibiotic use from 15% to 25% of patients, a total number of samples ranging from 10,000 to 15,000 was expected. Because the rate of acquisition of antibiotic resistant strains in patients under antibiotic therapy was not established, percentage was derived from retrospective case-control studies. According to these studies, the difference in the use of antibiotic therapy between

these groups ranges from 10% to 60%. For an estimated difference of 40%, a total of 525 courses of antibiotic therapies were required in each group for the study to have the ability to show 21 cases of patients with drug resistant colonisation/infection with an alpha level of 0.01 and beta-level of 0.95.

Chromogenic medium (MRSAid, bioMérieux, Marcy l'Étoile, France) was used for MRSA identification. Multilocus Variable Number of tandem repeat assay and multilocus sequence typing (MLST) analysis was used for molecular characterization of colonizing and infecting strains. The Multilocus Variable Number of tandem repeat assays and clustering was performed as previously described. Reference isolates presenting known MLVA profiles from already characterized MLST were used in order to identify ST or clonal complexes of all MRSA isolates. The reference strains were analysed by MLST analysis. Series of PCR allowing SCCmec element typisation, toxin content (tst, pvl, exfoliatins A and B) were performed to specific representative isolates.The genetic relatedness of the ESBL-harbouring *E. coli* and *Klebsiella* from screening and clinical samples, isolated after starting antibiotc therapy from the same patient was determined by PFGE following digestion with *XbaI* and *SmaI* digestion.

Summary of the main steps/phases of the study

Collection data (M1-M6): After acquiring the approval from the local ethical committees, questionnaires were sent to all centers to collect data regarding infection control measures and epidemiological data in the clinical centers (UCSC, CCS, IDMB). Clinical and microbiological protocols as well as data collection forms for the prospective, observational longitudinal study were discussed and agreed. The clinical study was organised with the enrolling centers through repeated local visits and periodic conference calls. Advertisement and informative materials were elaborated and provided to the recruiting wards.

Cohort study and nested case-control studies (M7-M48): During this study period, recruitment was completed and overall 10197 individual patients were enrolled, 4395 (43.1%) from IMDB, 3824 (37.5%) from UCSC and 1978 (19.4%) from CCS. 4160 patients entered the antibiotic cohort. They provided 58804 swabs, 30482 nasal and 28322 rectal.

Data analysis (M48-M60): The statistical analysis has been performed at the EKUT. After analyzing the cohort as a whole patients were stratified according antibiotic therapy and target bacteria (MRSA and ESBL-positive gram negative). The application of interpretable classifiers (supervised machine learning methods) led us to favor simpler classifiers. Since the medical data were highly correlated, the method of choice was the Support Vector Machine (SVM) that tries to find the function separating the data points, using a higher dimensional space. The applied SVM uses also a function (kernel) to increase the dimensionality of the given data enabling a direct interpretation of the outcome coefficients to find the most important features to distinguish patients negative at hospital admission and acquiring colonization due to MRSA and ESBL after starting in hospital antibiotic therapy. The effectiveness of the classification was the ratio of the true positives to the true negatives (maximizing the sensitivity). The Platt scaling was used to obtain probability estimates. The analysis was performed for the combination of epidemiological variables (e.g. gender, age) and their encoding (antibiotics usage and co-morbidities). The SVM classifier was fed with the matrix of data represented in the values ranging from 0 to 1 and was tested for including all of diseases represented in the database. The rows represented patients and the column the features. The original database consisted of the 467 columns

(features) and over 10,000 patients. The model that produced the highest area under receiver operating characteristic curve (AUROC) was chosen. The analysis was completed as planned using using MongoDB, Python and the scikit-learn Python package implementing the machine learning methods.

Main scientific results

Overall 10197 individual patients were included in the study. During the study period 2980 patients had a positive swab (29.2%), 2450 were positive for ESBL (24%) and 530 were positive for MRSA (5.2%). At admission the screening yielded 1102 positive samples for ESBL (11% of the samples performed) and 293 positive samples for MRSA (2.9% of the samples performed). These 1395 patients were not allowed to enter the follow-up study but, according to the protocol, they were offered a second screening at discharge that was accepted and performed in 905 patients (65%). The remaining patients entered the cohort study once antibiotic treatment was started. Overall 4160 patients were enrolled in the cohort, representing 40.8% of the patients screened at admission. Among these patients, 173 (4.2%) had a positive MRSA sample during follow-up, between T0 and discharge screening (DS) and 976 (23.5%) had a positive sample for ESBL. The patients who were negative at admission screening and did not enter the cohort study, having not been started on antibiotics, were offered to be screened again, according to the protocol, at discharge. Of the 3674 patients screened at discharge for MRSA, out of 5857 patients, (62.7%), 64 were MRSA positives (1.1% over 5857, and 1.7% over 3674). Of the 3401 patients screened at discharge for ESBL, out of 5465 patients, (62.2%) 372 were ESBL positives (6.8% over 5465, and 10.9% over 3401).

Objective 1 - To determine the rate of acquisition of target antibiotic-resistant bacteria by 1,000 antibiotic-days according to different classes of antibiotics, duration of therapy, and antibiotic combination (monotherapy versus combination therapy):

Overall, 52775 days of antibiotic therapy were observed. In particular, the 976 ESBL positive patients in the cohort were treated with 1271 antibiotic treatments (11961 days of observation). Time of observation before acquiring new colonisation was 7373 days of therapy. The exposures to antibiotics and the rates of colonization for the more frequently used antibiotics and combinations are shown in the following table:

	IDMB (N:553)		**UCSC (N:131)**		**CCS (N:292)**		**Total (N:976)**	
Antibiotic	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*
MONOTHERAPY								
CEPHALOSPORINS	5.3	*60.3*	4.4	*29.1*	5.9	*58.3*	5.3	***54.8***
Beta-lactams	5.7	*21.2*	5.5	*15.6*	7.4	*43.7*	5.7	***20.9***
Quinolones	6.2	*21.8*	6.1	*18.9*	7.7	*37.5*	6.2	***24.0***
Carbapenems	5.7	*16.7*	6.0	*11.7*	6.5	*35.7*	5.7	***18.5***

	IDMB (N:553)		**UCSC (N:131)**		**CCS (N:292)**		**Total (N:976)**	
Antibiotic	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*
AMINOGLYCOSIDES	5.8	*21.4*	4.0	*14.6*	5.6	*63.7*	5.8	***42.9***
Macrolides	6.7	*26.0*	7.0	*14.6*	2.5	*15.9*	6.7	***20.0***
GLYCOPEPTIDES	5.5	*47.6*	6.7	*14.6*	6.9	*48.4*	5.5	***29.2***
ANTI-ANAEROBICS	6.2	*51.8*	5.1	*29.7*	7.2	*50.4*	6.2	***43.7***
COMBINATIONS								
Aminoglycosides-Beta-lactams	7	*26.7*	3.3	*17.0*	2	*90.9*	3.3	***34.7***
Carbapenems-Quinolones	7.3	*7.2*	1	*10.3*	4	*25.6*	5.2	***10.2***
Carbapenems-Beta-lactams	3	*54.1*	1	*74.1*	2.5	*55.5*	2.2	***60.0***

The 173 MRSA positive patients in the cohort were treated with 281 antibiotic treatments (1429 days of observation). Time of observation before acquiring new colonisation was 853 days of therapy. The exposures to antibiotics and the rates of colonization for the more frequently used antibiotics and combinations are shown in the following table.

			UCSC (N:18)		**CCS (N:75)**		**Total (N:173)**	
Antibiotic	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*
MONOTHERAPY								
CEPHALOSPORINS	5.3	*6.6*	5.8	*5.0*	6.8	*11.0*	6.0	***7.8***
Beta-lactams	6.5	*5.3*	8.3	*0.9*	7.4	*9.8*	7.0	***3.5***
Quinolones	4.1	*2.1*	10.3	*2.2*	5.4	*9.8*	6.4	***3.8***
Carbapenems	6.0	*4.2*	4.0	*1.9*	5.0	*15.6*	5.3	***5.2***
AMINOGLYCOSIDES	21	*2.4*	0	*0*	8.2	*12.7*	9.2	***7.1***
MACROLIDES	5.7	*4.6*	2.0	*2.2*	7.0	*21.9*	5.7	***9.6***
Glycopeptides	7.2	*6.1*	5.5	*1.5*	3.0	*3.0*	6.3	***3.4***
Anti-anaerobics	6.0	*2.7*	11	*2.1*	7.4	*9.0*	7.8	***5.6***
COMBINATIONS								
Aminoglycosides-Beta-lactams	0	*0*	0	*0*	5.0	*17.5*	5.0	***3.6***

			UCSC (N:18)		**CCS (N:75)**		**Total (N:173)**	
Antibiotic	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*	Days of therapy (mean)	*Colonized pts per 1000 days of treatment*
CARBAPENEMS-QUINOLONES	4.5	*4.0*	0	*0*	3.7	*37.7*	4.0	***8.4***
Quinolones-Beta-lactams	0	*0*	5.5	*3.0*	0	*0*	5.5	***1.9***

