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PROJECT FINAL REPORT

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[1] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

4.1 Final publishable summary report

Executive Summary

In the EvoTAR project the human gut microbiome was primarily used as a starting point for the study of antibiotic resistance. The gut harbours the most complex and abundant microbiota of our body and here antibiotic-resistance may emergence readily through selection of pre-existing resistant bacteria and gene transfer events for which there are ample opportunities in this environment. To study the dynamics of the resistome (*i.e.* the total antimicrobial resistance determinants (ARD) in the human gut the consortium exploited different culture independent technologies, such as full metagenomic sequencing, functional metagenomic selections and resistance genes capture platforms as well as novel high throughput culture methods. Metagenomic sequencing revealed that a relatively short-term exposure altered both the richness (a decrease of 67.8%) and the ARD abundance (a decrease of 65.2%) greatly. There was thus no enrichment of the ARDs, presumably because of the inability of most species to withstand the harsh antibiotic treatment. In contrast, a long-term (chronic) exposure altered the relation between the richness of the microbiome, which was decreased and the abundance of the ARDs, which was increased. Clearly, such exposure selects for the species than can strive in constant antibiotic presence, due to the ARDs they encode. Also functional selections showed that hospitalization and antibiotic treatment has profound effects on the gut resistome, with a vast expansion of the resistome in some patients. This expansion of the resistome during hospitalization could lead to an increased risk of transfer of antibiotic resistance genes to infecting pathogens. Importantly, preliminary data indicate that 6 months after discharge, the abundance of antibiotic resistance genes return to the same level. Also with the newly developed gene capture platform (described below) important changes in the composition of antibiotic-resistance genes in samples form hospitalized patients were observed, with remarkable gains and loss in the recovery of certain families of genes.

In addition to the culture-independent methods optimized cultivation methods for the human gut microbiota were developed that can capture a representative majority of the cells present in a sample by both abundance and overall community structure. These novel culture technologies in combination with whole genome sequencing revealed important reservoirs of antibiotic resistant organisms and antibiotic resistance genes in soil and marine environments. One example was the identification of 17 new carbapenemases with 30-76% amino acid identity to previously confirmed carbapenemases. Our results show that the environment including soil and water is a source of highly diverse carbapenemases that are produced by a variety of bacterial species and have not yet emerged in clinical settings. These carbapenemases may constitute potential carbapenem-resistance determinants of clinical relevance if acquired by pathogenic bacteria, as they were functionally expressed in *E. coli*.

Dedicated research on the evolution and spread of resistance in Enterobacteriaceae and enterococci revealed, among others, low-likelihood of recent transmission of ESBL-*E. coli* between poultry and humans but that virtually identical ESBL-carrying plasmids were shared by genetically unrelated human and poultry isolates, strongly suggesting that ESBLs are mainly disseminated via epidemic plasmids that can spread between different reservoirs. The latter was demonstrated using the PLACNET tool mentioned below.

A major objective of the EvoTAR project was to develop generic and predictive models that allow a detailed description of the within-host dynamics and between-host dynamics of antibiotic resistance modules (genes, genetic elements, clones) and that will quantify the probability and rate of emergence and spread of resistance-conferring genes/mutations under various environmental conditions, different selective pressures and in different genetic backgrounds. To study the between host transmission of antibiotic resistance three generic model frameworks have been developed. With these models it is possible to study how different diseases and antibiotic resistances spread over a network of connected hosts (i.e. persons, hospitals, farms, etc.), to identify hosts at risk of becoming infected and for identifying hotspots for the emergence of multidrug resistant bacteria. Furthermore, results of the models could be used to aid in the development of nationwide surveillance programs by informing policy makers where to concentrate efforts. The use of *in silico* pharmacokinetic-pharmacodynamic (PKPD) models based on

data from *in vitro* time-kill experiments can provide valuable information to guide dosing of antibiotics. Experimental work on fitness costs has generated some general implications of importance for understanding and predicting resistance development. One important conclusion from this is that it is at present very difficult to predict the magnitude of the fitness effect of particular resistance mechanism in a particular genetic background.

Finally, novel interventional strategies to tackle antimicrobial resistance were developed and thoroughly evaluated. DAV132, developed partly in the context of EvoTAR by Da Volterra, is the first product with a clinically-demonstrated protection of intestinal microbiota from disruption during antibiotic treatments.

Apart from its scientific outcomes during the course of the EvoTAR projects important novel tools for the analysis of resistance genes, and the natural history of plasmids via a tool called PLACNET. A new method that we name pairwise comparative modelling (PCM) was developed to identify ARDs in large complex datasets. With ARD many novel resistance genes were identified and expanded very significantly the list of resistance known genes. Furthermore, the EvoTAR consortium successfully developed the experimental and computational workflows to use PacBio for reading out results of functional selections. This development enabled unprecedented quality and throughput of functional selections. A majority of the functional selections for this project rely on this approach. A novel *Targeted capture approaches* was developed in EvoTAR enabling cost-effective and high-throughput resistomes analysis. An antibiotic resistance gene-capture platform was designed that uses the SeqCap Ez technology of Roche NimbleGene. This platform consisted of 80,000 targets involving allelic forms of resistance genes and genes associated with the backbone of mobile genetic elements able to contribute to the spread of resistance. Furthermore, a core genome MLST (cgMLST) scheme was developed for *E. faecium* to standardize current intra-laboratory surveillance of this nosocomial pathogen. cgMLST transfers genome-wide single nucleotide polymorphism (SNP) diversity into a standardized and portable allele numbering system that is far less computationally intensive but with the resolution of SNP-based analysis of whole-genome sequencing (WGS) data.

Summary of Project context and Objectives

This project addresses the problem of antibiotic resistance in bacteria. Antibiotics are one of the most apparent success stories of modern medicine and have saved the lives of countless people that suffered from bacterial infections. However, the use of antibiotics has also led to the emergence of antibiotic resistance in bacteria, which is a major threat to human health as therapeutic options for treating infections by antibiotic-resistant bacteria are increasingly limited. It is generally appreciated that the emergence of antibiotic resistance is a complex problem accelerated by the overuse of antibiotics. However, antibiotic resistance is a natural biological phenomenon with many facets that are still poorly understood: what are important reservoirs of antibiotic resistance? How do resistant and non-resistant bacteria interact in these reservoirs? Which conditions promote the evolution and transfer of resistance? Expanding our knowledge on these aspects will provide novel leads to combat the emergence of antibiotic resistance.

The overall purpose of EvoTAR was to increase the understanding of the evolution and spread of antibiotic resistance in human pathogens. More specifically, EvoTAR aimed to characterise the human reservoir of antibiotic resistance genes ("the resistome") by investigating the dynamics and evolution of the interaction between resistant and non-resistant bacteria from the human microbiome and the interrelations of the human resistome with environmental, animal and food reservoirs of resistance genes. Novel methods were developed and used to quantify resistance transfer under controlled conditions in gene exchange communities. Mathematical modelling have been applied to predict gene flow between different reservoirs and, consequently, to make a prognosis of future resistance trends. Novel *in vitro* and *in vivo* models of antibiotic resistance evolution and transfer allowed the study of the efficacy of novel intervention approaches aimed at reducing selection and spread of antibiotic resistance.

To reach its main objective, the multi-disciplinary EvoTAR consortium pursued the following five research themes:

1 Dynamics & Evolution	To elucidate the dynamics and the evolution of the interaction between resistant and non-resistant bacteria from the human microbiome.
2 Reservoirs	To characterize antibiotic resistance genes from the human microbiome and to elucidate the interactions of the human microbiome with environmental, animal and food reservoirs of resistance determinants.
3 Transfer	To determine the transfer potential of antibiotic resistance genes to human pathogens and to assess the contributions of the environment and the genetic elements on which the antibiotic resistance genes are carried on the efficiency of transfer.
4 Modelling	To generate integrated mathematical models, using data on the evolution, transfer and spread of antibiotic resistance genes, to describe the flow of antibiotic resistance genes between different reservoirs and to predict future resistance trends.
5 Novel interventions	To explore novel intervention approaches aimed at reducing the spread of antibiotic resistance.

To study objective 1 "*To elucidate the dynamics and the evolution of the interaction between resistant and non-resistant bacteria from the human microbiome*" the EvoTAR consortium performed the following three studies:

A. Metagenomic sequencing of the human microbiome during and after administration of antibiotics (WP1)
B. Population dynamics of resistant and susceptible enterococci and *Enterobacteriaceae* during and after administration of antibiotics (WP2)
C. Experimental adaptive evolution of resistance genes (WP3, WP6).

Ad A. Metagenomic characterization has revealed changes in both the phylogenetic diversity and the total gene repertoire of the human microbiome during and after cessation of antibiotic treatment. Understanding both short-term and long-term effects of antibiotic treatment on the diversity of the human microbiome is essential because it sheds light on which organisms of the human microbiome are resistant to antibiotic exposure during treatment and thus can potentially transfer their resistance(s) to other bacteria. Persistent perturbations of the gut microbial communities have been associated to numerous chronic diseases. Possibly, antibiotic treatments might lead to such perturbations. Our study of the dynamics of the gut microbiome exposed to antibiotics provided for the first time large-scale information about the longer-term effects of antibiotic therapy. Furthermore, by comparing the sequencing data with databases of antibiotic resistance genes we were able to quantitatively determine which resistance genes are present in the human microbiome before, during and after antibiotic treatment

Ad B. For the functional studies on antibiotic resistant organisms genes enterococci and *Enterobacteriaceae* as Gram-positive and Gram-negative marker organisms, respectively, were selected for this project. Both groups of bacteria are commensals of the gastrointestinal tract but can cause life-threatening infections in hospitalized patients. Enterococci and *Enterobacteriaceae* have been implicated in the transfer of important resistance mechanisms (for example vancomycin resistance in enterococci and Extended Spectrum β-Lactamases [ESBLs] in *Enterobacteriaceae*) between human and non-human reservoirs and to other pathogenic bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Enterococci and *Enterobacteriaceae* can therefore be considered as paradigms for the functional study of the emergence and spread of antibiotic resistance. By determining the changes in the population of these bacteria during exposure to antibiotics throughout hospitalization, we were able to determine the dynamics of the emergence of antibiotic-resistance genes and clonal lineages associated with multi-drug resistance of enterococci and *Enterobacteriaceae*. This has given detailed information on the dynamics

and interactions between resistant and non-resistant bacterial populations during and after antibiotic exposure.

Ad C. In the EvoTAR project, we have also studied evolution of resistance genes and the stability of resistance genes in bacterial hosts. This has provided important information on the risks that these resistance genes spread easily through a bacterial population, which would significantly increase the risk of widespread dissemination of these resistance determinants, or that these resistance genes will be inherently limited to a small range of hosts.

For objective 2 "*To characterize antibiotic resistance genes from the human microbiome and to elucidate the interactions of the human microbiome with environmental, animal and food reservoirs of resistance determinants*" the following three studies were performed:

A. Functional metagenomics of antibiotic resistance genes in the human microbiome (WP3)
B. Detection and quantification of antibiotic resistance genes in human and non-human reservoirs (WP4)
C. Genomic characterization of antibiotic resistant bacteria from different reservoirs (WP2, WP5)

Ad A. The functional repertoire of antibiotic resistance genes in the human microbiome using functional metagenomic approaches was studied and revealed great dynamic. This has lead to an in-depth description of the effects that hospitalization and antibiotic therapy have on the repertoire of antibiotic resistance genes that are harboured by the bacteria from the human gut.

Ad B. Using a gene sequence capture technology we have detected and quantified the load of antibiotic resistance genes and the genes associated with their transfer in both human and non-human reservoirs in a high-throughput fashion. This revealed the extent by which resistance genes from the human reservoir are also found in non-human niches, indicating a possible transfer of antibiotic resistance genes between human and non-human reservoirs.

Ad C. By high-throughput genome sequencing of enterococci and *Enterobacteriaceae*, which are important vectors for the spread of antibiotic resistance (Livermore, 2009) and by high-throughput culturing to select antibiotic-resistant bacteria, followed by subsequent characterization by genome sequencing we have identified and characterized bacteria that play a central role in acquiring and transferring antibiotic resistance genes between bacteria and between different environmental reservoirs.

Objective 3 "*To determine the transfer potential of antibiotic resistance genes to human pathogens and to assess the contributions of the environment and the genetic elements which carry the antibiotic resistance genes on the efficiency of transfer*" included the following studies:

A. Determination of factors affecting the bacterial host range of resistance plasmids (WP4, WP6)
B. Contribution of environmental conditions to the efficiency of transfer of antibiotic resistance genes (WP6)
C. Analysis of fitness costs incurred by resistance mutations and resistance plasmid carriage and genetic adaptation of resistance plasmids to novel bacterial hosts (WP6, WP7)

Ad A. A critical issue for the understanding of plasmids as disseminators of antibiotic resistance is to define the bacterial host-range of each resistance plasmid. Plasmid maintenance in ecosystems (for example, the human gut) depends on the stability of the resistance plasmid in the different bacterial hosts, which is a crucial factor in assuring spread of the plasmid by horizontal gene transfer. This aspect has been studied in EvoTAR.

Ad B. In the EvoTAR project the conjugation efficiency of plasmids and their subsequent capability to propagate in different bacterial hosts has been determined by a variety of methods. The environmental signals that trigger the induction of conjugation has been identified, which ead to insights into the ecological circumstances in which horizontal gene transfer takes place.

Ad C. Considerable attention has been given to determine the fitness costs that are incurred by resistance mutations and the carriage of resistance plasmids and to determine the efficiency by which different plasmids can adapt to their bacterial hosts.

Studies for Objective 4 "*To generate integrated mathematical models, using data on the evolution, transfer and spread of antibiotic resistance genes, which can be used to describe the flow of antibiotic resistance genes between different environments and bacterial hosts and to predict future resistance trends*" involved:

A. Development of mathematical models that describe the probability and rate of resistance development taking into account several levels of modular trait interactions (WP7)
B. Development of generic and predictive models which will lead to a detailed description of the within-host dynamics and between-host dynamics of antibiotic resistance modules and spread of antibiotic resistance at the population level (WP7)

Ad A. The success of an antibiotic resistant clone is largely determined by the competitive fitness of that clone in comparison to susceptible ones in a number of different environments. This overall fitness will be determined by many modular traits (the host bacterium, the plasmids, transposons or integrons with their resistance genes in the bacterial cell and the resistance gene itself) that act at different levels and which are studied as part of the other objectives in this proposal. EvoTAR studied how traits influence the survival, growth and transmission success of the various genetic elements that can influence the trait or are influenced by the trait using mathematical models that describe the probability and rate of resistance development taking into account several levels of modular trait interactions.

Ad B. A major objective of the EvoTAR project was to develop generic and predictive models which will lead to a detailed description of the within-host dynamics and between-host dynamics of antibiotic resistance modules (genes, genetic elements, clones) and which will quantify the probability and rate of emergence and spread of resistance-conferring genes/mutations under various environmental conditions, different selective pressures and in different genetic backgrounds. This modelling-based approach has proven essential for the prediction of the risk that a given antibiotic resistance gene may successfully spread to pathogens and thereby contribute to future resistance problems and how changes in the selective pressures influenced rates of spread of antibiotic resistance at the population level.