Objective 2 - To study the virulence and fitness of the isolates (i.e. new colonising strains) causing subsequent nosocomial infections

Determination of immune response of clinical isolates of *S. aureus:* Six MRSA isolates from two patients were selected based on the clinical history (hospital acquired pneumonia). All isolates were re-cultured and subjected to bacterial identification by means of Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). All six strains were resistant to methicillin, and all other beta-lactams, fluorochinolones, gentamicin, rifampicin, and tetracycline. Two strains were also erythromycin and clindamycin resistant. There were no other differences between the isolates of the same lineage. All six clinical isolates were grown in poor medium (RPMI) and rich medium (MH medium) for 20 h (RPMI) and 23 h (MH), respectively. All strains grew in a *S. aureus* typical manner with lag-, log-, and stationary phase. The difference between the strains during the first 10 h in RPMI were marginal, later five out of the six strains grew with an almost identical doubling time, only one strain showed reduced growth rate during stationary phase. In MH medium all six strains showed no significant difference in growth rates. Virulence of clinical strains was investigated in an infection model using larvae of the wax moth *Galleria mellonella*. The larvae were infected with three different doses of each strain $5x10^6$, $1x10^7$, and $5x10^7$. Infection of *S. aureus* was performed by inoculation of 25 µl of the respective dose into the 9th segment of larvae followed by incubation at 37°C for 48 h. PBS injected larvae served as control. The highest dose of $5x10^7$ CFU led to death of all larvae within 24 h, medium dose of $1x10^7$ CFU resulted in death of 80-90% of all larvae within 48 h and the lowest dose of $5x10^6$ CFU resulted in death of 40-70% of larvae. There was no significant difference in virulence between the strains indicating no major changes in expression of virulence mechanisms during the colonization period of the selected clinical strains. SNP analysis revealed that the strains from the patients show very high homology with only a few single SNP. These alterations were found in genes mostly belonging to general metabolic functions.

Identification and analysis of E. coli virulence and fitness factors based on in vitro studies and an animal model of infection: Four ESBL-producing *E. coli* pairs of isolates representing one “colonizing” fecal isolate and one “infecting” isolate from the blood or urine of the patients were prepared for competitive growth experiments. Due to their already multi-resistant phenotype, the use of antibiotic resistance in order to distinguish between both isolates of the individual strain pairs was difficult. Finally, a zeocin resistance cassette derived from pEM7/Zeo (Life Technologies) was amplified and inserted via recombineering into the bacterial chromosome.

Objective 3 - To determine genotypic relation between colonising and infecting strain in the same patient and patients' and hospital staff colonising strains

All patients entering the cohort study were followed for a 30-day period. Among the 173 patients who became colonized with MRSA after starting antibiotic and being followed-up in the cohort, 10 developed hospital acquired infections. Five were respiratory tract infections, four wound infections and one, skin and soft tissue infection. Infections were diagnosed on average 13.8 days (range 7-30 days) after starting antibiotic treatment. Mean age of patients was 54.5 years (SD, 18.5 years), BMI was 26.7 (SD, 3.5). Median length of hospital stay was 30.2 days (SD, 10.8). Six of the patients reported underlying conditions: cardiovascular diseases in four patients and diabetes in the remaining two. One patient had a history of previous hospitalization, one of home nursing care; two were bedridden, four had no control of bowel movements. Two patients had open wounds, one had a central venous catheter and one a urinary catheter. None had a history of antibiotic treatment in the month preceding hospitalization and none were admitted on antibiotics. Phenotypically the colonising strains (17 strains) and infecting strains (11 strains) were the same. By MLVA all these 10 patient harbored strains were identified as clonal isolates ST239, characterized by multiple antibiotic resistances and highly associated with the staphylococcal chromosomal cassette *mec* (SSC*mec*) type III genetic element. Among the 976 patients who became colonized with ESBL five developed a hospital acquired infection in the 30-day follow up, namely a bacteraemia and 4 urinary tract infections. Infections were diagnosed on average 16.9 days (range 9-30 days) after starting antibiotic treatment. Mean age of patients was 59.5 years (SD, 17.1 years). Median length of hospital stay was 24 days (SD, 6.1). Underlying conditions were neoplastic diseases in three patients, HIV and cirrhosis in one patient, respectively. Four patients had a history of previous hospitalization. None had a history of antibiotic treatment in the month preceding hospitalization and none were admitted on antibiotics. Phenotypically and by PFGE the colonising and infecting strains (5 strains) were the same.

Objective 4 - To predict the risk for nosocomial acquiring of the target bacteria after a single treatment therapy adjusted by length of hospitalization, patients comorbidities, BMI, and ward colonisation pressure

This analysis was performed through the Support Vector Machine Analysis. The average AUC for predicting ESBL positivity in patients negative at hospital admission including all epidemiological variables and antibiotics treatments was 0.73, while for predicting MRSA positivity it was 0.59, The highest coefficients for acquiring ESBL strains were observed for the following combinations: carbapenems and macrolides (12.56), linezolid and quinolones (11.00), penicillins and quinolones (8.80), metronidazole and piperacillin (7.10). Linezolid was the antibiotic used as monotherapy associated with the highest risk of developing ESBL colonization (5.79), followed by amynoglycosides (5.06). The SVM also showed that the risk was also associated to the sequential use of the drugs. In particular, glicopeptydes following piperacillin (3.42) was associated to the highest risk.

The highest coefficient for risk of acquiring MRSA strains was observed for the combination of a cephalosporin and piperacillin (2.59) followed by the following sequential therapies: cephalosporins after daptomycin (2.26), metronidazole after cotrimoxazole (2), penicillins after aminoglycosides (2), macrolides after penicillins or linezolid.

Conclusion

This is the largest prospective study designed to assess the acquisition of antibiotic-resistant microorganisms (MRSA and ESBL-producing gram negative) in hospitalized patients on antibiotics. This is also the first study in the field of nosocomial acquired infections, in which supervised learning models (support vector machines) were used, associated with learning algorithms to analyze data and recognize patterns. Overall, including more than 10000 patients with more than 55000 samples, the study shows that 4.2% of patients were newly colonized by MRSA and 23.5% by ESBL-producing gram negative after starting antibiotic therapy. The rate of acquisition is still significantly higher also after adjusting for colonisation pressure and rate of acquisition in patients not undergoing antibiotic therapy. The combinations of antibiotics most associated with the acquisition of ESBL were carbapenems and macrolides, linezolid and quinolones while cephalosporin and piperacillin significantly increased the risk for MRSA development. These results clearly underline that the new acquisition of colonisation due to MDR bacteria should be considered as a common (according to the WHO definition) adverse event of antibiotic therapy. The information about the risk related to single, combination and sequential therapy has also an immediate clinical application in selecting therapies with the lowest risk to produce resistance according to patients´ comorbidities, duration of hospitalisation and previous antibiotic therapy (free app almost completed to be available on the SATURN website). Importantly the study also provides microbiological and molecular evidence that the infections in patients developing new colonisation after antibiotic therapies are the same of those responsible for infections in the same patienst. These data could be used to design new studies with interventions (i.e. screening or colonisation) that could prevent the development of infections in high risk hospitalised patients.

3.4 WP5 – Observational AMR carrier study

Scientific approach of the study / the procedures, methods used

The overall objective of this study was to examine the effect of various antibiotics on selection and amplification of antimicrobial resistant organisms and resistance genes among patients carrying extended spectrum beta-lactamases (ESBL) and Carbapenem-resistant Enterobacteriaceae (CRE) at hospital admission. The trial included ESBL and CRE carriers in 4 centers (UCSC-Italy, CCS-Serbia, IDMB-Romania and TASMC-Israel) where AMR is a prevalent problem.

The clinical study and data collection was carried out over 3 years and were successfully completed in all centers. All strains and samples (3722 samples were taken for 639 informative patients), were shipped to the laboratory analysis centers in Tel-Aviv (CRE) and Antwerp (ESBL).

Data were examined and informative patients and samples were selected based on antimicrobial exposure. The lists generated were sent to the above laboratories for work-up of samples and strains.

New methodologies were developed and validated to facilitate the performance of this study; using rectal swab for quantitative sampling of carriage of resistant organisms and quantification of ESBL and KPC-producing Enterobacteriaceae from stool and rectal swabs have been established and validated.

At this point, Quantification of KPC and ESBL-producing Enterobacteriaceae from informative patients collected in the clinical study is being completed. Also, the molecular data is analyzed.

Summary of the main steps/phases of the study

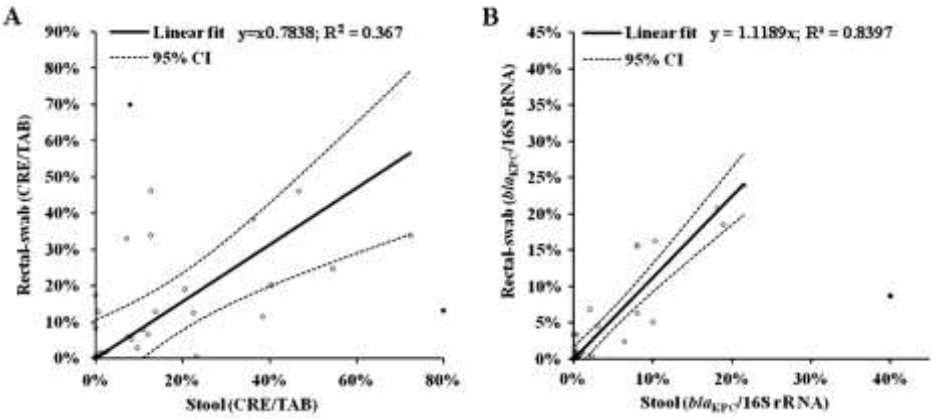
Preparations for the clinical study occurred during 2010 and included: 1) creating a Combined Clinical and Microbiological Protocol for WP4&WP5; 2) developing detailed data collection forms; 3) developing a website and electronic CRF for both WP4 and WP5; 4) developing a template IRB application that all centres could adapt according to the requirement of their local ethical committees; 5) organizing training visits at at CCS and IDMB together with WP4 (UCSC).

The clinical study began enrolling patients in 2011 and ended in 2013. Processes for the clinical study include patient recruitment, identification of carriers, clinical data collection and microbiology sample collection. A total of 693 informative patients were enrolled and 3722 samples were taken.

Main scientific results

We developed methodology to detect and quantify resistance organisms and genes using rectal swabs. We focused on the relative concentration of resistant organisms and resistance genes as compared to other gut flora. These methods assist and ease the study of resistance in large clinical and epidemiological studies. We found that molecular analyses are more reliable than culture based methods.

We used qPCR and culture-based method to compare two sampling methods, rectal swabs versus stool samples from 37 KPC-producing CRE known carriers. In the culture-based method, the CRE/total aerobic becteria showed positive linear correlation between stool samples and rectal swabs (r^2 = 0.367, P < 0.0002). A higher correlation was observed between the bla_{KPC}/16S rRNA genes (r^2 = 0.8397, P < 0.0001).



Rectal swabs are suitable for quantifying the carriage load of KPC-producing carbapenem-resistant Enterobacteriaceae (CRE). A. Lerner, J. Romano, I. Chmelnitsky, S. Navon-Venezia, R. Edgar and Y. Carmeli. Antimicrobial Agents and Chemotherapy, 2013 Mar;57(3):1474-9.

Three agar-based methods for direct CRE detection from rectal swabs were compared: CHROMagar-KPC (Chrom); MacConkey agar with imipenem at 1 µg/ml (MacI); and MacConkey plates with imipenem, meropenem, and ertapenem disks (MacD). First, we

compared the levels of detection (LODs) of 10 molecularly characterized carbapenemase-producing Enterobacteriaceae strains by the three methods. Second, we compared their performance in a surveillance study using rectal swabs (n = 139). The LODs of carbapenemase-producing Enterobacteriaceae strains were influenced by their MICs to carbapenems and were best for MacI, followed by Chrom. In the surveillance study, both Chrom and MacI had greater sensitivity (85%) than MacD (76%).

Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant Enterobacteriaceae from surveillance rectal swabs. Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y. J Clin Microbiol. 2011 Jun;49(6):2239-42.

We analyzed and described the clonal dissemination and success of various carbapenem resistant enterobacteraiceae. We established the important role of KPC producing klebsiella pneumoniae epidemic clone (ST258) examined published on its genome and plasmids and on its fitness and virulence.

The *bla*$_{KPC-3}$-carrying plasmid, pKpQIL, harbored by the carbapenem-resistant *Klebsiella pneumoniae* ST258 in Israel is a 113,637-bp, self-transmissible plasmid that belongs to the incompatibility group IncFII. It consists of a large backbone of a pKPN4-like plasmid and carries the *bla*$_{KPC-3}$-containing Tn*4401*a transposon of a pNYC-like plasmid.

Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic Klebsiella pneumoniae sequence type 258. Leavitt A, Chmelnitsky I, Carmeli Y, Navon-Venezia S. Antimicrob Agents Chemother. 2010 Oct;54(10):4493-6.

We reported the draft genome sequence of the *K. pneumoniae* ST258 XDR clinical strain from Israel.

Draft Genome Sequence of an Extremely Drug-Resistant KPC-Producing Klebsiella pneumoniae ST258 Epidemic Strain. Chmelnitsky I, Doniger T, Shklyar M, Naparstek L, Banin E, Edgar R, Carmeli Y. J Bacteriol. 2012 Nov;194(21):6014.

We demonstrated that carbapenem-susceptible *E. coli* strain isolated from a patient has acquired the main KPC3 carrying plasmid pKpQIL from *K. pneumoniae* in the patient's gut under antibiotic pressure.

Transfer of carbapenem-resistant plasmid from Klebsiella pneumoniae ST258 to Escherichia coli in patient. Goren MG, Carmeli Y, Schwaber MJ, Chmelnitsky I, Schechner V, Navon-Venezia S. Emerg Infect Dis. 2010 Jun;16(6):1014-7.

We detected 9 ertapenem-resistant, carbapenemase-negative *K. pneumoniae* (ERCNKP) belonged to ST-258. These strains carried the *bla*$_{CTX-M-2}$ or the *bla*$_{CTX-M-25}$ ESBL gene. Plasmid analysis of these isolates showed absence of the Tn*4401* transposon and the pKpQIL plasmid. Our results suggest that ERCNKP ST-258 evolved by loss of the *bla*$_{KPC}$-carrying plasmid pKpQIL. ERCNKP ST-258 appears to have low epidemic potential.

A Swordless Knight: Epidemiology and Molecular Characteristics of the blaKPC-Negative Sequence Type 258 Klebsiella pneumoniae Clone. Adler A, Paikin S, Sterlin Y, Glick J, Edgar R, Aronov R, Schwaber MJ, Carmeli Y. J Clin Microbiol. 2012 Oct;50(10):3180-5. Epub 2012 Jul 18.

We described the molecular epidemiology of Carbapenem-resistant *Escherichia coli*. The strains were multidrug resistant, all carried *bla*$_{KPC-2}$, and six of them were also ESBL producers. Six genetic clones were detected; within the same clone, similar transferable *bla*$_{KPC-2}$-containing plasmids were found. Plasmids differed between clones, however, all had similar backbone containing Tn*4401* elements.

Carbapenem-resistant KPC-2-producing Escherichia coli in a Tel Aviv Medical Center, 2005 to 2008. Goren MG, Navon-Venezia S, Chmelnitsky I, Carmeli Y. Antimicrob Agents Chemother. 2010 Jun;54(6):2687-91.

We described carbapenem-resistant KPC-2-producing *K. pneumoniae*. Isolates varied in their additional beta-lactamase contents and belonged to three different sequence types: ST340, ST277, and a novel sequence type, ST376. The KPC-2-encoding plasmids varied in size and differed among each of the STs. Two of the *Klebsiella bla*$_{KPC-2}$-carrying plasmids were identical to plasmids from Escherichia coli, suggesting a common origin of these plasmids.

Molecular epidemiology, sequence types, and plasmid analyses of KPC-producing Klebsiella pneumoniae strains in Israel. Leavitt A, Carmeli Y, Chmelnitsky I, Goren MG, Ofek I, Navon-Venezia S. Antimicrob Agents Chemother. 2010 Jul;54(7):3002-6.

We found reduced susceptibility to chlorhexidine among ST258 carbapanemase-producing isolates. 99% of ST258 isolates had MICs >32 µg/mL, compared with 52% of other *K. pneumoniae* sequence types ($P < 0.0001$). Reduced susceptibility to chlorhexidine appeared to be independent of the expression of *cepA*, *acrA* and *kdeA* efflux pumps.

Reduced susceptibility to chlorhexidine among extremely-drug-resistant strains of Klebsiella pneumoniae. Naparstek L, Carmeli Y, Chmelnitsky I, Banin E, Navon-Venezia S. J Hosp Infect. 2012 May;81(1):15-9.

Suppressive subtractive hybridization (SSH) library yielded 42 fragments (50 proteins) specific to the ST258 isolate tested, 30 of them located on various plasmids. The ST258 strains examined could be divided into two groups, one in which all 50 genes were ubiquitous and another group that lost 11 fragments, all located on one of the plasmids. This group of 50 genes was absent among other STs tested. Nineteen genes were unique to ST258 strains and 17 to CC258 (where CC stands for clonal complex). Most of the deduced proteins belonged to two major functional groups: 15 to the cell motility and secretion group, and 14 to the DNA repair and modification group.

Unique genes identified in the epidemic extremely drug-resistant KPC-producing Klebsiella pneumoniae sequence type 258. Chmelnitsky I, Shklyar M, Hermesh O, Navon-Venezia S, Edgar R, Carmeli Y. J Antimicrob Chemother. 2013 Jan;68(1):74-83.