Finally studies indicated below were executed for Objective 5 "*To explore novel intervention approaches aimed at reducing the emergence and spread of antibiotic resistance*":

A. Assessment of the efficacy of compounds that absorb and inhibit residual antibiotics in the colon to minimize emergence of antibiotic resistance (WP1, WP8).
B. Assessment of the efficacy of compounds that impede the conjugative transfer of resistance genes among bacteria to minimize dissemination of antibiotic resistance (WP6, WP8)
C. Identification of novel targets for therapeutic interventions (WP2)

Ad A. A novel intervention approach was conducted in EvoTAR aimed at administering a compound that absorb and inhibit residual antibiotics in the colon. It was anticipated and proven correct that this approach minimize selective pressures leading to the emergence of antibiotic resistance in the commensal flora without changing the fate of absorption of the antibiotic and its potential to treat the infection for which it has been administered.

Ad B. Horizontal gene transfer is widespread in the environment, where antibiotics are present at concentrations lower than those used in medicine, and where they perform other functions than those related to their therapeutic applications. The continuous presence of antibiotics and frequency of gene transfer make environmental microorganisms a good source for compounds capable of inhibiting transfer of genes associated with the response (including resistance) to different classes of antibiotics. In order to explore this possibility, a detailed characterization of natural and chemically synthesized compounds was tested for their capacity to inhibit conjugation.

Ad C. Using functional genomic approaches genes contributing to resistance that could serve as targets for the development of new therapeutic interventions were identified. The focus of these studies was oriented towards common gastro-intestinal commensals (enterococci and *Enterobacteriaceae*), that are a major hub for antibiotic resistance, by functional genomics-based approaches. The identification of the full complement of genes involved in antibiotic resistance in these groups of nosocomial pathogens will open up new avenues for the development of novel therapeutic interventions.

Main results

The sensitivity of identifying antimicrobial resistance determinants (ARD) in large complex datasets like metagenomic datasets is low often resulting in identifying only a fraction of the genes that are actually present. Currently, assigning a protein a given function relies on the shared identity of the sequence (*i.e.* letters corresponding to amino acids) with a protein for which the function is known and certain (reference protein). Setting an identity threshold is problematic since many of the proteins identified in metagenomic datasets share a low identity with reference proteins. A low identity threshold would increase sensitivity yet leads to false positives while a high identity threshold would be specific but insensitive. Previous studies identified between 100 and 1093 ARDs in the Human intestinal microbiota by BLAST coupled with an identity threshold varying from 50% to 80% with a reference protein of the ARD family.

Census of ARDs of the human gut microbiome

As opposed to primary-sequence similarities, comparison of the folded structures of proteins is expected to be more specific and sensitive. However, computing structures from primary sequence for a large number of proteins is challenging and was not carried out in a microbiome field. We developed in workpackage (WP)1 a new method that we name pairwise comparative modelling (PCM). PCM aims to predict protein functions with more specificity than one-dimensional method, and includes a large increase in sensitivity by allowing the functional assignment of proteins with low identity to known references. It relies on a highly efficient modelling algorithm, using an established ARD structure from the RCSB protein data bank as a positive template and the most closely related non-ARD structure as a negative template. We modelled the structure of all proteins encoded by a 3.9 million human gut microbial catalogue. A custom pipeline was developed to assess the fit of an unknown protein structure to a positive and a negative template. The approach was validated using a set of ARDs found in a search selecting antibiotic resistance from the soil microbiome; out of 1390 ARDs the pipeline correctly predicted 1374, a success rate of 96.6%. The average sequence identity was only 37.6%, well beyond that acceptable for BLAST-based searches.

The pipeline identified 6095 ARDs among the 3.9 million human gut microbial genes. They belong to various families. PCM outperformed other methods for all the families but the very well studied class A β-lactamases, where Resfams was more efficient.

Some 72% of ARDs could be assigned to a phylum; a majority were from *Firmicutes* (2962/6095, 48.6%%) and from *Bacteroidetes* (858/4405, 14.1%) while only 3.7% (225/6095) ARDs were from *Proteobacteria*,, that include most pathogens and were most studied previously. About 60% belonged to gene clusters denoted metagenomics units (that is, microbial genomes or sub-genome genetic elements); 95.6% of the clusters contain >500 genes and correspond to bacterial species, indicating that the majority of ARDs have chromosomal location.

In conclusion, identified a large number of novel ARDs and thus expanded very significantly the list of these important genes. Future studies will address the experimental validation of the ARD function.

With the establishment of an optimized approach to identify ARD we set out to study the dynamics of the resistome, *i.e.* the total of ARD, in the microbiota of patients upon hospitalization.

Impact of antibiotic treatment on the resistance determinants of the human gut microbiome

We (WP1) analyzed the resistome upon two types of antibiotic exposures: patients suffering from cystic fibrosis (a chronic pulmonary disease that predisposes to bacterial infections and thus to a high, chronic antibiotic exposure) and patients receiving a selective digestive decontamination (SDD) antibiotic cocktail. As control, we analysed individuals studied in the MetaHIT consortium, which were not exposed recently to antibiotics. Among the control individuals (n=663) the abundance of the ARDs was significantly correlated with the overall gene and species richness of the microbiome. This fits the conclusion reached while establishing the list of the ARDs from the human gut, that most ARDs reside on bacterial species (see above) – the higher the number of species an individual harbours, the higher is the level of the ARDs he carries. The same relation between gut microbiome richness and ARD abundance was also found for the EVOTAR individuals not exposed to antibiotics (n=45) or present in a hospital ward but not treated with antibiotics (n=32).

A short-term exposure (SDD; n=10) altered both the richness (a decrease of 67.8%) and the ARD abundance (a decrease of 65.2%) greatly. There was thus no enrichment of the ARDs, presumably because of the inability of most species to withstand the harsh antibiotic treatment (Figure 1).

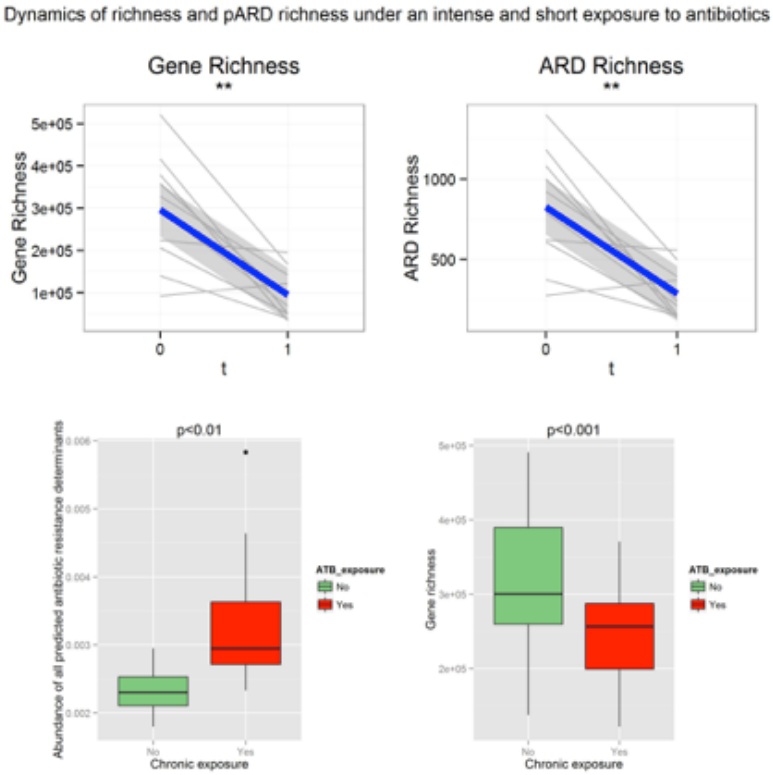

Figure 1. Dynamics of gene richness and ARD richness under an intense and short exposure to antibiotics.

In contrast, a long term (chronic) exposure altered the relation between the richness of the microbiome, which was decreased and the abundance of the ARDs, which was increased. Clearly, such exposure selects for the species than can strive in constant antibiotic presence, due to the ARDs they encode.

In addition, to mapping ARDs from metagenomic datasets resistance genes in partly the same sample set were also identified using functional selections, work performed in WP3.

Resistome mapping

Our efforts to characterize the resistome in hospitalized patients and its dynamics using functional selections can be grouped into three distinct efforts: (1) To develop new techniques for more thorough and higher throughput characterization of resistance genes from gut microbiomes, (2) To catalogue the

collection of resistance genes in the gut microbiome of hospitalized individuals, and (3) To assess the dynamics of the gut resistome during hospitalization and antibiotic treatment.

New techniques for more thorough and higher throughput characterization of resistance genes from gut microbiomes.

Parfums: High throughput metagenomic functional selections in E. coli
During the course of the EvoTaR project new techniques have been developed that increase the throughput of metagenomic functional selection in *E. coli.* Our first development was to develop a experimental and computational workflow for utilizing the short reads that result from the Illumina sequencing platform. This approach developed together with the Dantas Lab is termed Parfums and was published in Science (Forsberg *et al* (2012) Science) along with the first application of the methodology to study the resistome of soil bacteria.

PacBio based metagenomic functional selections in E. coli.
Following the development of Parfums sequencing platforms continued to improve and we switched to use the PacBio sequencing platform as it provides long reads that span the entire insert of functional selection libraries. Working together with the sequencing facility at University of Oslo we successfully developed the experimental and computational workflows to use PacBio for reading out results of functional selections. This development enabled unprecedented quality and throughput of functional selections. A majority of the functional selections for this project rely on this approach.

Metagenomic functional selections in Lactococcus lactis
Most functional metagenomic studies deploy *E. coli* as the expression host due to its amiability for cloning. Using a Gram-negative host strain is likely to introduce bias in the specific genes that are identified in functional metagenomic selections. To assess the extent to which this bias is causing significant problems we developed an experimental workflow for using *L. lactis* as our cloning host. Notably, this required substantial optimization to achieve sufficient library sizes. However, the results of these experiment remained discouraging as no resistance determinants other than those found in an *E. coli* small-insert library (specifically against the antibiotics tetracycline and D-cycloserine) could be detected in the *L. lactis* metagenomic libraries. Importantly, resistance determinants against the antibiotics linezolid, vancomycin and daptomycin (which are active against Gram-positive bacteria, but not against Gram-negatives) have not been identified. We conclude that there is currently no added value in further developing functional metagenomic libraries in *L. lactis* and have consequently terminated this particular research line.

Catalogue the collection of resistance genes in the gut microbiome of hospitalized individuals

Cataloging resistomes from E.coli *small insert functional selections*
Metagenomic expression libraries from over 60 patient samples have been constructed in the Gram-negative host *E. coli*. Screening for resistant clones from these libraries have resulted in the identification of tens of thousands different clones. All metagenomic inserts from resistant clones have been sequenced and annotated using the new PacBio based approach described above. In addition a novel annotation pipeline has been created to rapidly analyse metagenomic insert sequences derived from PacBio sequencing. A catalogue of resistance genes from the ICU patients has been constructed on the basis of these efforts. This catalogue contains over three thousand genes. Interestingly, a majority of these resistance genes are closely related to previously identified resistance genes. Analysis of the context of these resistance genes revealed that over 30 % of them have been previously identified within human clinical isolates.

Cataloging resistomes from E. coli *fosmid functional selections*
Using fosmid libraries we have identified genes that confer resistance to the disinfectant benzalkonium chloride (BC) from the human gut microbiota. Two of the genes that conferred BC-resistance to *E. coli* were predicted to be involved in membrane transport or efflux and to originate from Gammaproteobacteria and Bifidobacterium respectively, whereas the third gene was predicted to function

as an UDP-glucose-4-epimerase, originating from *Eggerthella lenta*. Two BC-resistant clones exhibited reduced susceptibility towards the antibiotics erythromycin and tobramycin, with one of these clones also showing reduced susceptibility to ampicillin. These data show that the human gut microbiota is a reservoir for genes that confer resistance to disinfectants. The reduced susceptibility to antibiotics in two BC-resistant clones indicates that, in gut bacteria, resistance to BC can be genetically linked to resistance against antibiotics.

The dynamics of the gut resistome during hospitalization and antibiotic treatment

Resistome dynamics from fosmid libraries and real time PCR
A preliminary analysis of the resistome dynamics was conducted for a subset of the patient samples for which samples existed from the first sampling point at the hospital, during hospitalization and after discharge. The results revealed that the resistome was highly dynamic and expanded during hospitalization in terms of abundance (e.g. more antibiotic resistant clones where selected from the libraries). These results are published in Journal of Antimicrobial Chemotherapy (Bulow *et al* (2014), JAC).

Resistome dynamics of key resistance genes based on microfluidic real time PCR
We (WP3) set up a high-throughput nanolitre-scale real-time PCR assay (using the 96.96 BioMark™ Dynamic Array for Real-Time PC, developed by Fluidigm Corporation, San Francisco, CA, U.S.A) to detect and quantify the presence of 85 resistance determinants in metagenomic DNA. We used this platform to characterize the dynamics of the resistome of the gut microbiota of patients during hospitalization and to assess the spread of resistance genes through sewage. This technique allows high-throughput characterization of key constituents of the gut resistome during antibiotic treatment and provides a low cost alternative to using functional metagenomics to characterize resistome dynamics.

Resistome dynamics based on comprehensive small insert libraries
Finally, we analyzed the dynamics of the gut resistome on the basis of the small insert functional selection libraries described above. This analysis revealed that the gut resistome is subject to substantial dynamics during antibiotic exposure and hospitalization. Two main conclusions where drawn from this analysis:

(i) The gut resistome expands during hospitalization, yet recovers after discharge. The abundance and diversity of antibiotic resistance genes identified in functional selection experiments more than double from the first sampling point (admission to ICU) to the subsequent sampling points during hospitalization. Then following approximately 6 months after discharge, the abundance of antibiotic resistance genes return to the same level.

(ii) The gut resistome becomes more enriched in resistance genes that are also shared with human pathogens during hospitalization. This is consistent with the hypothesis that the gut resistome acquires pathogenic resistance genes during hospitalization.

In conclusion we can see that hospitalization and antibiotic treatment has profound effects on the gut resistome. Furthermore, the expansion of the resistome during hospitalization could lead to an increased risk of transfer of antibiotic resistance genes to infecting pathogens.

In another culture independent method to analyse the resistome of human reservoirs (hospital and community) and reservoirs with a direct link to humans (foodborne animals) we (WP4) developed and used a next-generation *one-step* high-throughput targeted platform that was designed and validated during the EvoTAR project. Analysis of *resistome* uses metagenomic approaches, either "open" (target gene sequencing, shotgun metagenomic sequencing, metatranscriptomic sequencing) or "closed" formats (targeted and/or functional gene arrays) show poor sensitivity and specificity, and limited quantitation possibilities. *Targeted capture approaches* are the more cost-effective and high-throughput alternatives to obtain large data sets of orthologous genes from many individuals and was chosen to enhanced the resistomes analysis in the EvoTAR project. Advantages of targeted platforms, especially the new generation of *in-solution* targeted capture platforms, over array-based platforms or other genome-

partitioning are scalability, cost-effectiveness, and enhanced data quality (lower variance in target coverage, more accurate SNP calling, higher reproducibility and longer assembled contigs). Furthermore, current resistome analyses do not usually take into consideration either the genetic elements involved in gene mobility or the genes that contribute to co-selection of AR as those encoding resistance against biocides or heavy metals. We developed an AR gene-capture tool based on current knowledge on the genetic structure of resistance genes and the platforms to be propagated (about 80,000 allelic forms, including genes associated with the backbone of mobile genetic elements able to contribute to the spread of resistance), with the aim of reaching the desirable level of comprehensivity and curation required to analyze in depth the resistomes of different ecosystems. The platform uses the SeqCap Ez technology of Roche NimbleGene and involves both academic and industry collaborations. The company has expressed interest in the commercialization of this custom platform. We conclude that WP4 provided significant scientific and technological outcomes.

Development of a custom targeted capture platform (TCP) to analyze resistomes.
A customized SeqCap EZ platform, a solution-based capture system that allows the enrichment of genes or genomic regions in a single test tube, was designed to capture the resistome and mobilome of fecal microbiomes of humans (hospitalized and non-hospitalized) and foodborne animals. This large subtask involves different steps: i) analysis of current databases, ii) design of a custom SeqCap EZ platform, iii) validation of the platform. These activities were fully accomplished in 2014 and further optimized in 2015.

Analysis of DBs and creation of a curated DB.
A deep analysis of DBs for antibiotic resistance (CARD, http://arpcard.mcmaster.ca/; ARGannot http://en.mediterranee-infection.com/article.php?laref=283&titre=arg-annot-), antimicrobial biocide and heavy metals (BACMET, http://bacmet.biomedicine.gu.se/), and relaxases was performed to build a homemade non-redundant database of well-known genes. The workflow consisted on building a homology network (Blast All-to-All of protein sequences), and performing a cluster analysis and further manual annotation of AbR families. All proteins of each cluster were aligned to obtain profile Hidden Markov Models (HMMs) for each family. Then, two phylogenetic analyses were done. One included the proteins of each AbR family, and the other included proteins of each AbR family and their associated profile HMMs against Uniref100 database (http://www.uniprot.org). The last step is essential to improve annotation and identify both false positives and potential undescribed genes.

Design and manufacturing of the platform.
The number of targets included in the platform was significantly increased in comparison with the initial contrive described in the DoW (from 1,000 to 81,000 targets) taking advantage of the development of one-step targeted capture methods in the last three years, the bioinformatic tools and the improvement in the gene DBs (curated DBs used as template for our design became available after 2012). The final version of the SeqCap EZ capture platform consists of 7,963 non-redundant AbR genes, 47,806 manually curated genes from the results of profiles HMMs against Uniref100; 30,740 genes from BacMet-Antibacterial Biocide & Metal Resistance database, and 2,517 non-redundant genes from the home-made relaxase database provided by partner 10 (UC). All genes assemble a platform capture of approximately 81,000 non-redundant genes. This SeqCap Ez custom platform for capturing AbR, Metal and MGE has been manufactured in collaboration with RocheNimbleGen Inc (Madison, USA; www.nimblegen.com). A full bioinformatics workflow was developed as a complement of the design of the SeqCap EZ platform (Data processing and Functional annotation). First, the reads from the sequencing are filtered using the capture platform as a reference to remove the rubbish sequences. Then the filtered reads are assembled to make contigs. Finally, we use GeneMark to ORF prediction. Functional annotation consists of three steps. Annotation against capture platform, selected genes are double annotated against general database (i.e. Uniprot or RefSeq). If the candidate genes still pass the double filter as resistant gene (AbR, biocide or Metal) or as relaxase, the gene is studied phylogenetically to establish if this gene are a novel resistance gene or a known gene.

Validation of the platform.

A pilot to estimate the coverage of genes included in the final version of the customized SeqCap EZ platform, the suitability of different protocols for library preparation (Kappa vs Nextera approaches), NGS sequencing (NextSeq 2x100 changing the number of samples per run) and also the bioinformatic analysis of metagenome.
On-target capture ranges from 30 to 50% of the sequencing reads in the samples analyzed to date, data being within the range obtained for other SeqCap EZ platforms used in human genetics. In comparison with traditional metagenomics methods, the EvoTAR platform improves *sensitivity* (250-fold increase), *specificity* and detection of gene *diversity* (see Figure 2, 3, 4, and 5).

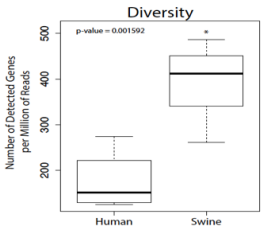

Figure 2. Box plot showing the number of detected genes per million of reads using the EvoTAR TCP of the samples form animals and hospitalized analysed. The two panels represent the number of gene alleles coding for resistance to antibiotics, which are plotted as box plots.

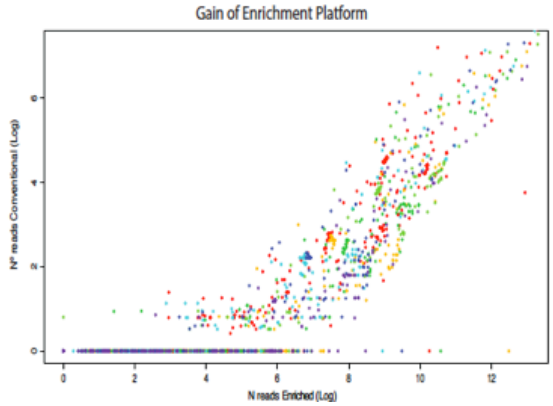

Fig. 3. Dot-plot of the genes-based alignment corresponding to the number of reads captured by conventional metagenomics (y-axis) and to the number of reads captured in the experiments with the current EvoTAR platform prototype using SeqCap EZ Illumina technology (values x-axis). Reads corresponding to the same gene sequence are aligned and represented by dots. Each color represents different metagenomes analysed (in this figure represented by samples from swine). An average of 15.0 million 100 bp paird reads were obtained for each individual sample in the SeqCap EZ Illumina experiments.

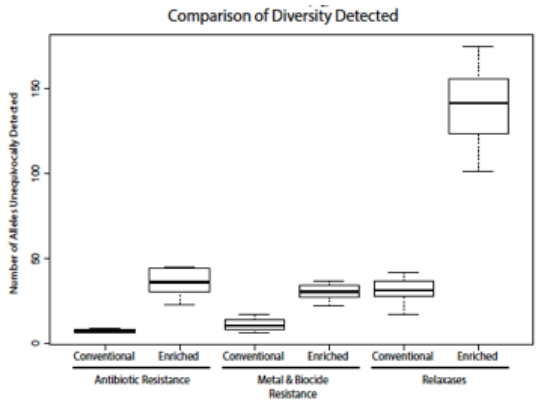

Figure 4. Box plots showing the differences in specificity and sensitivity of gene detection using conventional metagenomics (pre-capture) versus the EvoTAR TCP (post-capture), in this figure represented by samples from swine. The three panels represent the number of gene alleles coding for resistance against antibiotics, metals and biocides, or genes associated with mobile genetic elements (relaxases), respectively, which are plotted as box plots.

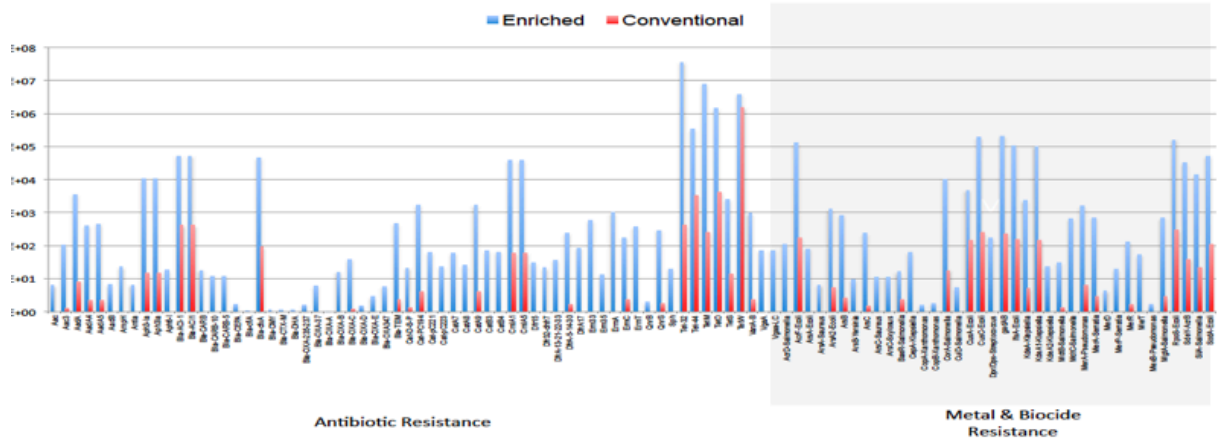

Figure 5. Diversity of the genes conferring resistance to antimicrobials using shotgun metagenomics vs targeted capture platform

A comprehensive outline of the AbR genes in animals and humans.
Samples from humans and animals contain genes coding for resistance to nine antibiotic families, namely beta-lactams (33 groups), aminoglycosides (33 groups), macrolides (23 groups), tetracyclines (23 groups), fluoroquinolones (5 groups), sulfonamides (3 groups), trimethoprim (11 groups), glycopeptides (5 groups), and chloramphenicol (13 groups). For the first time, we can identify with high sensitivity a wide diversity of resistance genes using metagenomic samples.
The diversity of AbR genes found in animals (consider that this set only represents a small sample) is higher than that found in samples from hospitalized patients (Fig. 2), with remarkable diversity of genes conferring resistance to tetracycline. Many AbR were found in both hosts but others, which include genes of all antibiotic families, were more abundant or associated with any of them.

Dynamics of the resistome.
Changes in the composition of AbR genes in samples form hospitalized patients were observed, with remarkable gains and loss in the recovery of certain families of genes.

Next to do culture-independent methods described above work performed in the framework of WP5, concentrated on developing and applying innovative high-throughput culturing strategies, including the MicroDish platform, for the isolation of antibiotic resistant microbial populations from the human intestinal tract as well as from other gut and non-gut environments. In addition, tailored narrow-spectrum high throughput cultivation has been employed to target microorganisms with specific phenotypes of interest, such as resistance to vancomycin and carbapenem.

Optimized cultivation methods for the human gut microbiota
Optimized cultivation methods for the human gut microbiota were developed that can capture a representative majority of the cells present in a sample by both abundance and overall community structure. To this end, a new method was established to perform profiling of antibiotic resistance of the gut microbiota in a high-throughput multiplex fashion allowing simultaneous profiling of 16 types of antibiotic resistance in gut bacteria. These resistance profiles provided the means to target bacteria identified as of great interest by the Human Microbiome Project (HMP) and culture several previously uncultivated bacteria. The genomic analysis of several strains from the Human Microbiome Projects most wanted list confirmed the multidrug resistance profiles of the strains as well as the novelty of the strains in relation to previously sequenced organisms. The organisms are currently being characterized to ensure proper phylogenetic classification including naming of new species.

High throughput cultivation screens to retrieve antibiotic resistance bacteria from humans, animal and the environment
High throughput cultivation screens have been used to retrieve antibiotic-resistance bacteria from human and animal intestinal tracts as well as from marine environments known for their prolific production of

antibiotics, and thus can be expected to also represent natural hubs for antimicrobial resistance. Previously uncultured bacteria could be cultivated using conventional plating, and application of antibiotics in the media can serve to capture a greater bacterial diversity. Moreover, we developed criteria to address an important caveat of the plate scraping method whereby bacteria may be detected that did not actually grow. Furthermore, genomic DNA was isolated from the regrown isolates in order to allow for a functional screen for resistance genes using small insert library screening. The cultivation study in which antibiotic-resistant bacteria were isolated from sponge samples yielded >200 strains that are *Pseudovibrio* spp. There is special focus on *Pseudovibrio* isolates since they are multidrug resistant, have the potential to produce antimicrobial compounds, and were isolated in large numbers from the different sources. Based on GTG-5 genomic fingerprinting and resistance profiles, 25 different strains were selected for whole-genome sequencing. All four type strains (*Pseudovibrio ascidiaceicola* DSM 16392, *Pseudovibrio axinellae* DSM 24994, *Pseudovibrio denitrificans* JCM 12308 and *Pseudovibrio japonicus* NCIMB 14279) belonging to this this genus were also sent for whole-genome sequencing.

A second cultivation-based study using the MicroDish platform was done wherein anaerobic antibiotic-resistant bacteria were isolated from faecal samples (n=20) obtained from SDD patients received from Partner 1, with the aim to broaden our knowledge regarding the role of little-studied and/or novel anaerobic bacteria as a reservoir for antibiotic resistance.

Furthermore, we examined the potential for antibiotic production by assessing the expression of associated secondary metabolite biosynthesis gene clusters. Metatranscriptome datasets from intestinal microbiota of four human adults, one human infant, 15 mice and six pigs, of which only the latter have received antibiotics prior to the study, as well as from sea bacterioplankton, a marine sponge, forest soil and sub-seafloor sediment, were investigated. We found that resistance genes are expressed in all studied ecological niches, albeit with niche-specific differences in relative expression levels and diversity of transcripts. For example, in mice and human infant microbiota predominantly tetracycline resistance genes were expressed while in human adult microbiota the spectrum of expressed genes was more diverse, and also included β-lactam, aminoglycoside and macrolide resistance genes. Resistance gene expression could result from the presence of natural antibiotics in the environment, although we could not link it to expression of corresponding secondary metabolites biosynthesis clusters. Alternatively, resistance gene expression could be constitutive, or these genes serve alternative roles besides antibiotic resistance.

Role of soil microbiota in the origin and evolution of resistance

Investigations on the role of the soil microbiota in the origin and evolution of resistance to two critically important antimicrobial classes in human medicine, glycopeptides and carbapenems led to i) description of a new glycopeptide resistance operon in *Rhodococcus equi* and ii) discovery of multiple new carbapenem-hydrolyzing enzymes produced by environmental bacteria.

Glycopeptides such as vancomycin and teicoplanin are last resort drugs for treatment of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* and enterococci. Although progenitors of vancomycin resistance genes have been described in soil bacteria, elucidation of the evolutionary trajectories leading to the presence of vancomycin resistance genes in clinical isolates is still lacking. We characterized the vancomycin resistance operon of a *Rhodococcus equi* isolated from soil in Denmark in 2004 and displaying a vancomycin-resistance phenotype inducible by glycopeptides. The vancomycin resistance operon in this strain had an unique genetic organization and was designated as *vanO*. This operon has low homology to enterococcal *van* operons and harbors a *vanHOX* cluster transcribed in opposite direction to the *vanS-vanR* regulatory system and comprised between three open reading frames with unknown function. This finding has clinical interest since glycopeptides are used to treat *R. equi* infections and resistance has been reported in clinical isolates.