We performed comparative sequence analysis of 3 *bla*$_{KPC-2}$ encoding plasmids to examine evolution of these plasmids and their dissemination. We found that all of them have an IncN replicon with a newly determined IncN plasmid sequence type (ST), ST15. The 2 *Klebsiella pneumoniae* (KPN) plasmids also harbor an IncF2A1-B1- replicon. The *bla*$_{KPC-2}$ is located in the Tn*4401*c transposon with a newly discovered mutation in the P2 promoter. In addition to the *bla*$_{KPC-2}$ gene, all 3 plasmids carry at least 1 additional resistance gene. Screening of the 27 additional *bla*$_{KPC-2}$ carrying plasmids from *Enterobacter cloacae*, *Escherichia coli* (EC), and *K. pneumoniae* showed that: all KPN and EC plasmids are IncN plasmids belonging to

ST15; 4/7 KPN and 1/6 EC plasmids contain an additional IncF2A1-B1- replicon; all *Enterobacter* plasmids belong to neither IncN nor IncF2A1-B1- replicon plasmids; 6/7 KPN and 2/5 EC plasmids carry the mutated P2 promoter. Study of the bla_{KPC-2} environment, transposon, pMLST, and Inc group suggests transposon and plasmid inter- and intra-species dissemination and evolution.

Mix and match of KPC-2 encoding plasmids in Enterobacteriaceae- comparative genomics Inna Chmelnitsky. Chmelnitsky I, Shklyar M, Leavitt A, Sadovsky E, Navon-Venezia S, Ben Dalak M, Edgar R, Carmeli Y. Diagn Microbiol Infect Dis 2014 Jun ;79(2):255-60.

Presumably unique genes of KPC-KP ST-258/512 were examined in a large international collection of strains: KPC-KP isolates (n = 160) that included both ST-258/512 (group A, n = 114) and non–ST-258 (group B, n = 46) strains were collected from the following countries: Greece, 20; Israel, 93; Italy, 19; USA, 25; and Colombia, 3. Group B included 30 different STs from various lineages. The *pilv-I* gene was present in 111/114 of ST-258 isolates, including KPC-negative isolates (97% sensitivity). Using primers for a unique ST-258 *pilv-I* allele resulted in a specificity of 100%. The sensitivity values of *is-66* and *prp* genes for detecting KPC-KP ST-258 were 83 and 89%, and the specificity 67 and 93%, respectively.

Development and validation of a multiplex PCR assay for identification of the epidemic ST-258/512 KPC-producing Klebsiella pneumoniae clone Adler A, Khabra E, Chmelnitsky I, Giakkoupi P, Vatopoulos A, Mathers AJ, Yeh AJ, Sifri CD, De Angelis G, Tacconelli E, Villegas MV, Quinn J, Carmeli Y. Diagn Microbiol Infect Dis 2014 Jan ;78(1):12-5.

We characterized the prevalence of carbapenemase-producing Enterobacteriaceae (CPE) carriage in post-acute-care hospitals (PACH). In the first survey the CPE carriage was 184/1147 (16%); all of the isolates were KPC-KP. The prevalence of CPE carriage in the second survey was 127/1287 (9.9%); of these isolates, 113 (89%) were KPC-KP, 9 (7%) were other KPC-producing species and 5 (4%) were NDM- and OXA-48-producing CPE.

Persistence of Klebsiella pneumoniae ST258 as the predominant clone of carbapenemase-producing Enterobacteriaceae in post-acute-care hospitals in Israel, Adler A, Hussein O, Ben-David D, Masarwa S, Navon-Venezia S, Schwaber MJ, Carmeli Y. J Antimicrob Chemother. 2015 Jan;70(1):89-92.

We described the clonal structure and resistance mechanisms of *Klebsiella pneumoniae* carbapenemase-producing *Escherichia coli* (KPCEC) in a multicenter study. The study included 88 isolates from four medical centres. Twelve (14%) KPCEC were from clinical sites and 86% from surveillance cultures. The clonal structure was studied by PFGE and MLST, and was highly diverse, with 79 and 45 different PFGE types and STs, respectively. The most common clones were ST-131 and ST-410, identified in 21 isolates (23%). Dominant clonal complexes (CCs) were CC131 (n = 16), CC410 (n = 14), CC10 (n = 17), and CC-69 (n = 6). The bla_{KPC-2} and bla_{KPC-3} genes were identified in 68 and 20 isolates, respectively. Sixteen of the 20 bla_{KPC2}-harbouring plasmids studied were of identical type, IncN-pMLST ST-15.

A multicenter study of the clonal structure and resistance mechanism of KPC-producing E. coli isolates in Israel Adler A, Miller-Roll T, Assous MV, Geffen Y, Paikin S, Schwartz D,

Weiner-Well Y, Hussein K, Cohen R, Carmeli Y. Clinical Microbiology and Infection. Clin Microbiol Infect. 2014 Oct 29 : S1198-743X(14)00070-6.

We studied and described the natural history of carriage of CRE, length of carriage and progression to infection

Sixty-six CRE carriers were surveyed for CRE rectal carriage at the next hospital encounter; screen-positive patients (23) were compared with 43 screen-negative control patients; Predictors continued carriage were (1) prior fluoroquinolone use (odds ratio [OR], 4.27), (2) admission from an institution or another hospital (OR, 4.04), and (3) time interval less than or equal to 3 months since the first positive CRE test (OR, 3.59). Among patients with no predictor variables, the likelihood of having a positive screen test at the next hospital encounter was 1/7. If they had at least 1 predictor, the likelihood increased to 1/2.

Predictors of rectal carriage of carbapenem-resistant Enterobacteriaceae (CRE) among patients with known CRE carriage at their next hospital encounter. Schechner V, Kotlovsky T, Tarabeia J, Kazma M, Schwartz D, Navon-Venezia S, Carmeli Y. Infect Control Hosp Epidemiol. 2011 May;32(5):497-503.

We determined who among newly identified CRE rectal carriers is prone to have a subsequent clinical specimen with CRE. A matched case-control study was conducted: cases with a primary positive CRE rectal test and subsequent CRE clinical specimens were matched in a 1:2 ratio with CRE rectal carriers who did not develop subsequent CRE clinical specimens (controls). Matching was based on calendar time of primary CRE isolation, whether the primary CRE isolation was ≤ 48 h or > 48 h after hospital admission, and time at risk to have a subsequent clinical specimen. 132 newly identified CRE rectal carriers (44 cases, 88 controls) were included. The median time interval between screening and subsequent clinical specimens was 11 days (range, 3-27); 86% of the clinical specimens were classified as true infections. Independent predictors of subsequent CRE clinical specimens were: admission to the intensive care unit, having a central venous catheter, receipt of antibiotics, and diabetes mellitus.

Asymptomatic rectal carriage of bla(KPC) producing carbapenem-resistant Enterobacteriaceae: who is prone to become clinically infected? Schechner V, Kotlovsky T, Kazma M, Mishali H, Schwartz D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Clin Microbiol Infect. Clin Microbiol Infect. 2013 May;19(5):451-6.

A cohort of 125 KPC KP carriers was followed and rectal swabs collected monthly for 3 to 6 months after discharge. Acquisition time was regarded as the earliest date of KPC KP isolation. Resolution of carriage was defined as a negative by culture and direct bla_{KPC} PCR test in at least two consecutive samples. Analyses were separated for recent (<4 months) (REC, 75 patients) and remote (≥4 months) (REM, 50 patients) acquisition groups. Forty-six (61%) patients in the REC group and 14 (28%) in the REM group were persistent carriers ($p < 0.001$). A significant risk factor for persistent carriage identified in both the REC and REM groups was the presence of any catheter ($p < 0.05$). Unique risk factor groups included long-term care facility (LTCF) residence ($p < 0.01$) and a low functional status ($p < 0.05$) in the REC group and high Charlson's score in the REM group ($p < 0.05$). Out of 100 with at least one negative sample, only 65 remained negative on subsequent cultures.

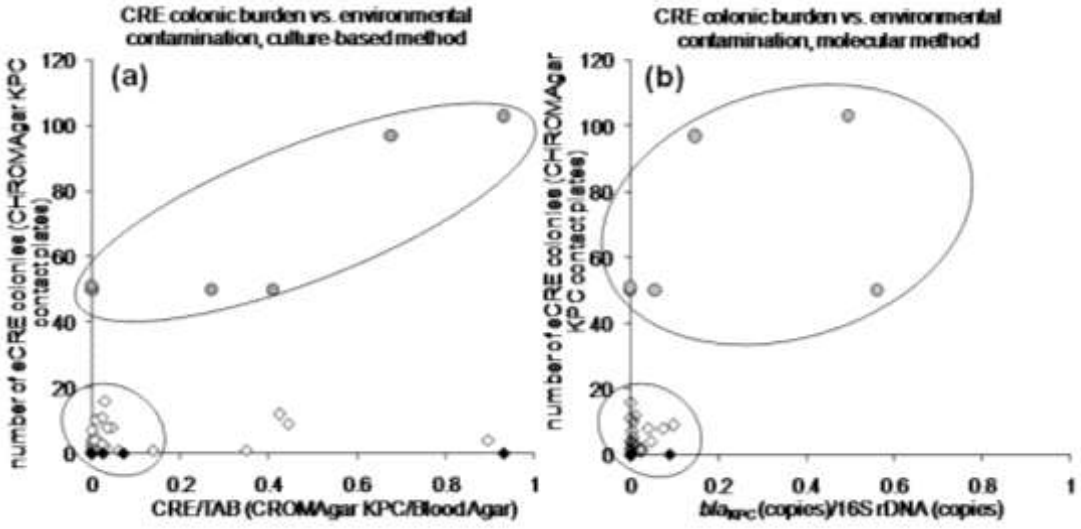
Gastrointestinal Colonization by KPC-Producing Klebsiella pneumoniae Following Hospital Discharge: Duration of Carriage and Risk Factors for Persistent Carriage. Feldman N, Adler A, Molshatzki N, Navon-Venezia S, Khabra E, Cohen D, Carmeli Y. Clin Microbiol Infect. 2013 Apr;19(4):E190-6.