Furthermore, detection and functional characterization of carbapenem-hydrolyzing ß-lactamase in environmental bacteria was achieved by antibiotic selective culture, functional metagenomics and sequence database mining. The culture-based approach yielded 29 bacterial isolates from 13 soil samples. Among these, we detected isolates belonging to genera or species for which MBL production was not reported prior to this study. Seven new metallo-ß-lactamases (MBLs) were discovered in *Pedobacter*

roseus (in which the produced CHß was annotated as PEDO-1), *Pedobacter borealis* (PEDO-2), *Pedobacter kyungheensis* (PEDO-3), *Chryseobacterium piscium* (CSP-1), *Epilithonimonas tenax* (ESP-1), *Massilia oculi* (MSI-1), *Sphingomonas* sp. (SPG-1) and *Epilithonimonas tenax* (ESP-1). Plasmid libraries were constructed from 10 of the soil samples used for the culture approach. We detected two subclass B1 MBLs (annotated as $bla_{\text{GRD23-1}}$ and $bla_{\text{SPN79-1}}$) and 7 subclass B3 MBLs ($bla_{\text{CRD3-1}}$, $bla_{\text{OSN5-1}}$, $bla_{\text{GRD33-1}}$ $bla_{\text{OSN49-1}}$, $bla_{\text{ALG6-1}}$, $bla_{\text{ALG11-1}}$, and $bla_{\text{DHT1-1}}$) in six of the 10 soil samples analyzed. The crude extract of the metagenomic MBLs showed significant imipenem hydrolysis. Taxonomic classification at the phylum level by RAIphy suggested that six enzymes originated from Proteobacteria, two from Bacteroidetes and one from Gemmatimonadetes. The sequence database mining approach allowed identification of three new resident carbapenemases in *Chromobacterium* sp. strain C-61 (CRS-1), *Chromobacterium haemolyticum* DSM 19808 (CRH-1) and *Chromobacterium piscinae* ND17 (CRP-1), which are species commonly found in aquatic environments. These carbapenemases showed between 68 and 76 % amino acid identity to *Klebsiella pneumoniae* carbapenemase (KPC), suggesting that bla_{KPC} may have evolved from possible ancestor genes resident on the chromosome of members of the genus *Chromobacterium*. Overall we isolated 17 new carbapenemases with 30-76% amino acid identity to previously confirmed carbapenemases. Our results show that the environment including soil and water is a source of highly diverse carbapenemases that are produced by a variety of bacterial species and have not yet emerged in clinical settings. These carbapenemases may constitute potential carbapenem-resistance determinants of clinical relevance if acquired by pathogenic bacteria, as they were functionally expressed in *E. coli*.

Culturing antibotic resistant isolates from the environment

Two hundred strains were cultured from marine environments. Phenotypic analysis suggests that some of the marine isolates (generally epiphytes) are exceptionally broadly resistant to antibiotics (both intrinsic and determined by individual genes) including beta-lactams, colistin (the basis of selection) but also chloramphenicol, kanamycin and derivatives and many others. Further, whilst sensitive to both rifampicin and erythromycin spontaneous resistance emerges at high frequency and in some cases (especially rifampicin resistance) correlated with changes in other phenotypes related to cell organization. One such strain, a *Flavobacterium*, is being focused on in depth. Additionally, resistance to non-clinical antimicrobials, accumulating resistant marine strains, was studied. A strategy of cloning functional resistance genes into *E. coli*, both from beta-lactam resistant strains and for antimicrobials of unknown mechanism (derived from our collection of marine bacteria) is being pursued with success in isolating resistant strains. Library construction is in progress from 10 strains. This effort uses co-culture using MDCC with producer and target strains, often working with limited quantities of antimicrobials. The aim is to identify resistance genes of unusual antimicrobials (antibiotic candidates) and provide insight into mechanism of action. The strain collection now stands at >250 strains. Finally, the ability of swarming bacteria to contribute to the spread of antibiotic resistance was studied. To this end, Swarms of the flagellated bacterium *Paenibacillus vortex* have been shown to collectively transport other microorganisms. It was found that *P. vortex* can invade antibiotic-rich environments by carrying antibiotic-degrading bacteria; this transport is mediated by a specialized, phenotypic subpopulation utilizing a process not dependent on cargo motility. Swarms of beta-lactam antibiotic (BLA)-sensitive *P. vortex* used beta-lactamase-producing, resistant, cargo bacteria to degrade BLAs in their path. In the presence of BLAs, both transporter and cargo bacteria gained from this temporary cooperation; there was a positive correlation between BLA resistance and dispersal. *P. vortex* transported only the most beneficial antibiotic-resistant cargo (including environmental and clinical isolates) in a sustained way.

In addition to the studies described above that aimed at identifying resistant organisms and/or resistance genes in complex ecological entities like the human gut or soil samples, the work performed in WP2 focused on understanding the evolution of Enterobacteriaceae and enterococci from commensal organisms to multidrug-resistant opportunistic pathogens. Bacteria can become resistant to antibiotics by the acquisition of resistance genes or by the accumulation of mutations in the target of an antibiotic, but in order to become successful nosocomial pathogens additional adaptations are often required, e.g. those that contribute to the efficient colonization (and infection) of hospitalized patients.

In this WP EvoTAR scientists worked to understand the evolutionary trajectories that contribute to the emergence of successful clones of multi-drug resistant nosocomial pathogens, using both comparative and functional genomic approaches. In comparative genomic studies, high-throughput sequencing approaches are used to sequence the genomes of a number of strains, in order to determine their evolutionary relatedness and their repertoire of antibiotic resistance genes, mutations associated with antibiotic resistance and other adaptive elements. In functional genomic studies, high-throughput approaches (e.g. transposon mutagenesis and RNA-seq) are used to efficiently study the function of genes in a bacterium. The work in WP2 was divided in three different tasks and the results of these will be summarized below.

Comparative phylogenomics
Work under this task focused on the multi-drug resistant nosocomial pathogens *Escherichia coli*, *Klebsiella pneumoniae* and *Enterococcus faecium*.

Comparative genomics of ESBL-*E. coli*.
A total of 24 ESBL-positive *E. coli* strains from chickens, chicken meat and humans and eight ESBL-positive *E. coli* strains that were isolated from pigs and from farmers that tended to these pigs, were sequenced and analyzed to assess whether resistant strains and/or antibiotic resistance plasmids are able to spread between these different niches. The results of these analyses revealed that genome sequencing could differentiate strains that are indistinguishable by classical sequence-based typing methods. Indeed, the differences within strains that were considered to be identical (i.e. had the same sequence type (ST)), were substantial (more than 4200 SNPs that could be identified), indicating considerable evolutionary divergence between strains in a single ST, and low-likelihood of recent transmission between poultry and humans. This observation is important as this contradicts statements in previous studies, which have claimed that identical strains were spreading from chickens to humans. Additional analyses of the genome sequence data, which were performed in collaboration with the group of Prof. Fernando de la Cruz (University of Cantabria, partner #10 in EvoTAR), have focused on the sequence-based reconstruction of plasmids that carry ESBL genes. This approach revealed that virtually identical ESBL-carrying IncI1/ST3 and IncI1/ST7, as well as AmpC-type β-lactamase-carrying IncK plasmid backbones were shared by genetically unrelated human and poultry isolates, strongly suggesting that ESBLs are mainly disseminated via epidemic plasmids that can spread between different reservoirs.

Comparative genomics of *E. faecium*
In a collaboration with groups at Harvard Medical School (Boston, USA) and the Broad Institute (Cambridge, USA), 51 *E. faecium* genomes were sequenced and analysed. This study revealed that the *E. faecium* population can be split into two major clades (termed A and B), in line with previous analyses. However, our data showed that clade A can be further split in two sub-clades (A1 and A2). Interestingly, the large majority of modern clinical isolates can be assigned to clade A1, while strains from food-producing animals are part of clade A2. Both strains from clade A1 and A2 can acquire resistance to vancomycin, while strains from clade B, which are typically isolated from healthy humans, are not resistant to vancomycin. Additional work on *E. faecium* specifically identified recent recombination events with the adaptation of *E. faecium* to novel niches, such as antibiotic-treated hospitalized patients. Interestingly, flows of recombination were largely congruent with the previously determined genetic sub-populations of *E. faecium*. Particularly, genes that are potentially involved in virulence (such as those coding for the biosynthetic machinery for capsule) or antibiotic resistance, specifically a D-alanyl-D-alanine carboxypeptidase that contributes to resistance to β-lactam antibiotics, are located in genomic regions that are of recombinogenic origin.

In another comparative genomic study, whole-genome sequencing was used to characterize how clinical *Enterococcus faecium* strains evolve during long-term patient gut colonization. To this end, the genomes of 96 *E. faecium* gut isolates, obtained over 8 years from 5 different patients, were sequenced. In addition to these 96 genomes, we also included publicly available genome sequences of 70 *E. faecium* strains to comprehensively describe *E. faecium* genome dynamics. All of the 96 patient isolates were grouped in *E. faecium* clade A, with only one strain clustering in clade A2. The remaining 95 strains were assigned to clade A1. The phylogenetic tree showed 5 clusters of closely related strains of patients, revealing the

microevolution of *E. faecium* strains during gut colonization. Evidence for direct transfer of strains between patients during hospitalization in the same ward was also obtained. In addition to core-genome based analyses, gene gain and loss was also studied, showing that loss and gain of prophages and plasmids is an important factor in generating genetic diversity during gut colonization. This study highlights the ability of *E. faecium* clones to rapidly diversify, which may contribute to the ability of this bacterium to efficiently colonize new environments and rapidly acquire antibiotic resistance determinants.

A core genome MLST (cgMLST) scheme was developed for *E. faecium* to standardize current intra-laboratory surveillance of this nosocomial pathogen. cgMLST transfers genome-wide single nucleotide polymorphism (SNP) diversity into a standardized and portable allele numbering system that is far less computationally intensive than SNP-based analysis of whole-genome sequencing (WGS) data. The *E. faecium* cgMLST scheme was built using 40 genome sequences that represented the diversity of the species. The scheme contained 1,423 cgMLST target genes and was tested using WGS analysis of 103 outbreak isolates from five different hospitals in The Netherlands, Denmark and Germany. The cgMLST scheme performed well in distinguishing between epidemiologically related and unrelated isolates, even between those that had the same 'traditional' sequence type, which denoted the higher discriminatory power of this cgMLST scheme over conventional MLST. The *E. faecium* cgMLST scheme's performance was found to be equivalent to a SNP-based approach. The cgMLST scheme will facilitate rapid, standardized, high-resolution tracing of *E. faecium* outbreaks.

Comparative genomics of *Klebsiella pneumoniae*.
Genome sequencing was performed of three pairs of KPC-producing *K. pneumoniae* strains which were isolated from patients. KPC stands for *K pneumoniae* carbapenemase; this enzyme confers reduced susceptibility to resistance to all β-lactam antibiotics including penicillins, cephalosporins, and carbapenems. The pairs of KPC-producing *K. pneumoniae* strains consisted of a colistin-susceptible strain that was isolated from a patient prior to colistin therapy and a colistin-resistant strain from the same patient that acquired colistin resistance during therapy. In colistin-resistant isolates a non-synonymous mutation in the *pmrB* gene and a disruption of the *mrgB* gene, which was proposed to be involved in the regulation of the PhoP/PhoQ two component system, were identified. These genome-based findings were corroborated by the construction of targeted mutants in *K. pneumoniae*, indicating that the upregulation of the PhoQ/PhoP system and activation of the *pmrHFIJKLM* operon which leads to resistance to polymyxins in *K. pneumoniae* through modification of lipopolysaccharides. The genome sequence data were used to assess how widespread the identified colistin resistance mechanisms are in a collection of 55 colistin-resistant clinical strains of KPC-producing *K. pneumoniae* from Italy and Greece. This analysis showed that *mgrB* inactivation is a common mechanism (detected in approximately 50% of cases), which highlights its clinical relevance. Moreover, the study also showed that different mechanisms of *mgrB* inactivation can be responsible for clinical resistance.

Species-selective metagenomic analysis
In this task we aimed to study the dynamics of the *Enterococcus* and *Enterobacteriaceae* sub-populations in the gut microbiota during hospitalization. Because metagenomic shotgun sequencing cannot accurately detect shifts in species that are present in relatively low levels in feces, culture enrichment needs to be performed. To determine which culture media were appropriate for this study, three enrichment media (Kanamycin Aesculin Azide broth, Enterococcosel broth and Enterobacteriaceae Enrichment broth) and a non-selective medium (Brain Heart Infusion broth) were used to enrich *Enterococcus* and *Enterobacteriaceae* sub-populations from fecal samples of healthy human donors. Enrichment was determined using multiplexed, high-throughput 16S rRNA sequencing on the Illumina MiSeq platform. While in all samples *Enterococcus* and *Enterobaceriaceae* were present at <1% of the population, enrichment led to >60% of *Enterobacteriaceae* in all four samples and >60% of *Enterococcus* in three out of four samples (enrichment for enterococci was unsuccessful in the fourth sample). Based on these data, Enterococcosel broth and Enterobacteriaceae Enrichment broth were chosen for further experiments. Enrichment cultures were performed with fecal samples from seven patients during hospitalization at the Intensive Care Unit. While enrichment for *Enterobacteriaceae* proved to be largely unsuccessful in this patient population (possibly because *Enterobacteriaceae* are eradicated from the gut

microbiota of these ICU patients due to prophylactic antibiotic therapy), enterococci could be enriched from nine out of sixteen samples. DNA was isolated from the fecal samples and these samples have been sequenced. However, analysis has been delayed because non-enriched samples from hospitalized patients and a trial in WP8 were prioritized. Data analysis is taking place currently.

Functional genomics of enterococci and *Enterobacteriaceae*

A system for the generation of a library of transposon resistance mutants in *E. faecium* was developed and initially coupled to a microarray-based transposon mapping approach. This approach led to the identification of three novel determinants of ampicillin resistance in *E. faecium*. The identified intrinsic ampicillin resistance genes are highly conserved among *E. faecium* strains, indicating that this organism has a high potential to evolve towards ampicillin resistance. Using the same high-throughput functional genomic screening, a two-component system was identified in *E. faecium*, which contributes to decreased susceptibility to the disinfectant chlorhexidine and the antibiotic bacitracin. Subsequently, a method for next-generation sequencing based screening of transposon mutant libraries (Tn-seq) was developed for the vancomycin-resistant isolate *E. faecium* E745. This method was used to identify genes that are important for vancomycin-resistance in *E. faecium*. As part of this project, long-read PacBio and Oxford Nanopore sequencing was used to complete the draft genome sequence of *E. faecium* E745, which was previously sequenced by short-read sequencing (Illumina). Several methods for the computational analysis of Tn-seq data were developed (including the quantification of transposon insertions in a 25-nt window and a gene-per-gene analysis). Practically all genes that were identified to be contributing to vancomycin resistance in *E. faecium* E745 belonged to the vancomycin resistance transposon that is present in this strain. A small number of additional genes, with borderline statistical significance, were found to be putatively involved in vancomycin resistance in *E. faecium* E745. However, the targeted deletion mutants that were generated in these genes did not exhibit an increased susceptibility towards vancomycin, compared to the wild-type strains. These data suggest that the vancomycin resistance transposon is solely responsible for vancomycin resistance in *E. faecium*. It can, however, not be excluded that genes that are essential to *E. faecium* (and which therefore cannot be disrupted by a transposon) may functionally contribute to vancomycin resistance.

Changes in biocide and antibiotic susceptibilities, metabolism, and fitness costs were studied in biocide-selected *E. coli* and *K. pneumoniae* mutants. Some strains that developed resistance to the disinfectant triclosan showed marked increases in MICs to several antibiotics, including ampicillin, ciprofloxacin and tetracycline. However, these mutants exhibited significant fitness costs in acquiring resistance. These various phenotypes suggest a trade-off of different selective processes shaping the evolution toward antibiotic/biocide resistance and influencing other adaptive traits.

A comprehensive transposon mutagenesis profiling was performed to identify genes that contribute to antibiotic resistance in the nosocomial pathogen *K. pneumoniae*. Effects on resistance (either increased susceptibility or increased resistance) have now been described and experimentally confirmed for 101 genes. Three mutants were found to exhibit increased susceptibility to cephalosporins and carbapenems. This result is relevant as *K. pneumoniae* is becoming increasingly resistant to these classes of antibiotics and it is of importance to understand the mechanisms by which resistance can emerge with the long-term goal to develop novel anti-infectives targeted against *K. pneumoniae.*

Expanding on the genomic analyses on colistin-resistant *K. pneumoniae* described above, loss-of-function mutations in *mgrB* were further characterized. These mutations are stable and occur without major consequences on fitness and virulence, leading to the efficient dispersal of this particular multidrug-resistant *K. pneumoniae* clone. Interestingly, *K. pneumoniae* ST512 was found to have a higher mutation frequency than other clones, potentially contributing to its ability to become colistin-resistant.

Evolution of antibiotic resistance and fitness costs

Studies in *Salmonella* were performed in WP2 to identify mutations that lead to antibiotic resistance (against streptomycin, colistin and meropenem) at concentrations 10-fold above the Minimum Inhibitory Concentration (MIC) and 0.2-fold below the MIC. Completely different mutations accumulate at low concentrations, compared to those that occur at high antibiotic concentrations. Nevertheless, even accumulated mutations at concentrations below the MIC lead to high-level resistance, indicating that

exposure to low levels of antibiotics may still lead to the emergence of highly resistant bacterial populations.

In another study, the compensation of fitness costs of resistance (severely impaired growth rate associated with resistance due to absence of two major outer membrane porins) was studied in *E. coli*. An evolution experiment was performed with 16 lineages of *Escherichia coli in which the ompCF* genes were deleted with reduced fitness and increased resistance to different classes of antibiotics, including the carbapenems ertapenem and meropenem. After serial passaging, the relative growth rate increased to near-wild-type levels, due to (a) compensatory mutations in genes leading to constitutive high-level expression of the PhoE porin or (b) mutations in *hfq* and *chiX* genes that disrupted Hfq-dependent small RNA regulation, causing overexpression of the ChiP porin. These findings may explain why porin composition is often altered in resistant clinical isolates, thereby providing new insights into how bypass mechanisms may allow genetic adaptation to a common multidrug resistance mechanism.

Measurements of fitness costs.
We (WP7) measured the fitness costs of mutational resistance mechanisms during growth under various types of *in vitro* and *in vivo* conditions and in different genetic contexts. Mutations were introduced into different bacterial species and clones to assess the impact of species and clone characteristics on fitness costs. Growth characteristics have been measured in single culture and during competitions between susceptible and resistant strains. Similarly, we have analysed potential epistatic effects between different types of resistance mutations by combining them in all possible combinations. Our main findings are the following:

a) We demonstrated that the fitness and resistance effects of any given resistance mutation is largely independent of the genetic background used. Thus, when introducing the same resistance mutations (*rpsL*-streptomycin resistance, *rpoB*-rifampicin resistance, *fusA*-fusidic acid resistance and *gyrA*-fluoroquinolone resistance) into four different Salmonella strains that vary in their DNA sequence, no differences could be seen with regard to how much fitness was reduced and how much resistance was increased. This indicates that epistatic effects are not very strong for these chromosomal resistances.
b) We analyzed potential epistatic effects between five different types of resistance mutations by combining these five mutations in all possible combinations. We observed few cases of strong epistatic interactions for these mutations and instead their combined fitness effects were largely additive.
c) We examined the fitness effects of mutations that confer resistance to tigecycline, mecillinam and colistin. In general, these resistance mutations conferred a reduction in fitness ranging from a few percent up to 50%.
d) We studied the fitness costs and distributions of different transposons carrying the *vanA* gene, contained in different plasmids (variety of genetic backgrounds) in a well-defined collection of vancomycin resistant *Enterococcus* responsible for hospital outbreaks around the world. Our results suggest that various Tn*1546* variants could spread in their plasmid vehicles across bacterial populations, but only a limited number of hosts ensure their stable maintenance and that differences in fitness might explain the particular association of particular genetic configurations with particular clones and species.

Evolutionary potential of low-level resistance genes.
To assess the evolutionary potential of resistance genes we (WP3) identified sets of genes conferring low-level resistance to either fluoroquinolones or beta-lactams. The two drug classes differ significantly in the ability of chromosomal mutations to lead to high-level resistance phenotypes. In these studies we focused on two main parameters that would influence the risk of such low-level resistance genes to become high-level resistance genes. First, we assessed the stability of plasmids expressing low-level resistance genes in absence of antibiotic selection pressure. Second, we assessed the extend to which evolution of the plasmids with low-level resistance genes could contribute to high-level resistance phenotypes in a host strain subjected to antibiotic selection pressure.

Stability of low-level resistance genes.

Stability of low-level flouroquinolone resistance genes
Using metagenomic functional selections we identified 20 novel *qnr* genes conferring low-level resistance towards the flouroquinolone antibiotics. The stability of 20 plasmids containing genes encoding different Qnr-like proteins was analysed. Eight of the plasmids where shown to be unstable, indicating that fitness costs associated to the presence of plasmid-encoded resistance genes are allele specific. The other 12 genes were stable, which and do not produce relevant fitness costs.

Stability of low-level beta-lactam resistance genes
In contrast to the *qnr* genes, previous work have shown that plasmid-encoded beta-lactamases can evolve *in vivo* under antibiotic selective pressure. To assess this possibility we selected genes from functional metagenomic libraries conferring low-level resistance to beta-lactams. We focused on clones containing putative beta-lactamases, PBPs or hypothetical proteins. We did not take into consideration clones containing genes encoding regulators as MarA, since the role of these genes on resistance depends on the presence in the host genome of the genes they regulate. In total we focused on 6 low-level beta-lactam resistance genes. Adaptive evolution of the lineages containing each of the 6 resistance genes in absence of selective pressure showed that the plasmids were very stable. This data indicates that the fitness cost of expressing the low-level beta-lactam resistance genes was low.

Evolvability of low-level resistance genes

Potential of low-level flouroquinolone resistance genes to become high-level resistance genes.
The 12 stable *qnr* genes from described above were subjected to evolution by sequential sub-culturing in increased concentrations of quinolones. After 25 days (around 200 generations), the MICs to quinolones of the strains carrying these plasmids increased by several fold. The evolved plasmids were extracted and used to transform a wild-type strain. The aim was to establish whether the mutations leading to quinolone resistance were in the low-level resistance genes or genomic. In parallel, the genes coding the Qnr-like proteins present in the evolved plasmids were sequenced. None of the evolved plasmids increased the MICs for quinolones to the level observed in the evolved strains when they were re-introduced in a wild-type strain suggesting that the mutations leading to quinolone resistance in the evolved strains were chromosomally encoded. In agreement with this hypothesis, none of the sequenced *qnr* genes presented any relevant mutation. Our results indicate that the risk that Qnr-like elements evolve towards high-level quinolone resistance is not high, likely because mutations at topoisomerase genes are easily selectable in the presence of quinolones. This result is in agreement with information concerning QnrA and SmQnr published at (Sanchez, M. B.; Martinez, J. L., PLoS ONE 2012, 7, e35149). If this hypothesis holds true, the different alleles of *qnr* genes currently present in human pathogens should not have evolved under quinolone selective pressure at clinics, but rather represent different acquisition events. Following this work we analysed the structure of plasmids containing *qnrA* and *qnrB* genes so far present at public databases. Our results indicate the different *qnrA* or *qnrB* alleles have polyphyletic origins suggesting that the *qnr* genes currently present in the plasmids of human pathogens are not the result of evolution under antibiotic selective pressure in clinics.

Potential of low-level beta-lactam resistance genes to become high-level resistance genes.
The 6 low-level beta-lactam resistance genes where introduced into an *E. coli* strain defective in *mutS* and *ampC* and evolved in ampicillin and cefotaxime. In all cases, strains evolved to acquire high-level resistance to the drug used. For the ampicillin-evolved clones, plasmids were rescued and an *ampC* minus, but *mutS* proficient strain was transformed with both the original and the evolved plasmids. In all cases, the evolved plasmid conferred higher resistance than the original one, indicating that mutation was in the plasmid. Noteworthy, the mutations present in the plasmid were synonymous and did not alter the amino acid sequence. This could indicate that evolution of elevated beta-lactam resistance could result from different protein expression levels resulting from differential codon usage.

As indicated above evolution and emergence of antibiotic resistance is not only the result of mutations but also of acquisition of resistance genes importantly through transfer of plasmids carrying resistance genes. In WP6 the dynamics of antibiotic resistance transfer within bacterial populations was studied and

novel methods were developed to quantify resistance transfer under controlled conditions in bacterial communities. To achieve these objectives, and according to EvoTAR technical annex, work was divided in six specific tasks.

Characterization of environmental factors that affect plasmid stability and propagation.
We compared the conjugation kinetics of representatives of five prevalent plasmid groups (after constructing derivatives labeled with fluorescent protein reporters), in an effort to gain insight about potential differences that can explain the prevalence of different plasmid groups. The repressed (wild type) versions of IncF and IncI plasmids, although very prevalent in enterobacterial populations (specifically *E. coli*), showed reduced conjugal infectivity. This result suggests that alternative fitness components are important in determining the success of these plasmids. One potential compensation probably originates from the decreased burden that repressed plasmids cause on the host bacterial population. More work needs to be done to define these compensatory effects more rigorously. In general, it can be concluded that each of the five tested plasmid groups has some specific properties that affect its conjugation kinetics and, thus, its infectivity of susceptible recipient populations. These differences can contribute to a rational explanation for the prevalence of different plasmids in enterobacterial populations. Obviously, infectivity rates are not the sole cause to explain differential prevalence. Nevertheless, our results show that infectivity rates can represent a significant contribution and, perhaps more importantly, that each plasmid group shows differential parameters that are experimentally testable. When combined with the analysis of differential stability in different hosts under different conditions, this type of analysis can lead to a sort of "specification sheet" that defines a set of relevant parameters for each plasmid group.

Identification of signals that trigger conjugation
Studies on plasmid conjugation kinetics led to a general hypothesis of the role of transcriptional overshooting in plasmid conjugation and genome rebooting. The main achievement was the elucidation of plasmid R388 transcription control system, and the finding that transcriptional overshooting probably provides the main systems-level control of plasmid conjugation. The signals that trigger conjugation are thus endogenous, rather than environmental (fitness changes of recipients themselves after conjugation cause the observed variations in apparent conjugation frequencies). Another significant result was that plasmid R388 is able to infect a recipient population based on privileged donor multiplication. The reason for this effect is not known. It seems plasmid R388 can cause some detrimental effect on the recipient population. This is comparable to the effect of bacteriocin, although R388 is not known to code for any bacteriocin. Other, more complex, causing mechanisms can be envisaged (conjugation-induced killing, etc.). Besides, the cost of gene amplification on fitness and stability was quantified for an IncI plasmid (Enterobacteriaceae, γ-proteobacteria) and for plasmids of different families (Enterococci, Firmicutes).

Quantitation of fitness costs incurred by plasmid carriage.
The results obtained, both in Gram-positive as well as Gram-negative bacteria, show that fitness costs of carrying resistant plasmids are strongly dependent on the particular plasmid, the genetic host and the growth conditions. Thus, fitness costs vary between low (undetectable) to up to 20% or even more, depending on conditions. Comparative genomics was used to analyze compensatory mutations in evolved strains. Results showed that adaptive mutations and indels occur either in the chromosome or in the plasmid. Several chromosomal mutations and indels were associated to genes involved in key cellular functions. The majority of the changes observed in evolved plasmids were due to deletions associated with key functions (replication, conjugation and maintenance) or accessory genes (antibiotic resistance, plasticity – IS elements). These genome alterations might be responsible for the fitness differences observed between evolved and non-evolved strains. Preliminary data show that evolved strains show improved fitness compared to their non-evolved counterparts. In a particular case studied in detail, when antibiotic selective pressure was applied, rapid amplification of the resistance-conferring gene to high copy numbers occurred. When selection was relieved, the amplified array rapidly disappeared. This mechanism provides a rapid and reversible adaptive mechanism for bacteria to increase their resistance under strong selective pressures. Because of the high instability of the amplified arrays, it is likely that the clinical microbiology laboratory will typically miss the importance of this type of mechanism. The complexity of factors involved in plasmid fitness cost is also supported by results obtained from studies

performed with two epidemic plasmids with a major role in the emergence and dissemination of CTX-M-type ESBLs in Bolivia. Despite the natural history of those plasmids (the IncA/C plasmid was displaced by the IncI1 plasmid), in vitro experiments performed in diverse experimental conditions did not provide evidence explaining such epidemiological change. Ongoing studies might contribute in identifying predictor markers for plasmid success.

Evolution of plasmid host-range

Important observations made by other authors (e.g., the group of Eva Top, University of Idaho) indicate that important changes in plasmid host range occur easily within a given backbone and depend on quick adaptive mutations. The host-range itself is not specific of a given plasmid group, but changes widely among very similar plasmids. These results, which were published between the writing of EvoTAR and the beginning of work, affected the type of experiments that were carried out by EvoTAR members and, in general, diminished the interest in finding specific mutations that affect host range. Nevertheless, some effort was put to analyze the mechanisms of evolution of plasmid host-range in specific cases. When using Gram-positive plasmids, results showed that multireplicons can be a strategy to broaden the host range of antibiotic resistant plasmids among opportunistic pathogens sharing common habitats. When using ColE1-like plasmids and studying their adaptation to *E. coli*, it was found that plasmids rapidly evolve increased stability, by either point mutations or IS-element insertions. When several plasmids coexist, the overall burden is the additive value of the individual burdens. As anticipated, the problem with these studies is the difficulty to generalize, due to the plasmid individuality in the changes that affect host-range.