We developed methodologies and established the importance and magnitude of environmental contamination with CRE in the hospital setting and established the relation between gut CRE concentration, antibiotic use, and dissemination.

We examined the extent of CRE contamination in various sites in the immediate surroundings of CRE carriers and evaluated the performance of two sampling methods; 1five out of 14 sites examined were found to be contaminated, were further studied. The environmental contamination decreased with distance from the patient; the bed area was the most contaminated site. The CHROMAgar KPC contact plate method was a more effective technique for detecting environmental CRE than were eSwab-based methods.

Environmental contamination by carbapenem-resistant Enterobacteriaceae (CRE). A Lerner, A Adler, J Abu-Hanna, I Meitus, S Navon-Venezia, Y Carmeli. J Clin Microbiol. 2013 Jan;51(1):177-81.

We studied CRE dissemination by quantifying environmental contamination from the vicinity of 34 carriers using selective contact plates. We examined rectal CRE concentrations (by using culture- and molecular-based methods) and clinical characteristics and correlated these with environmental contamination. Eight (24%) carriers were non-spreaders: no CRE was detected in their vicinity. Faecal continence was the only independent predictor of being a non-spreader. Among the 26 spreaders, we identified a distinct group of six (18%) super-spreaders who accounted for 79% of environmental colonies detected. Super-spreaders were likely to have high rectal CRE concentrations.



Correlation between environmental contamination with CRE and rectal CRE concentration. Rectal CRE concentration was measured from rectal swabs as a. the ratio of CRE to total aerobic bacteria (CRE/TAB) (r^2=0.175) and b. the ratio of KPC-producing bacteria to total flora (bla_{KPC} gene copies/16S rDNA gene copies; r^2=0.351). Grey circle dots represent super-spreaders; white rhombus dots represent low-level spreaders; and black rhombus dots non-spreaders.

Spread of KPC-producing carbapenem-resistant Enterobacteriaceae: the importance of super-spreaders and rectal KPC concentration Lerner A, Adler A, Abu-Hanna J, Cohen Percia S, Kazma Matalon M, Carmeli Y. Clin Microbiol Infect. 2014 Dec 26. S1198-743X(14)00168-2.

We examined and described the emergence and spread of new mechanisms of resistance to carbapenemes and their dissemination by gene, plasmid and clonal spread.

We identified the introduction of *bla*$_{OXA-48}$ gene into Israel, all isolated from non-Israeli patients (two Palestinian, one Jordanian and one Georgian). Three *Escherichia coli* strains belonging to different clonal complexes, one *Klebsiella oxytoca* and one *Klebsiella pneumoniae* sequence type 101; the *bla*$_{OXA-48}$ gene was located inside Tn*1999.2* and was carried on a 60 kb plasmid with an identical RFLP pattern. The plasmid was able to efficiently conjugate from *Klebsiella* spp. to *E. coli*, with a conjugation efficiency up to ~10000 times higher than that of pKpQIL.

Introduction of OXA-48-producing Enterobacteriaceae to Israeli hospitals by medical tourism. Adler A, Shklyar M, Schwaber MJ, Navon-Venezia S, Dhaher Y, Edgar R, Solter E, Benenson S, Masarwa S, Carmeli Y. J Antimicrob Chemother. 2011 Dec;66(12):2763-6.

We described three unusual *Enterobacteriaceae* species carrying the *bla*$_{KPC-2}$ gene: *Leclercia adecarboxylata* (one isolate), *Kluyvera cryocrescens* (2 isolates) and *Kluyvera ascorbata* (one isolate). The *bla*$_{KPC-2}$ gene was located on the Tn*4401* transposon and was typed by sequencing as Tn*4401c*. The plasmid incompatibility group (Inc) was identified as IncN.

Detection of the Plasmid-Mediated KPC-2 Carbapenem-Hydrolyzing Enzyme in Three Unusual Species of the Enterobacteriaceae Family in Israel. Geffen Y, Adler A, Paikin S, Khabra E, Gorenshtein S, Aronov R, Carmeli Y. J Antimicrob Chemother. 2013 Mar;68(3):719-20 .

We described an OXA-48-producing Enterobacteriaceae (OPE) outbreak in a neonatal intensive care unit (NICU). During the peak of the outbreak (January to August 2012), there were 49 patients who had proven or suspected acquisition of OPE in the NICU, including 16 with invasive infections, out of a total of 156 patients who were hospitalized during that period. Intervention included cohorting colonized patients, conducting frequent rectal-culture surveillance, and improving the implementation of infection control practices. As a result, the incidence of OPE acquisition declined to 5 cases in the first 4 months, followed by no new cases in the next 3 months. 31 patient-unique isolates were available for analysis: 29 *Klebsiella pneumoniae* isolates, all belonging to a single clone (sequence type 39), and 2 isolates from *Enterobacter cloacae*. All isolates possessed the *bla*$_{OXA-48}$ and *bla*$_{CTX-M-14}$ genes, which are located on the same plasmid. This plasmid, similar to the global *bla*$_{OXA-48}$-harboring vector, has now acquired *bla*$_{CTX-M-14}$, leading to resistance to all β-lactam agents.

Epidemiological and microbiological characteristics of an outbreak 1 caused by OXA-48-producing Enterobacteriaceae in a neonatal 2 intensive care unit in Jerusalem Israel. Adler A, Solter E, Masarwa S, Miller-Roll T, Abu-Libdeh B, Khammash H, Najem K, Dekadek S, Stein-Zamir C, Nubani N, Kunbar A, Assous MV, Carmeli Y, Schwaber MJ. J Clin Microbiol. 2013 Sep;51(9):2926-30.

Five VIM producing Carbapenem-resistant Aeromonas *caviae* belonging to four different pulsotypes were identified. The carbapenemase genes bla_{VIM-1} and bla_{VIM-35} were located inside a class I integron with two different sizes to its variable region. The 4650 bp integron included an IS*Pa21*-type transposase gene and was almost identical to a bla_{VIM}-harbouring integron that was identified in *Enterobacter cloacae* isolated in the United Arab Emirates.

Emergence of VIM-producing Aeromonas caviae in Israeli hospitals Adler A, Assous MV, Paikin S, Shulman A, Miller-Roll T, Hillel S, Aronov R, Carmeli Y, Schwaber MJ J Antimicrob Chemother. 2014 May;69(5):1211-4.

3.5 WP1 – Bacterial Genetics and Functional Studies

Scientific approach of the study / the procedures, methods used

The major objective of WP1 was to carry out microbiologic analysis of samples/strains collected during the community- or hospital-based clinical trials (WPs 2-5). These studies aimed to quantify the resistance burden of ESBL- or carbapenemase-harbouring or fluoroquinlone-resistant Enterobacteriaceae, and of viridans streptococci in stool samples/rectal swabs and oropharyngeal samples prior to antibiotic treatments and follow their dynamics under and after antibiotic treament. These studies utilized both phenotypic and genotypic méthods to realise this aim, and these results are presented in the clinical WP summaries.

WP1 also aimed to study known and novel/emerging mechanisms of resistance in the bacterial pathogens enumerated above. Except for basic screening on culture, these studies utilized state-of-the-art molecular techniques.

Finally the third major aim of WP1 was to study clonality, fitness costs of resistance gene carriage and virulence using comparative genomics, genome-wide arrays, high-throughput sequencing, in vitro competitive growth experiments, and mice models of infection

Summary of the main steps/phases of the study

Development of laboratory manuals, microbiologic support for trial setup occurred during 2010. Setting up of assays was done during 2010-2011. Sample analysis extended from 2011-2014 for WP1 partners.

Main scientific results

For objective 1, a large bulk of WP1 work consisted of microbiological and molecular analysis of the specimens and strains by WP1 partners for Wps 2-5 and these results are presented in the relevant clinical trial WP reports. Next to the microbiological support, an ongoing objective of WP1 partners consisted of setting up techniques for the microbiological analysis of SATURN samples/strains. UA optimized and developed detailed protocols and kits for sample acquisition, microbiological work-up at clinical sites, storage and shipment in specific transport/storage media at -80C. These were tailored to the respective clinical trials and are described therein. UA also set up techniques for phenotypic and genotypic quantification of ESBL-harbouring *E. coli* & *Klebsiella spp.* and FQ-resistant *E. coli*, and identification of resistance mechanisms (CTX-M, TEM, SHV, Qnr, and others) and determination of clonality and virulence factors. UA also analysed rectal swab samples for WP5, using the quantitative real-time PCR (qPCR) of a target gene (CTX-M) and marker for total bacterial (16SrDNA) quantification. TASMC performed a

molecular and bacteriological comparison between stools vs. rectal swabs. to answer whether rectal swab sampling could be considered to be a good representation of a stool sample. This limited study concluded that that rectal swabs are as efficient and accurate as stool samples in detecting CRE using both culture-based and qPCR methods. TASMC established a methodology for quantifying resistant Enterobacteriaceae from environmental samples and carbapenem resistance genes from swabs and from environmental specimens. TASMC and UA optimized qPCR primer-probe sets for the quantification of CRE and gut bacteria that were later utilized to screen the WP5 trial samples. TASMC also aimed to understand the role of the importance of super-spreaders and rectal KPC concentration in mediating the spread of KPC-producing CRE. Super-spreaders were likely to have high rectal CRE concentrations and to have been admitted with respiratory disease. CRE spread to the environment followed the 20/80 rule: 20% of carriers are responsible for 80% of shedding and may play a central role in CRE transmission. Finally, UA In collaboration with UCSC (WP4) and UniGe, selected patient cohorts based on MRSA acquisitions, infection with MRSA, and specific antibiotic combinations that might select for MRSA acquisitions. These strains were earmarked for studying virulence and clonality using whole genome sequencing (Illumina), and this work is being finalized.

For objective 2, molecular analysis done by WP1 partners dissected some of the resistance mechanisms emerging under pressure of specific antibiotic groups. Firstly at UniGe, MRSA collected as part of WP4/WP5 in Italy, Romania and Serbia were typed using High Throughput Multiple Locus Variable Number of Tandem Repeat Analysis (MLVA), multilocus sequence typing (MLST) and characterization of important molecular markers (toxin, agr, SCCmec cassette typing). These molecular tests allowed identifying the main clonotypes responsible for MRSA carriage and infection in the participating institutions and countries. Briefly, of the 595 MRSA screened, strains were identified as ST1, ST239, ST45, ST22, ST8, ST228, ST80, ST111, ST152, ST7 and ST5, belonging to CC1, CC8, CC45, CC22, CC8, CC5, CC111 CC7, and CC5 , respectively. In addition, as only a very limited number of MRSA clones, belonging to five clonal complexes, are responsible for the majority of MRSA infections worldwide, UA studied virulence determinants influencing biofilm formation in a highly prolific biofilm-forming *S. aureus* USA300 skin abscess isolate (ST80 belonging to CC80, see objective 3). IDIBAPS optimized a RT-PCR procedure for efflux pumps expression in both Pseudomonas aeruginosa and Acinetobacter baumannii that was utilized on strains from WP2. IDIBAPS also used isolates collected within the SATURN project to evaluate a loop-mediated isothermal amplification-based technology for the detection of carbapenemase-carriage among clinical isolates of *Acinetobacter* spp.

ESBL-harbouring *E. coli,* and *Klebsiella spp*. (ESBL-EN) selected from stool samples from WP3 that were plated on chromogenic media with antibiotic selections were studied further by UA. ESBL-EN were mostly resistant to amoxicillin (100%), piperacillin (99.6%), cefotaxime (92.4%) and trimethoprim-sulfamethoxazole (57.9%). Also, 36.7% and 24.4% of the ESBL-EN showed coresistance to ciprofloxacin and gentamicin. For each ESBL-EN positive stool sample, strains with unique susceptibility patterns were selected for detection of β-lactamase genes $bla_{CTX\text{-}M}$, bla_{SHV}, and bla_{TEM} by PCR. Most isolates (87.2%) were positive for CTX-M with CTX-M-15-like subtypes being the most prevalent (47.4%), followed by the CTX-M-1-like subtypes (28.4%). Among the SHV subtypes, the most prevalent one was SHV-12 (10.1%), whereas among the TEM subtypes, the most prevalent one was TEM-104 (33.0%).

Patients included in the WP3 study with a lower or upper uncomplicated or complicated UTI and treated with ciprofloxacin were also studied further for quantification and identification of fluoroquinolone resistant viridans streptococci in the oropharyngeal flora by APHP. The aim was to evaluate the impact of fluoroquinolone treatments on the oral flora taking as a marker viridans streptococcal species. All colonies picked up from patients and controls were identified by Maldi-Tof. *S.mitis* was identified as the predominant streptococcal species. Concerning resistance mechanisms to fluoroquinolones, APHP identified chromosomal mutations and very interstingly, found evidence of a high rate of antibiotic efflux. As this mechanism has not been explored in *S. mitis*, APHP carried out molecular studies that showed that an increase production of an analog of PatA/B (ABC transporter) was present associated with high efflux. Taking pairs of strains (wild-type and resistant strains selected during treatment) APHP obtained their complete genome sequences and carried out bioinformatics analysis to identify SNPs associated with gene overexpression.

For objective 3, UW set up in vitro systems, RNASeq, and animal models to study competitive fitness of S. aureus MRSA vs. MSSA, and growth systems of E. coli under clinical relevant in vitro conditions e.g. urine. Based on strain collections delivered by partner UCSC in WP4 and first characterization by partner UniGe, six MRSA were characterized regarding fitness applying genome, transcriptome, growth, and virulence analysis. In particular, three consecutive isolates from two patients gained within a three month period of hospitalization were compared by whole genome sequencing, RNAseq, biofilm formation, and virulence in a *Galleria* infection model and two mouse models including application of in vivo imaging techniques. All strains express multiple antibiotic resistances such as beta-lactam resistance, fluoroquinolone, aminoglycoside, and rifampicin resistance. There was no difference in growth behaviour, biofilm formation, hemolysis, and virulence of selected strains. Whole genome sequencing revealed accumulation of some single nucleotide polymorphisms (SNPs) in housekeeping genes such as oxidoreductases or fitness-associated genes such as ABC transporters. Moreover, RNAseq indicated differential gene expression e.g. in purine biosynthesis and citrate cycle genes. However, no common pattern could be identified probably due to the limited number of consecutive isolates and the short period between isolation time points. These results suggested that short term evolution of MRSA is not a linear process leading to a common pattern of events in a time-dependent fashion. The differential fitness gains, together with the turnover rate and the mutation rate, strongly affect the success of antibacterial treatment, reversibility, and long-term abundance of resistant strains.

UA identified and analyze *S. aureus* virulence factors with respect to epidemiology and biofilm formation, based on *in vitro* studies using optimized continuous shear-flow and static biofilm models as well as powerful comparative genomic typing techniques such as whole genome mapping and (OpGen, Gaithersburg, USA) (Figure 1) and whole genome sequencing (MiSeq, Illumina). Interestingly, among strains collected at the three sites, a majority belonged to ST239, which is the most widely disseminated hospital-associated clone in Asia, and harbours a novel surface-anchored virulence factor, *sasX* (SATW20_21850), which is absent from all other known *S. aureus* genomes. UA studied whether the *sasX* gene has spread towards Europe, both in ST239 and other STs belonging to the same or different clonal complex. Preliminary results show a restriction of *sasX* to solely ST239 in Europe. Remarkably, all tested *sasX*-positive ST types formed significantly more abundant biofilms than those not harbouring *sasX*, confirming its identification as important factor linked to virulence and colonisation.

UW and UA selected four ESBL-producing *E. coli* pairs of isolates representing one "colonizing" fecal isolate and one "infecting" isolate from the blood or urine of the patients were prepared for competitive growth experiments. Due to their already multi-resistant phenotype, the use of antibiotic resistance in order to distinguish between both isolates of the individual strain pairs was difficult. Finally, a zeocin resistance cassette derived from pEM7/Zeo (Life Technologies) was amplified and inserted via recombineering into the bacterial chromosome. Again, the multi-resistance of the isolates complicated the genetic engineering process. Competition experiments were performed.

IDIBAPS carried out studies on clonality and resistance mechanisms in Acinetobacter spp. and impact of the latter on the potential virulence of Acinetobacter spp. in a Caenorrhabditis elegans infection model.

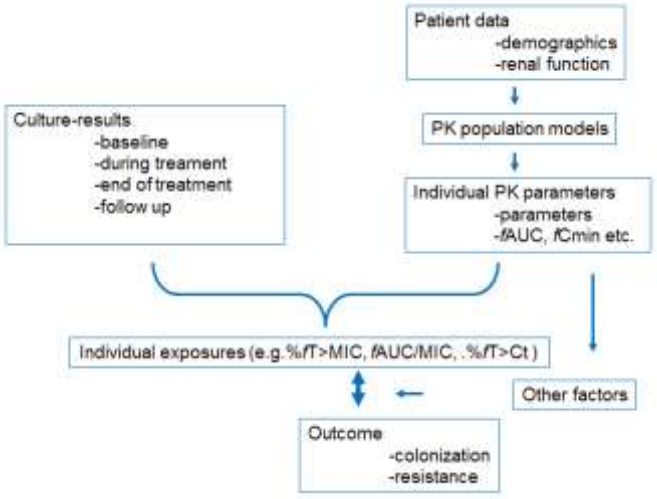
Conclusion

WP1 translational studies provided unprecedented knowledge on pathogen and antibiotic resistance genes dynamics at baseline and under pressure of various antibiotics both in the European community and in hospitalized patients. We also identified identify antibiotic efflux to be a major mechanism selected under ciprofloxacin treatment in vivo in the oropharyngeal flora. Molecular studies identified the overexpressed efflux pump as a novel ABC transporter in S. mitis strains. Studies on MRSA, ESBL-carbapanenemase-harboring, and fluoroquinolone-resistant Enterobacteriaceae, and MDR Acinetobacter and Pseudomonas spp. mapped important clones in European hospitals and fundamental work on these strains identified important genes involved in virulence, biofilm formation, and biological fitness which are important determinant of their pathogenetic success. Such a bedside-bench-bedside approach have made the WP1 studies truly translational, and in the long term likely to impact both antibiotic use guidelines and hospital policies on breaking transmission routes of these important pathogens.