The natural history of plasmid adaptation

As a general approach to the problem, we developed a plasmid reconstruction method for Illumina whole genome datasets, called PLACNET. PLACNET allowed us to achieve a giant leap in the analysis of natural history of plasmid adaptation, by providing massive data for comparative genomics. The implications of this method in the analysis of the natural history of plasmid adaptation (both in Gram-negatives and Gram-positives) is exemplified in several publications of high impact. Among other results, we select two important conclusions as examples of the applications of this bioinformatics technology: the turbulent plasmid flux in *E. coli* ST131 (a measure of the speed of plasmid evolution compared to core-genome evolution), the transfer of epidemic plasmids from animal to human *E. coli* isolates as the cause for dissemination of ESBLs, and the characterization of the Firmicutes plasmidome. Besides the grand scenario, EvoTAR also tackled the natural history of specific examples of antibiotic resistance dissemination. Analysis of *E. coli* plasmids encoding cephalosporin resistance from various origins, indicates that spread of ESBL-encoding plasmids occurs by HGT among *E. coli* lineages. The finding of nearly identical plasmids in different Enterobacteriaceae and in epidemiologically unrelated individuals suggests that such plasmid lineages possess traits involved in host adaptation (e.g., IncHI1 plasmids carrying a sugar metabolic element likely enhancing *E. coli* fitness in the equine gut). Findings show that specific plasmid lineages contribute to global ESBL spread within host species with limited overspill between hosts. With respect to specific studies on Gram-positive plasmids, the persistence of VanA-type VanR 15 years after the ban of avoparcin was shown. *vanA* was mainly linked to a specific plasmid lineage that was non-typeable and did not carry genes conferring resistance to antimicrobials used in poultry production. Findings suggest adaptation of such plasmid to *E. faecium* in the avian gut. An additional study comparing clinical and avian VRE isolated in Denmark is ongoing. Preliminary results suggest that vanA-encoding plasmids in human and avian *E. faecium* are not directly linked.

Analysis of antibiotic resistance gene transfer in controlled environments

We attempted to construct a device that could be used as a universal conjugation sensor by taking advantage of the SOS-response provoked by conjugation. All attempts failed, so we are still trying today to achieve this goal, which we consider will be a useful addition for the antibiotic-resistance analysis toolbox. Therefore, we returned to more classical means for studying antibiotic-resistance transfer in controlled environments mimicking natural ecosystems. We developed two such systems: a freshwater microcosms and a mouse societal model. In both systems, proof of principle was obtained that we can track antibiotic-resistance transfer. This will allow detailed studies of the effects of system and

environmental parameters on the dissemination of antibiotic-resistance. Experiments to test the efficacy of conjugation inhibitors on both systems are underway.

A major objective of the EvoTAR project in general, and of WP7 in particular, was to develop generic and predictive models that allow a detailed description of the within-host dynamics and between-host dynamics of antibiotic resistance modules (genes, genetic elements, clones) and that will quantify the probability and rate of emergence and spread of resistance-conferring genes/mutations under various environmental conditions, different selective pressures and in different genetic backgrounds. This modelling-based approach is essential for the prediction of the risk that a given antibiotic resistance gene may successfully spread to pathogens and thereby contribute to future resistance problems and how changes in the selective pressures influence rates of spread of antibiotic resistance at the population level. To provide parameter values for the various models, experiments were performed to determine fitness of various single and multi-drug resistances in combination and in different genetic backgrounds. Below we summarize the major findings from this work under four different headings—within host level models, between host level models, mixed level models and measurements of fitness costs.

Within host (individual) level models

In silico pharmacokinetic-pharmacodynamic (PKPD) models can be developed based on data from *in vitro* time-kill experiments and can provide valuable information to guide dosing of antibiotics. We developed a mechanism-based *in silico* model that can describe *in vitro* time-kill experiments of *E. coli* wild type, and six isogenic mutants, exposed to ciprofloxacin and to identify relationships usable to simplify future characterizations in a similar setting. The developed model includes susceptible growing bacteria, less susceptible (pre-existing resistant) growing bacteria, non-susceptible non-growing bacteria and non-colony-forming non-growing bacteria. A common model structure with different potency for bacterial killing for each strain successfully characterized the time-kill curves for both wild type and the six *E. coli* mutants. Our results show that the model-derived mutant-specific EC_{50} estimates were highly correlated with the experimentally determined MICs, implying that the *in vitro* time-kill profile of a mutant strain is predictable by the MIC alone based on the model.

Between hosts (population) level models.

Efficient national surveillance for healthcare associated infections. A general model framework for the spread of a disease over a network of connected nodes was developed. To validate this model and illustrate the potential use of it, we simulated the transmission of a novel HCAI that spreads predominantly by direct patient movement between Scottish hospitals as a result of patient movements, i.e. with little or no transmission in the community, and extend it by comparing existing surveillance programs with a (putative) optimal program to see if, with easily acquired information on the network of patient transfers, existing national surveillance schemes can be made more efficient. The model enables us to prioritise hospitals for inclusion in a laboratory surveillance system and thereby to address two key questions: 1) What is the optimal distribution of surveillance effort across hospitals and is the current system maximally efficient; and 2) Would there be benefits from increasing or decreasing the number of hospitals engaged in surveillance? Our analyses show that the current surveillance system, as it is used in Scotland, is not optimal in detecting novel pathogens when compared to a gold standard. However, efficiency gains are possible by better choice of sentinel hospitals, or by increasing the number of hospitals involved in surveillance. Similar studies could be used elsewhere to inform the design and implementation of efficient national, hospital-based surveillance systems that achieve rapid detection of novel HCAIs for minimal effort.

Multistrain network model. The first model was extended by incorporating the possibility to simulate the spread of multiple "strains" on a network, leading to a generic model to study the spread of different resistances (genes or plasmids) between hosts. To illustrate the potential use of this model a similar approach was used as with the first model. With this model it is possible to predict where potential hotspots are for the emergence of multidrug-resistant strains. To illustrate this, we calculated the frequency of each individual hospital to be the first hospital to be co-colonised with the two strains over 20000 simulations. The results show that some hospitals are (on average) more frequently the first to be

co-colonised then others. Identification of these hospitals show that mainly (large) teaching hospitals are hotspots for the emergence of multidrug-resistant strains (there are seven teaching hospitals in Scotland and the seven most frequently co-colonised hospitals are those seven hospitals, followed by large community hospitals).

The effects of population structure on the long-term prevalence of antibiotic resistance.
To study the impact of population structure on the long-term prevalence of an antibiotic resistant strain a novel model was developed. The results of this model show that having a batch-structure might be beneficial for keeping antibiotic resistance levels low. The main finding of this model is that having a continuous structured population (like a human population) might lead to a higher long term prevalence of an antibiotic resistant strain than a batch-type structured population if all other parameters are kept the same and that a batch-structure population is much harder to invade than a continuous birth-death population, i.e. to reach comparable prevalence levels between the two population structures the "spontaneous creation rate" of an antibiotic resistant strain needs to be much higher in the batch-structure population compared to the continuous birth-death population.

A hospital-level risk factor analysis of Staphylococcus aureus bacteraemia in Scotland. The outcomes of the first model (specifically the connectivity of hospitals) were also used in a hospital-level risk factor analysis for *Staphylococcus aureus* bacteraemia cases in Scotland. The aim of this study was to identify risk factors for the presence and rate of MRSA bacteraemia cases in Scottish mainland hospitals. Specific hypotheses regarding hospital size, type and connectivity were examined. In Scotland, although hospital size is a significant predictor of the presence and rate of MRSA, it does not fully explain all the observed variation among hospitals. In this study we found that in Scotland, there is a certain level of connectivity above which the majority of hospitals, regardless of size, are positive for MRSA. Higher levels of MRSA are associated with the large, highly connected teaching hospitals with high ratios of patients to domestic staff.

Antimicrobial prescribing and its relationship with antimicrobial resistance in MRSA. The specific aims of this study were two-fold. Firstly, to examine spatial and temporal trends in Scottish primary and secondary care prescribing rates. Secondly, to investigate whether or not there were any associations between primary or secondary care prescribing rates and antibiotic resistance in the MRSA population. To address this, there have been calls for improved antimicrobial stewardship to better regulate drug usage, as well as improved surveillance, monitoring and regulation. Firstly, it was found that antibiotic usage of several antimicrobials increased and the rate of this increase should be monitored to prevent extreme over-use and drugs potentially becoming obsolete. Secondly, the rate of prescribing of different antimicrobials differed between HBs and over years which could be due to several factors but likely mirrors differing HB-specific prescribing guidelines but also represents a lack of consistency in treatment. Thirdly, resistance was found to be associated with prescribing rates for three antimicrobials over this study period, although there are also likely to be other factors contributing to resistance (for example historic prescribing).

Global disease burden due to antibiotic resistance – state of the evidence. The absence of comprehensive and reliable estimates of the global health burden due to antibiotic resistance makes it difficult to assess trends and harder to justify the allocation of adequate resources to deal with the problem. Quantification of the burden of resistance requires data on the incidence of clinical conditions appropriately treated with antibiotics, the frequency of treatment failures due to resistance and their impact on clinical outcome. Treatment failures in turn depend on the level of resistance in the aetiological agent to the antibiotic used. These data are not easily obtained, as illustrated by a case study of neonatal sepsis. One obstacle is that global health statistics as currently collected do not provide the necessary information. Improving this situation will require changes to the ways in which global health statistics are collected. The primary benefit will be more accurate assessment of the global disease burden due to antibiotic resistance and its forward trajectory, helping make the case for investment in combating the problem.

The utility of Whole Genome Sequencing of Escherichia coli *O157 for outbreak detection and epidemiological surveillance.* This study assessed the utility of whole genome sequencing (WGS) for

outbreak detection and epidemiological surveillance of *Escherichia coli* O157, and the data was used to identify discernible associations between genotype and clinical outcome. The results show WGS data can provide higher resolution of the relationships between *E. coli* O157 isolates compared with MLVA. The method has the potential to streamline the laboratory workflow and provide detailed information for the clinical management of patients and public health interventions.

Intercontinental exclusion of community-associated MRSA subtypes with distinct antibiotic resistance gene profiles. We investigated the evolution and global spread of ST59, a pandemic, community-associated clone of *S. aureus*, which is found globally and is a major cause of skin and soft tissue infections in south-east Asia. We showed that two distinct ST59 clades emerged independently, one in Taiwan and the other in the USA. Whilst both clades also contained sequences from Australia and Europe, no exchange of strains between Taiwan and the USA was observed in either direction. We also found that strains in the Taiwan clade possessed a greater number of both antibiotic resistance and virulence determinants than the USA clade. Using growth experiments in the laboratory, we demonstrated that ST59 strains from the Taiwan and USA clades were able to out-compete USA-300, the dominant community-associated strain in the USA. Our findings are consistent with the hypothesis that differences in antibiotic usage in the USA and Taiwan, and competition with other *S. aureus* strains including the dominant community-associated strain in the USA (USA-300), could explain the lack of transfer of ST59 between Taiwan and the USA.

Novel transmission model. We have established an innovative, cheap, and reproducible experimental model of transmission of information about antibiotic resistance using two large (>50 individuals) populations of the cockroach *Blatella germanica* placed in two compartments, one with frequent antibiotic exposure (mimicking a hospital), and the other with minimal antibiotic exposure (the community), with a certain rate of migration between the environments. This experimental model can be used to address how population structure, density, migration, antibiotic pressure etc. influences the transmission of resistant bacteria in a population and between different environments and compartments.

Mixed level models.
We developed a computational multi-scale modeling to connect performances arising at distinct scales. These models are known as nested or embedded models and have been used to address specific questions involving within-host dynamics enclosed in a model of between-host epidemiological scenarios. This approach requires a nested model because the different units involved in resistance are nested units of selection across distinct scales of the subcellular, cellular and supra-cellular environmental levels of the ecosystem, including communities of hosts. Thus, any alteration of the carriers of any particular resistance trait, or its mechanisms of variation and mobilization (mutation, recombination, transposition, lateral gene transfer, migration) may influence the dynamics of other units of higher and lower hierarchy and thus have evolutionary and/or ecological consequences on a bacterial population. The difficulty for modeling this type of ecosystem scenarios with nesting has been an important limitation to convincingly study processes of AR evolution, but exciting new opportunities have recently arisen from a natural computing formalism inspired on the structure and functioning of biological cells, called membrane computing. Membrane-computing considers that any biological system is a hierarchical construct where the flow of materials can be interpreted as computing processes. In particular, membrane computing offers a versatile framework known as P-system that consists of a hierarchical membrane structure of nested compartments (regions) where multisets of objects are located and can move across the resulting "membranes" and evolving according to a finite number of given rules. Using this approach, we developed a new computational approach designed for computing at more than three levels of organization, subcellular, as genes or plasmids, cellular, and, supra-cellular through the software implementation of a simulator Antibiotic Resistance Evolution Simulator (ARES). ARES will facilitate predictive computational models on the potential trans-hierarchical response of antibiotic resistance to particular interventions in specific scenarios.

Two different approaches were pursued in EvoTAR WP8 to interfere with the selection and dissemination of antibiotic resistance. The first approach aimed to reduce the free antibiotic concentration

in the gut, thereby reducing the risk antibiotic resistance selection. The second approach aimed at interfering with conjugative transfer of antibiotic resistance genes.

Reducing antibiotic concentrations in the gut.
The vast majority of orally administered antibiotics are only partially absorbed into the blood reaching the intestinal tract, and for some of them, a significant part of the administered drug remains intact before reaching the colon. A similar phenomenon occurs for parenterally administered antibiotics that are recycled, via the hepatobiliary route, from the blood into the small intestine. Thus, for both oral and parenteral antibiotics, active residues reach the colon at doses that are lethal for most commensal bacteria. These residues thereby provoke serious collateral damage amongst the intestinal microbiota of patients: their gut microbiota balance is disturbed; several bacterial populations are erased whereas other strains proliferate. Antibiotic-treated patients' microbiota will need several months to recover. This phenomenon brings along harmful consequences as antibiotic resistant bacteria are selected in the gut. It indeed plays a key role in the onset of *Clostridium difficile* infections (CDI) by allowing resistant *Clostridium difficile* bacteria to outnumber other intestinal bacteria, causing very painful and deathly diarrhea. In addition, selection of resistant bacteria in the gut also leads to increase global antibiotic resistance as resistance genes are passed on to the many strains of bacteria present in the commensal flora via exchange mechanisms.

In EvoTAR, partners in WP8 contributed to the development of two microbiota-focused interventional products: DAV132 for human health and DAV133 for animal health, helping innovation to translate into marketed products.

DAV132 is a groundbreaking approach whereby a highly efficient adsorbent, with a specific intestinal delivery technology, can be co-administered with virtually any antibiotic. DA132 is developed to prevent CDI, to avoid the havoc that antibiotics wreak upon the gastrointestinal tract as they are eliminated from the body, and eventually dramatically reduce antibiotic resistance development. DAV132 inactivates the unwanted fractions of the antibiotic that reach the lower digestive tract and remediates it through stool, after the antibiotic has been systemically absorbed to fight infections. In EvoTAR, clinical batches of DAV132 were manufactured in compliance with all standard regulations and then used in a clinical study performed in France with 44 healthy volunteers. The study was randomized with 4 groups: 14 volunteers received oral moxifloxacin (a widely used quinolone antibiotic), 14 volunteers received moxifloxacin associated with DAV132, 8 received DAV132 alone and 8 received no treatment at all. The study demonstrated that, in humans, DAV132 was well tolerated. The metagenomic analysis performed in EvoTAR also illustrated that DAV132 protects humans from fecal microbiome disruption after oral moxifloxacin treatment, without decreasing the efficacy of the antibiotic (no change in the plasma concentration). It is the first time a product successfully achieves this result and it opens the way for a novel use of antibiotics with much less harmful consequences. The work of the EvoTAR program was immensely helpful in the clinical development of DAV132. Upon these foundations, the development work will continue with the hope DAV132 product will be introduced on the market in some years.
DAV133 relies on a similar mechanism of action to preserve animals' microbiota from antibiotic disruption and particularly avoid the selection of resistant bacteria. The medical interest of this approach is to limit the carriage of resistant bacteria in livestock and companion animals to thwart the transfer of antibiotic resistant bacteria from animals to humans. In this regard, DAV133 is much in line with the One Health approach stating the importance of taking into account animal health questions to improve human health. In the context of the EvoTAR program, DAV133 was optimized for animal use (special formulation, specific administration scheme), manufactured and then tested in an animal clinical study. This also vastly contributed to the development of the product.