3.6 WP6 – Pharmacodynamics

Scientific approach of the study / the procedures, methods used

In each of the 4 clinical studies, patient data were collected as complete as possible that were required to estimate exposure using demographic characteristics, including age, weight and creatinin. A diagram showing the analysis is shown in the figure below:



Strains were collected and susceptibility determined, as well as mechanisms of resistance in WP1. For selected strains, MICs were to be determined for a number of antimicrobials to further quantify effects. A computer program was to be developed that could handle covariates and estimate exposures in individual patients using pharmacokinetic parameter estimates, in particular the time the concentrations of the antimicrobial remained above the MIC (Minimum Inhibitory Concentration) of the micro-organism, or other specified threshold concentration (Ct). Population models from the literature where screened and evaluated for their use to estimate pharmacokinetic parameters of individuals, if not available they were developed. The databases of the clinical trials were, at the end of each trial, merged, rebuild and validated. The exposures of selected antibiotics – those of which enough patients had received the drug to warrant further analysis – were subsequently calculated using demographics, information on dosing regimens received, the population models available and the program developed to calculate individual exposures. Finally, the relationship between exposure and colonization and emergence of resistance was evaluated using (multiple) logistic regression analysis and classification and regression tree (CART) analysis.

Summary of the main steps/phases of the study

(1) ensure data collection (2) inventorize population pharmacokinetic models and where appropriate build these models of specified antimicrobials (3) build software and validate software that allowed the calculation of individual exposures of large numbers of patients (4) explore exposure response relationships.

Main scientific results

In order to determine the individual exposure in each patient, a program needed to be developed that could simulate concentration-time profiles of individual patients using pharmacokinetic parameter estimates, either obtained from general models or, preferably, from population pharmacokinetic models and covariates. In addition, the program should be able to calculate pharmacodynamics indices for a large number of patients on an individual basis without the cumbersome need to re-enter data for each individual run – the way most, if not all, programs run at present. The program developed fulfilled these requirements. It is based on Excel and a library of subroutines. It allows the user to enter individual patient primary pharmacokinetic parameters as well as the MIC of the relevant micro-organism or Ct value. It then automatically calculates the secondary pharmacokinetic parameters and the pharmacodynamics indices (PDI). Among the PDIs implemented in the program are *f*AUC/MIC, *f*Cmax/MIC and %*f*T>MIC. The program was validated and used to determine pharmacodynamics indices in a population of intensive care (ICU) patients and in a population with community acquired pneumonia (CAP), for ceftazidime (Muller, Punt et al. 2013), ceftobiprole (Muller, Punt et al. 2014) and ceftriaxone (Muller, Punt et al. Manuscript in preparation). Figure 1 shows an example of an exposure response relationship with ceftazidime based on the output of the program, the relationship between %*f*T>MIC and clinical cure using logistic regression.

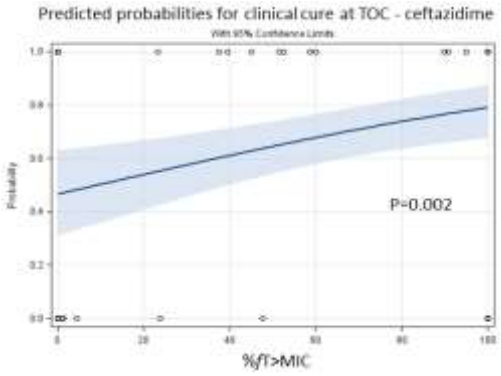


Figure 2

The clinical studies most suited to perform exposure response (ER) analysis were WP4 and WP5 and WP4 in particular because of the large number of patients (close to 10000) involved. Response was defined as becoming colonized with an ESBL. Since there were a variety of antimicrobials and regimens employed by the different centres, the number of patients in each group was large enough to determine ER. The complexity of the original databases – one of the reasons being the amount of data collected for each patient related to infection, such as positive cultures, more than one micro-organism per positive culture, different resistant mechanisms and susceptibility testing results meant that specific algorithms needed to be developed and the final database to analyze results validated. During the process of analyzing results this was continued. From the final database, ER initially focused on those drugs with more 100 patients i.e. ceftriaxone (N=975), amoxicillin/lavulanic acid (N=263), ciprofloxacin (N=228), levofloxacin (N=208). In the initial analysis, to maintain the number of patients in each group, MIC breakpoints of EUCAST were chosen a surrogate target values. All four antibiotics showed significant correlations between exposure and ESBL colonization. For ceftriaxone, multivariate regression analysis that the fT>MIC ($p<0.0001$), fAUC/MIC ($p=0.039$) and fCmin/MIC ($p=0.007$) were all independent risk factors whereas ward was also found to be significant contributing factor of colonization ($p<0.0001$) using an MIC of 4 mg/L. Subsequent CART analysis showed that the ESBL colonization rate was significantly higher between 75% and 99% and there by resembled an inverted U-shape related to the %*f*T>4 mg/L for ceftriaxone.

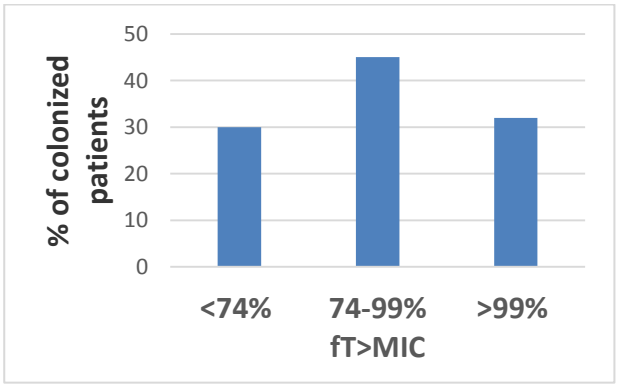


Figure 3.

The cut-off values for fAUC/MICs was 34.9, with larger values having a lower rate, 22% versus 34%. Of the patients that became colonized there was also a strong association between exposure and whether or not the micro-organism was susceptible or resistant. Similar associations were found for the two quinolones ciprofloxacin and levofloxacin. For

both antimicrobials, there appeared to be an association between exposure and degree of ESBL colonization. Interestingly, in the ciprofloxacin group the colonization ratio was significantly higher than in the levofloxacin group, 25% versus 13%. For both drugs, there was a correlation with *f*AUC/MIC, but it differed for each drug in the initial analyses, with a lower exposure resulting in a higher colonization rate of 63% versus 23% – but very small numbers in the low exposure group – and for levofloxacin the opposite was found. This may relate to the dosing regimens as levofloxacin is given once a day and ciprofloxacin twice a day in most cases. Currently, these data are analyzed more in depth. For amoxicillin-clavulanic acid, higher exposures resulted in a slightly but significantly elevated colonization rate.

It is concluded that there is a significant association between exposure and colonization of ESBL, but further in depth analysis needs to be performed. The results may be used to design dosing regimens that decrease the probability here-of.

4. Potential impact and main dissemination activities/exploitation of results

4.1 Potential impacts & Future directions

Deeper understanding of antibiotic selection pressure and judicious antibiotic use

The gaps between scientific knowledge and current practices of misuse of antimicrobial agents are enormous. AMR represents a particular challenge, because it touches upon several aspects of care, from basic knowledge to antibiotic prescribing. SATURN addressed this knowledge gap through a variety of research platforms focusing on the effect of various antibiotic agents and prescribing patterns on selection and spread of ARB.

SATURN results provide (1) a deeper understanding of the effect of various antibiotic classes, duration of treatment, order of treatment and dosage used on AMR in the community, general hospital wards and in ICUs and (2) a basis for better treatment decisions regarding antibiotic choices in various settings to minimise AMR, without compromising patient outcomes. This unprecedented approach will allow development of guidelines on antibiotic use and formulary interventions at the local, regional and European level.

Knowledge base for better treatment decisions

Antibiotic use is one of the most important drivers in the development and selection of antibiotic resistance. As only a limited number of new drugs are to be expected shortly and unnecessary side effects should be avoided, well-informed control measures need to be taken. In order to contain the development and selection of antibiotic resistance we need to improve antibiotic prescribing in everyday's practice. The availability of credible, high-quality clinical data about specific aspects of the relationship between antibiotic use and resistance is important in influencing physicians' antibiotic prescribing practices. The SATURN consortium is confident that the evidence generated from the clinical studies and WP6 (pharmacodynamics) will provide clinicians and policy makers with crucial information to improve use of antimicrobial agents in the future.