Interfering with conjugative transfer of antibiotic resistance genes.
Transfer of antibiotic resistance plasmids from one bacterium to another relies on the use of different conjugation systems that were identified as potential targets for the development of inhibitory treatments that could reduce the spread of antibiotic resistance. Instituto Biomar participated in the Evotar project with the aim of establishing the proof of concept for this approach, focusing on the inhibitor of conjugation AD0149, previously identified in collaboration with other Evotar partner, Universidad de

Cantabria. Loss of the conjugation inhibitory properties of the compound upon purification over 95% indicated that the inhibitory activity was associated with minor components of the original sample that could not be identified despite strong efforts in that direction. This barrier resulted in an intense screening campaign, that led to the identification of several inhibitors, being the family of the Tanzawaic acids the most potent. These results will be published in the next months.

Characterization of the new inhibitors highlighted the need for chemical modification of the structure to improve water solubility, a very relevant property, as the intended system to evaluate the compound was an aquatic microcosm, and also for the potential application of the inhibitors in aquaculture. Finally, through characterization of the modified compounds and some additional candidates in the screening system, a demonstration of the capacity of some inhibitors of conjugation of blocking transfer of antibiotic resistance in a bacterial population was provided. These results, obtained in collaboration with Universidad de Cantabria, support the potential application of conjugation inhibitors in the control of antibiotic resistance spread. Some challenges, related to the potency requirement, cost and regulatory issues will need to be resolved to allow the establishment of conjugation inhibitors as real tools in the control of the resistance problem. Future work with models resembling use of antibiotics in hospitals or farming settings will help resolving these pending challenges.

Summarizing conclusions of the main results

The aim of EvoTAR was to characterize humans, animals and environmental reservoirs of antibiotic resistance genes to study the dynamics within and interactions between these reservoirs. In five figures, we have tried to summarize important findings of the EvoTAR with respect to its aim. In these figures numbers indicate data generated within the EvoTAR project. Below every figure this is explained in more detail. Figure 10 summarizes all findings that are detailed in figures 6-9.

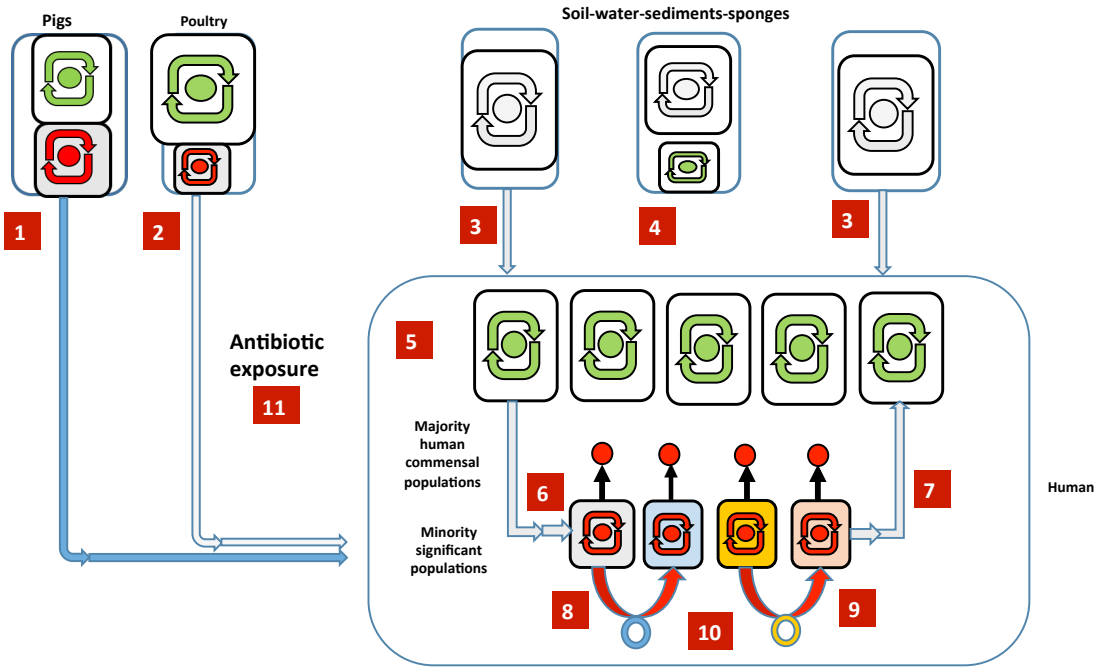

Figure 6. Studied reservoirs and observed links between reservoirs of antibiotic resistance genes, mobile genetic element and strains. Red coloured and numbered squares refer to data generated within the EvoTAR project.

1. Only few cases of *E.coli* or *E. faecium* clones shared by humans (farmers) and food animals, pigs can be documented (WP2). However, pigs and humans share a number of antibiotic-resistance

genes as detected by gene capture, and pigs are highly enriched in a high diversity of resistance genes (WP4). Clones and resistance genes are frequently maintained within the pigs. Meta-transcriptomic datasets from intestinal microbiota from pigs demonstrate that genes are locally expressed.

2. Whole genome sequence studies reveal low-likelihood of recent transmission of ESBL-*E.coli* clones between poultry and humans (WP2). Also *vanA*-encoding plasmids in human and avian *E. faecium* are not directly linked (WP6). However, some plasmids (IncK plasmid backbones) are shared by avian and human Enterobacteriaceae (WP6); in general promiscuous plasmid transfer among bacteria of different reservoirs is possible (WP2) but infrequent.
3. Isolates from marine environments are infrequently resistant, but some of them are multi-resistant. In soil there are bacteria with resistance againt vancomycin and also with resistance against carbapenems that can be expressed in *E.coli*; there is no evidence of transfer to human isolates (WP5).
4. Isolates from water bacterioplankton, sponges, water sediments might contain resistance genes that are locally expressed, suggesting a role in ecological adaptation unrelated with antibiotic resistance (WP5). Transfer to humans was not documented.
5. Bacteria of the predominant commensal taxons in the intestinal human microbiota contain a wealth of genes (intrinsic resistome) able to provide resistance to the bacterial host (WP4). These genes are locally expressed under antibiotic exposure, which explain the maintenance of richness during therapy (WP1), but are very unfrequently transmitted to significant bacteria for public health. Low-level antibiotic resistance genes are frequently detectable (WP3), but not clear evidence of evolution to high-level resistance was found.
6. Significant bacteria (significantly pathogenic) for human health, as *Enterobacteriaceae* or *Enterococcus* are present in <1% of the microbiome population. These organisms have genes of the intrinsic resistome (WP2), and are the populations with a higher density of significant resistance genes (WP4). Under therapy these populations reach high densities significantly increasing the number of resistance genes in the human intestinal microbiome (WP1).
7. There is no clear evidence of significant rate of transfer of these genes from potentially pathogenic populations to majority taxons, but that might happen (genetic exchange communities, for instance *Enterococcus-Lactobacillus-Clostridium*) (WP4).
8. The increase of the number of resistant organisms resulting from antibiotic selection facilitates the spread of plasmids containing antibiotic-R, among closely-related clones or taxons (WP5 and 6).
9. There is a solid link of plasmids and host lineages, suggesting epistatic genome-plasmid robustness and/or contribution to local adaptation (WP6, WP7).
10. Some inhibitors of plasmid transfer by bacterial conjugation, as tanzawaic acid derivatives, have been found (W8) which might limit plasmid spread under antibiotic selection.
11. Short-term antibiotic exposure do not produce a significant enrichment of antibiotic resistance genes in the microbiome (WP1). Very long-term exposure significantly increases overall level of resistance genes, even if the richness is decreased. Standard (hospital) antibiotic exposure do not decrease richness, but the number of resistance genes is increased, probably because of the selection of minorities (WP1, WP4). Reduction of antibiotic exposure can be achieved by adding novel charcoal-based products aiming to adsorb residual antibiotics in the colon while not interfering with the duodenal/jejunal absorption (WP8).

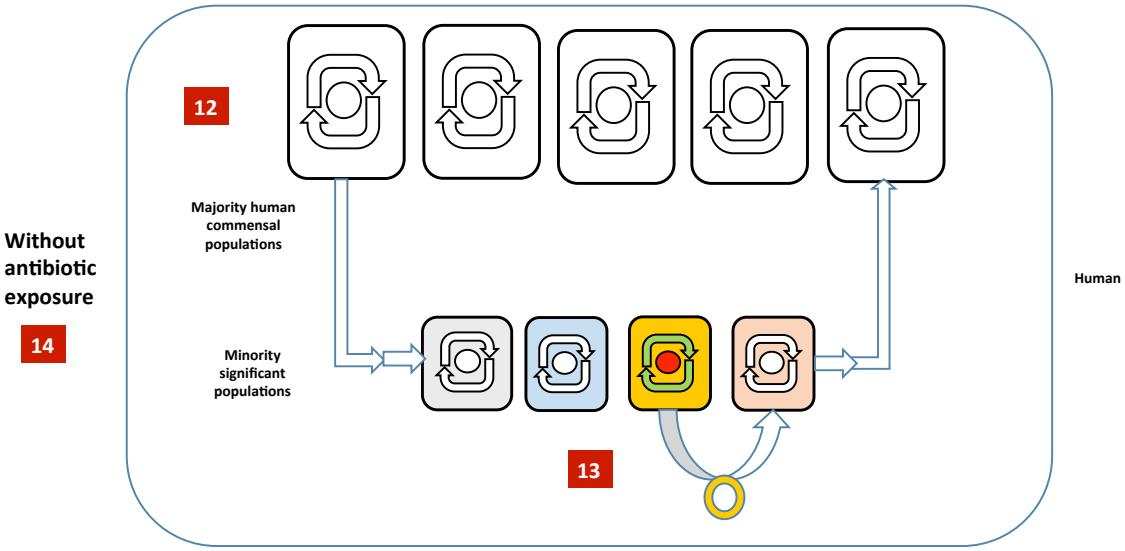

Figure 7. The human resistome in the absence of antibiotic exposure.

12. Upon discontinuation of antibiotic exposure, the intrinsic resistome of the majoritarian taxons is less active; the minority populations returns to their normal abundances, with decrease of the total density of detectable antibiotic resistance genes (WP3).
13. Some mobile genetic elements encoding resistance traits imposes a very small fitness cost to their hosts, and the consortium is stably maintained over time (WP7) and suggest influences on host ecological adaptation (WP6).
14. Starting after one month, after three-six months of antibiotic discontinuation the populations of the intestinal microbiome reach the equilibrium point, returning to the original population structure (WP1).

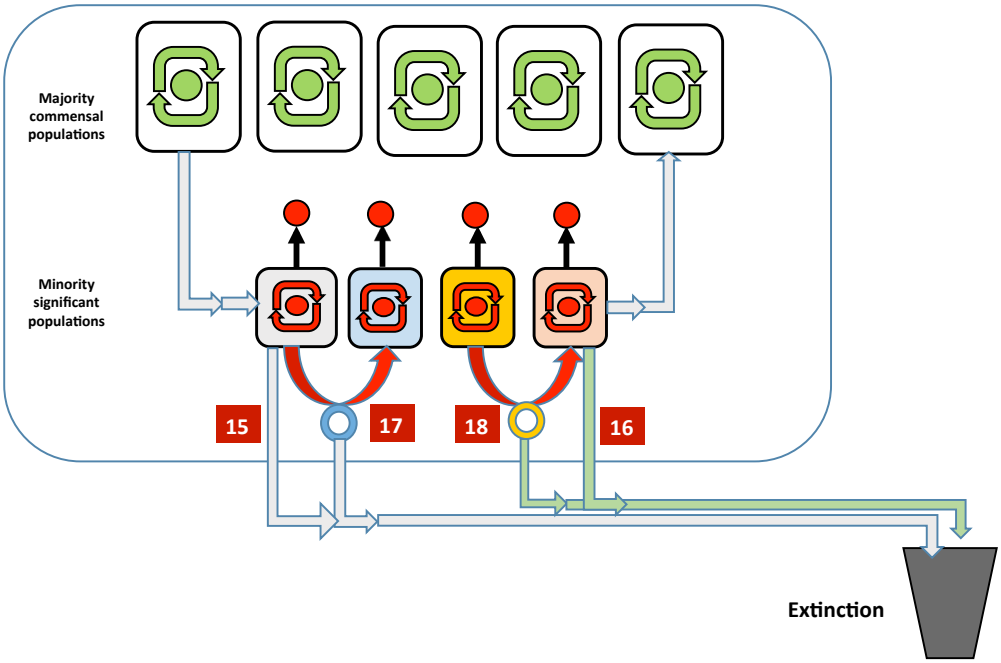

Figure 8. Maintenance of antibiotic resistance genes and plasmids.

15. Most clones and species harboring resistance genes and plasmids are stably maintained even without antibiotic exposure, suggesting a resistance-host long-term adaptation (WP4, WP3, WP7).
16. Fitness costs of harboring (and expressing) particular mobile genetic MGE elements might remove some resistant organisms from the microbiome (WP7).
17. Fitness costs of mutations in resistance genes are variable and depends on the host genetic background (WP7).
18. Some MGE are able to spread but only within a limited number of hosts (WP7, WP4).

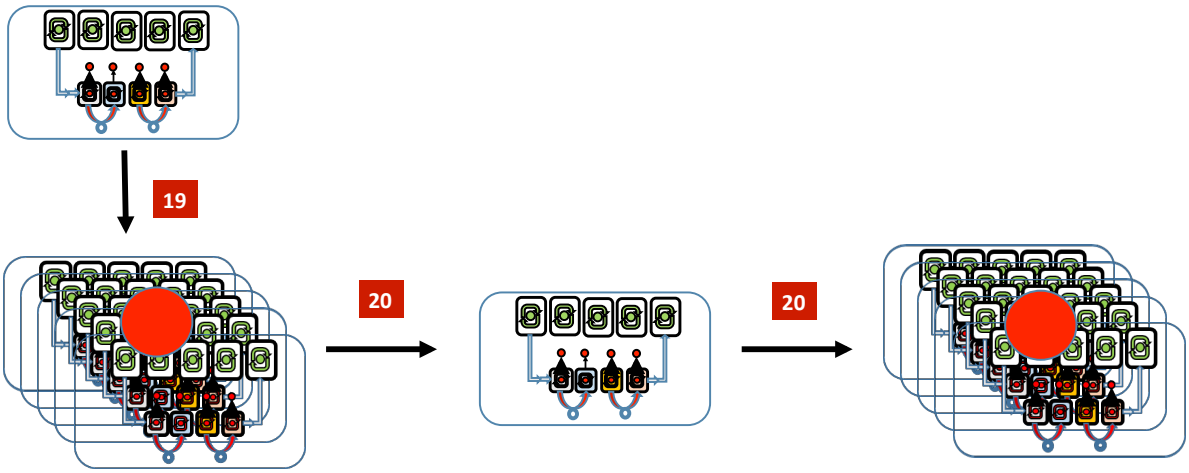

Figure 9. Dissemination of antibiotic resistance

19. Antibiotic selection and the resulting epidemic-endemic spread of resistant clones increases the variability and adaptation of hosts and is a major factor in the enrichment of resistance genes (WP2). Small antibiotic concentrations might be selective and amplify resistance plasmids and genes (WP2).
20. A major factor contributing to the expansion of resistance genes is the transfer of colonized patients between *connected* hospitals (WP7).