Improved knowledge on the molecular epidemiology of antibiotic exposure

Moreover, SATURN studies, provide as a result improved knowledge on molecular epidemiology and the effect of antibiotic exposure on virulence and survival fitness of AMR in key pathogens. These data provided the following impacts:

- Information on the impact of antibiotic cycling and antibiotic mixing on overall antibiotic resistance levels in ICUs;
- Information on the impact of antibiotic cycling and antibiotic mixing on the rates of endogenous and exogenous acquisition of ARB in ICUs;
- Quantification of the relevance of epidemiologic factors associated with carriage of ARB
- Information on the spread of fluoroquinolone-resistant clonal groups of *E. coli* and ESBL-producing Enterobacteriaceae in the community of heterogeneous European regions;
- Better understanding of the transmission of AMR in the community without antibiotic selective pressure;

- Proposal for appropriate interventions to limit the spread of multi-drug resistant *E. coli* in the community and the healthcare setting;
- Proposal for optimal ecological antimicrobial regimens in common infectious conditions;
- Support for the rationale for shorter antibiotic treatment duration for common community- or hospital acquired infection;
- Insight on the role of selection versus clonal spread in the emergence and dissemination of AMR;
- Proposal for thresholds for selecting antimicrobial agents based on individual outcome and population antibiotic resistance prevalence;
- Explanation of the role of the host's own faecal flora and transmission among households in the emergence of antibiotic resistant *E. coli*;
- Prediction for fluoroquinolone resistance of *E. coli* in the community based on individual level exposure and selection of resistance to fluoroquinolones;
- Important data on the underlying basis for the global dissemination of certain clones of MDR Gram-negative bacteria by studying virulence, clonality and the fitness costs of resistance gene carriage in these successful clones;
- Further clarification of the complex mechanisms of AMR in key pathogens selected by antibiotic use;
- Explanation on the persistence of clinically important ARB over time following antibiotic use.

Economic impact

The results of the proposed molecular and pharmacodynamic studies provide microbiologists, clinicians and hospital epidemiologists new data on the differences between various antibiotic agents in their propensity to amplify AMR and lead to dissemination of resistance genes, among carriers of these strains. The study provides also data on PK/PD indices and duration of therapy on amplification of AMR, and the relationship between amplification of AMR and environmental contamination. These data will allow better decision making on antibiotic prescription to reduce AMR without compromising patients' safety. The results of the proposed study serve the pharmaceutical industry on the development of safer antibiotic agents with improved profile in preventing resistance.

Public Health impact – European dimension

Results of the SATURN project should be generalised and applied to settings and healthcare institutions in other European countries. Practical recommendations might be used by others, after taking into account differences in baseline resistance rates and patient case-mix. Furthermore, this program provides a sound basis for improved antibiotic prescribing practices in the future and can generate a direct impact on clinical practice and appropriate administration of broad-spectrum antimicrobial agents.

The comprehensive knowledge provided by SATURN can be directly used to improve antibiotic usage policies. Thus, the results of this project is of value to policy makers, by providing data useful for evaluation of different national antibiotic stewardship strategies. Assessment of the usefulness of the recommendations derived from the results of this study is also valuable help for those in charge of infection control, for hospital administrators, and for those managing budgets of large healthcare organisations. Ultimately, the results of this work contributes to the prevention of antibiotic-resistant infections and improve quality of patient care in Europe.

4.2 Dissemination activities and exploitation of foreground

The dissemination and communication about the project and its findings to the relevant target audiences is crucial for the success of the project. An information dissemination plan was set up at the start of the project, which detailed the project dissemination and communication activities, their objectives, targets, tools and associated budget. The objectives of the SATURN dissemination and communication activities were to divulge knowledge generated by the project and to promote scientific and technical progress on the understanding of the impact of antibiotic exposure on antimicrobial resistance to the scientific community and the stakeholders in infectious diseases, public health, policymakers, drug manufacturers, and health care workers. It also aimed to inform the public about ongoing research activities and their possible impact on society.

Communications

Communication was fundamental to SATURN since awareness of the results from SATURN is one of our major concerns. To this end a number of communication and dissemination activities were carried out. For instance, a fully functional and user friendly website was developed and maintained throughout the duration of the project and will even be maintained beyond it (at least 2 years after the end of the project). This web site includes information about the vision, objectives and outcomes of SATURN.

In addition, a poster, a leaflet as well as 4 newsletters, presenting the partners and the project with its aims, actions and results, were prepared to support the dissemination activities of the project to a wider public. The poster, leaflet and the newsletters were distributed to patient associations, industries and scientists from a wide range of research areas during conferences and workshops. These documents are also available for download on the public website of the project, to allow the general public to access the information.

Publications

A large number of publications were presented both at conferences and in journals based on the results of the project. The major papers reporting results from the project were and will be published in peer-reviewed, prestigious scientific journals. Most of the partners already have an established tradition for joint publications in high impact journals. Furthermore, the results were and will be presented at national and international conferences (ECCMID, ICAAC, ICPIC, etc.) depending on the stage of the project.

Exploitation of foreground

As foreseen, the research in SATURN has not resulted in patentable new knowledge. However, the results will allow all participating institutions to pursue internationally competitive high-quality research and facilitate future collaborative projects.

Specifically, the following plans for exploitation and use of the project results have been identified:

- The results of the WP3 community study will allow to guide local antibiotic polices, screen high-risk patients for ESBL carriage and encourage further research on acquisition and transmission of ARB causing UTI in the community setting. Furthermore, follow-up projects may include intervention studies that will attempt to decrease infection risk in carriers of FQ-resistant or ESBL-producing bacteria.

- The results of the WP6 pharmacodynamic study will improve rational use of antibiotics. It will provide clinicians and policy makers with guidance on how to improve antibiotic use in the hospital setting, and limit the spread of ESBLs and CRE from colonised patients. The results of this study will provide a rationale for antibiotic policies, both in general guidelines as well as for hospital formularies. The PK/PD relationships found will serve as a basis to test specific hypotheses on emergence of AMR in the future.
- The results of WP5 AMR study will allow rational use of antibiotics based on patients' carrier status, thus will allow better exploitation of screening culture results. Linking the results of WP5 with other WPs will allow a comprehensive approach to formulary interventions aimed at limiting the spread of AMR.
- The functional and genetic studies on antibiotic-resistant and antibiotic-sensitive bacteria (WP1) will generate new evidence about the changes effected by antibiotic therapy on commensal organisms or opportunistic pathogens in the oropharyngeal, nasal and gastro-intestinal flora and study known and emerging AMR mechanisms in fluoroquinolone-resistant, carbapenem-resistant or extended-spectrum beta-lactamase harboring Gram-negative bacteria, MRSA and fluoroquinolone-resistant viridans streptococci. Studies on the population biology, clonal fitness, and virulence potential will uncover the mechanisms underlying the successful dissemination of major AMR clones. Together, these studies will provide a comprehensive pan-European picture of AMR.
- The results of the ICU clinical study described in WP2 will allow guiding antibiotic policies in ICUs and will elucidate the effects of different strategies on selection and transmission of nosocomial ARB. As the antibiotics may have different effects on these parameters, follow-up projects may include more targeted antibiotic intervention studies focussing on pathogens with certain AMR pheno- or genotypes.
- The results obtained from WP4 nosocomial acquisition study will allow physicians to improve hospital antibiotic usage. It will provide useful information for targeting screening for ARB in patients undergoing antibiotic therapy and for defining epidemiological and clinical variables associated with the development of infections due to ARB in patients previously colonised. This may result in a reduction of hospital spreading of ARB and related morbidity and mortality.

5. SATURN partners and contacts

The SATURN project, coordinated by Prof. Stephan Harbarth (University of Geneva) united the best expertise in the field of antimicrobial resistance in Europe. The consortium brought together 14 partners from 11 countries which are members or associated states of the EU. In addition to these partners, the project interacted with hospitals (subcontractors) throughout Europe for the SATURN ICU-trial.

- University of Geneva – Stephan Harbarth (Switzerland)
- Medical University of Lodz – Maciek Godycki-Cwirko (Poland)
- Tel-Aviv Sourasky Medical Center – Yehuda Carmeli (Israel)
- Universiteit Antwerpen – Herman Goossens, Surbhi Malhotra (Belgium)
- Università Cattolica Sacro Cuore – Evelina Tacconelli (Italy)
- University Medical Centre Utrecht – Marc Bonten (Netherlands)
- ARTTIC – Carlos Triay, Andrea Kuperberg (France)
- Institut D'Investigacions Biomediques August Pi i Sunyer – Jordi Vila, Ignasi Roca (Spain)
- Assistance Publique – Hôpitaux de Paris (Hôpital G Pompidou) – Laurent Gutmann (France)
- Universität Würzburg – Knut Ohlsen (Germany)
- Clinical Centre of Serbia – Biljana Carevic (Serbia)
- Institute for Infectious Diseases 'Matei Bals' – Liliana Preotescu (Romania)
- Radboud University Nijmegen Medical Center – Johan W Mouton (Netherlands)
- University of Tübingen – Evelina Tacconelli (Germany)

SATURN public website: http://www.saturn-project.eu/