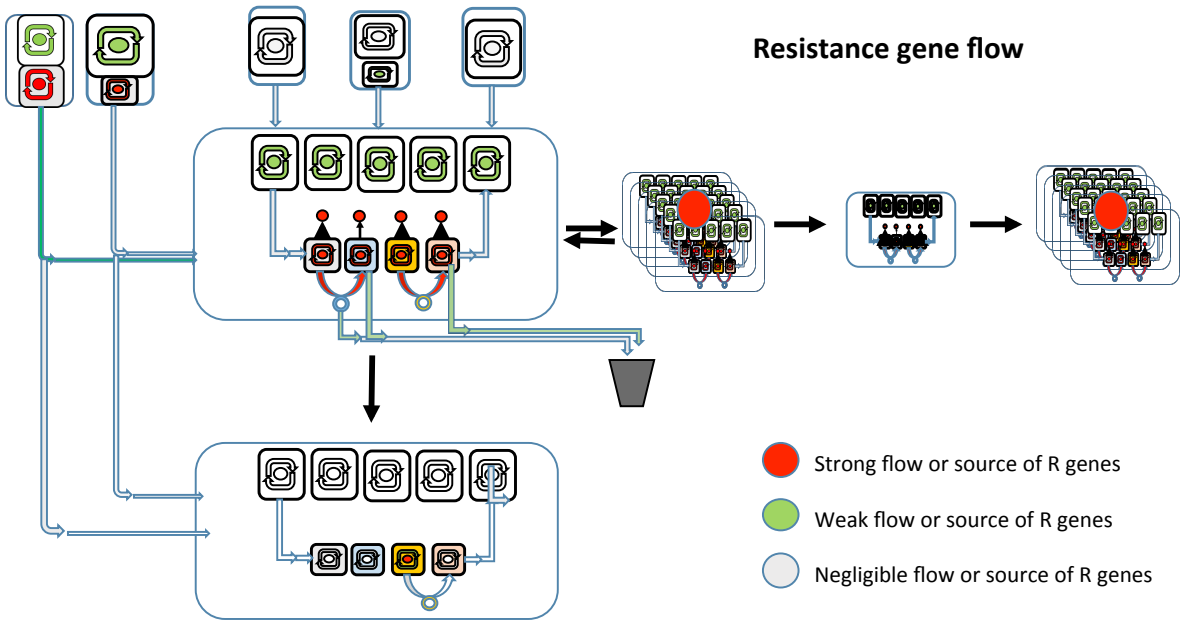

Figure 10. Summary of reservoirs and observed links between reservoirs of antibiotic resistance genes, mobile genetic element and strains in the presence and absence of antibiotic induced selective pressure, and describing aaintenance of antibiotic resistance genes and plasmids and dissemination of antibiotic resistance. The used three colour codes in the figure indicate strong/weak/negligible flow or source of resistance genes.

Potential impact, main dissemination activities and exploitation of use

Novel approaches to identify antibiotic resistance determinants (ARDs) from complex, metagenomic datasets in WP1 allowed the construction of a large list of the ARD from the human gut genome. This lays grounds for detailed investigations of the resistome dynamics under different conditions – active treatments with antibiotics or passive exposure due to residues in the food or environment. These investigations can open avenues to reducing the overall load of ARDs, by adjusting treatments and implementing practices to reduce the residues in the environment. Beyond humans, reservoir of the ARDs in domestic and farm animals could be followed and the transfer from the zoonotic sources to humans explored.

The methodology developed to identify ARDs, based on structure modelling, has a potential to be also applied to identification of other functions of interest in the human gut microbiome that could be associated to health and disease. Indeed, a majority of the millions of genes that are known have no clearly identified function. Our comprehension of the overall microbiome function is greatly hampered by this lack.

Studies in WP2 have uncovered multiple pathways that can lead to the evolution of antibiotic resistance and successful clones of nosocomial pathogens. These findings have had considerable impact and may

contribute to novel screening methods and/or interventions aimed to detect and stop the spread of high-risk antibiotic-resistant bacteria. Some of the most noticeable pathways to impact for EvoTAR WP2 are outlined below.

- Multi-drug resistant clinical *E. faecium* isolates form a specific sub-population which has emerged from human commensal and animal strains. Several genetic elements are present in clinical *E. faecium* strains while being absent in human commensal and animal strains. These genes may be used to develop rapid PCR-based strategies to distinguish clinically relevant *E. faecium*, which may have the potential to spread rapidly in hospitals, from strains that are not adapted to cause outbreaks among hospitalized patients.
- The study on the dissemination of cephalosporin-resistant *E. coli* strains from poultry, chicken meat and hospitalized patients provided an important correction on previous studies, which used low-resolution traditional typing methodologies to study the relatedness of strains from these different reservoirs. Our study could not substantiate previous claims on direct transfer of resistant *E. coli* strains from chickens via meat to humans but highlighted the important role of identical plasmids spreading between *E. coli* strains from different reservoirs. As plasmids can spread promiscuously between bacteria from different species, this may considerably complicate containment of resistance plasmids from diverse reservoirs. In addition, this study highlighted the superiority of whole-genome sequencing for bacterial typing, compared to other methods like MLST and PFGE.
- While many research groups appreciate the usefulness of whole-genome sequencing for bacterial typing, there is often a hurdle on the implementation of this technique due to the requirement of bioinformatics analyses. To open up whole-genome sequencing as a typing approach for laboratories without dedicated bioinformatics support, a core genome MLST scheme for *E. faecium* was developed, which can be used with minimal bioinformatics expertise.
- Several studies in EvoTAR have highlighted the rapid emergence of colistin resistance in *K. pneumoniae* through multiple evolutionary trajectories. Worryingly, resistance to colistin can come at no detectable fitness cost. Our findings should lead to guidelines that minimize the non-essential use (e.g. in farming and, potentially, in prophylactic antibiotic therapies) of colistin, an antibiotic of last resort, to minimize the emergence of resistance.
- Studies in WP2 have expanded on previous observations that exposure to low levels of antibiotics can lead to high-level resistance. This mechanism may lead to the selection for resistance among bacterial populations in the environment. These findings may lead to interventions aimed at minimizing the release of antibiotics into the environment (e.g. through wastewater).

Dissemination of research data in WP2 was mainly performed through publications in scientific journals and presentation at microbiology conferences, including large international meetings like those organized by the Federation of European Microbiology Societies and the American Society for Microbiology. The majority of articles published in WP2 are available under a ‘gold’ or ‘green’ open access license, which has contributed to the visibility of the project.

The results of WP3 are expected to have a significant impact on the research field of antibiotic resistance. The impact here is both in the form of creating new and enabling technologies that accelerate research also beyond antibiotic resistance as well as building scientific knowledge about antibiotic resistance.

Enabling technologies:
Higher throughput functional metagenomics: In WP3 we have developed several improved methodologies for performing high throughput functional selections. These methodologies (ParFUMS as well as its extension based on PacBio sequencing) have increased with throughput of functional metagenomics by 10-100 fold. Accordingly, we have in the EvoTAR project generated the largest dataset of antibiotic resistance genes which is equivalent in size to the current largest databases of antibiotic resistance genes (CARD maintained by McMaster University). These tools allow much more comprehensive characterization of resistomes also outside the clinical setting. Furthermore, these

developments can be applied to the area of industrial biotechnology where functional metagenomics can be used to improve bioprocesses (Forsberg et at. 2015 AEM).

New hosts for functional metagenomics: We developed a methodology for construction of functional metagenomic libraries in *L. lactis* a Gram-positive organism. While we where not able to identify specific Gram-positive resistance genes using this approach, it still enables new paths for research within functional metagenomics, including the critical testing of expression bias for functional metagenomics.

Rapid diagnostics for resistome dynamics: We develop a methodology based on the Fluidigm Biomark system to interrogate a large set of clinically relevant resistance genes from any sample. With more testing and validation this approach could potentially be used as a rapid diagnostic for resistance genes in infecting pathogens.

Scientific knowledge:

Substantially expanded catalogue of resistance genes: Through the EvoTAR project we have identified several thousand new variants of resistance genes using the improved functional metagenomic methods that we developed. This has enabled us to construct a better catalogue of resistance genes that will be made publicly available. Accordingly, we have uncovered more of the biological dark matter, e.g. the unknown resistance genes of the human gut microbiome.

Improved understanding of resistome dynamics: We have characterized the temporal dynamics of the resistome during ICU stay (including massive drug treatment). We have found that the resistome expands during hospitalization, both in terms of abundance of resistance genes as well as in terms of diversity of resistance genes. Furthermore, our findings suggest that the resistome during hospitalization is more enriched in resistance shared by human pathogens.

Limited potential for evolution of low-level resistance genes: We have tested resistance genes conferring low-level resistance towards both fluoroquinolone and beta-lactam antibiotics. Our results showed that there was a substantial interaction between the host genome and the low-level resistance gene that determined the evolutionary potential of the low-level resistance gene. For fluoroquinolones high-level resistance can more easily evolve through chromosomal mutations in *E. coli* compared to beta-lactams. Accordingly, fluoroquinolone low-level resistance genes cannot readily evolve into high-level resistance genes in such hosts. In contrast, beta-lactamases conferring low-level resistance to cefotaxime could be evolved to become high-level resistance genes in *E. coli* due to the relative paucity of chromosomal mutations conferring resistance to cefotaxime.

Two main outcomes will result from the work performed in WP4. First, the development of novel tools for metagenomic detection and characterization of minority bacterial populations present in complex samples, contaminated samples, or samples with low concentration of DNA. Second, an effective methodology for early detection and monitoring a major threat in Global Health as antibiotic resistance (Biomedicine, Food Protection, Food Safety, Environmental damage). Especially, the analysis of minority populations constitutes a major bottleneck that limits the use of current metagenomics for a broad set of applications in Biotechnology and other fields of societal interest. It is expected that the advances made in this WP will importantly:

- impact the outcomes of metagenomics applications by allowing bioprospection and characterization of minority or rare populations, significant for human, animal, food, and health environmental health.
- impact the way metagenomics is applied to solve major societal threats in Public Health, focusing in our case on a key health problem recognized as such by regulatory agencies (FMI, G8).

Due to the generic nature, this tool developed in WP4 will be (i) replicable, for verified applications, in other health institutions outside the consortium, and (ii) applicable to other health, environmental and biotechnological applications, subsequent to suitable adaptation of the detection technology (after the end of EvoTAR). Contact with RocheNimblegen has been established to improve the technology and to commercialize the design.

We foresee that the technology developed in this WP will provide measurable improvements for specific applications in Biomedicine, Agro-Food, and Environment. They include:

- *Reduction of costs* for the end users by: i) avoiding unnecessary delays in detecting threats for food safety (adoption of executive interventions); ii) improving diagnosis of Public Health threats (treatment failures, length of hospitalization stays), iii) providing biomarkers (Health) and biosensors (environment) oriented to risk assessment.
- Increasing the *quality and functionality of field and reference laboratories* by implementing standardized metagenomic procedures in routine practices (diagnosis, personalized medicine, detection of threats, management, biosurveillance, food safety, food protection, environmental risk analysis and protection).
- Increasing efficiency in development and application of *antimicrobial drugs*, and novel *decontamination and sanitation procedures* in environments, influencing global health.
- Increasing the *accuracy of predictive analysis*, assuring early containment of health threats, allowing the *definition of risks* for health related with antibiotic, metal and biocide resistance and virulence genes[2]

The EvoTAR partners are key-opinion leaders in the fields of metagenomics and genomics, informatics and computational biology, veterinary, environmental biotechnology, infectious diseases and plasmid biology. They will draw attention to peers of EvoTAR assets, to widen the scope of the usage of this platform and to recruit future research groups for further validating of the technical approach and products. Collaboration with other research consortiums to further exploitation of the platform (environment, food production, sanitation, health, drug development) has been established. Two project proposals have been submitted, including the H2020-LEIT call which is pending of final decision, to further exploit this technology

WP6 was overall an academic work package, intending to study the dynamics of antibiotic resistance transfer within bacterial populations and to develop novel methods for this type of analysis. Therefore, results of WP6 led principally to a wide number of scientific publications, published in high-ranking journals, as their main dissemination activity. The item with the highest potential impact was the development of the bioinformatic tool called PLACNET, a method to extract and reconstruct plasmid sequences from Illumina whole-genome datasets. This is for utmost importance when studying plasmid-derived antibiotic resistance whole genome sequencing (WGS) based epidemiology. WGS is more and more considered the "gold-standard" for molecular typing providing the most optimal level of resolution. However, with the use of popular short-read technologies like Illumina-based sequencing it is very difficult to reconstruct plasmids from the many assembled contigs. With the developed PLACNET tool this is now possible. The method has been made freely available for the scientific community and other potential users. We are presently working in a more use-friendly version that can be run from personal computers by using a dedicated web page.

WP7 has overall been very successful and we have generated a wealth of novel data that has been presented in publications and at various symposia, conferences, public outreach activities and seminars. With regard to impact and future use we want to point out the following broader implications of this work.

To study the between host transmission of antibiotic resistance three generic model frameworks have been developed. With the first two models it is possible to study how different diseases and antibiotic resistances spread over a network of connected hosts (i.e. persons, hospitals, farms, etc.). The framework fits well with existing data on MRSA bacteraemia cases in Scotland, thereby validating the model. These models can be used to identify hosts at risk of becoming infected and for identifying hotspots for the emergence of multidrug resistant bacteria. Furthermore, results of the models could be used to aid in the development of nationwide surveillance programs by informing policy makers where to concentrate efforts. The study on hospital level risk factors shows that, although large hospitals are important, size

alone does not fully explain the number of bacteraemia cases in a hospital. This is of high importance for hospitals in Scotland, as this is where most of the data was collected, but also for other hospitals worldwide. Hospital systems are remarkably similar and conclusions from this study can easily be extrapolated to other hospitals or countries.

Regarding the utility of Whole Genome Sequencing of *Escherichia coli* O157 for outbreak detection and epidemiological surveillance it is demonstrated that WGS offers the potential to streamline reference laboratory processes by the use of a single diagnostic tool to generate the information required to support clinical management of cases, and Public Health investigations and interventions to control spread. It has the potential to transform the way we assess relatedness of strains and the risk of development of severe complications. However, issues relating to ease of performance and standardisation, as well as IT infrastructure and data storage need to be addressed before it is introduced routinely. Also the study on intercontinental exclusion of community-associated MRSA subtypes with distinct antibiotic resistance gene profiles demonstrates the power of whole genome sequencing for resolving longstanding questions about the origin and transmission routes of bacteria in the community setting. Through in vitro experiments inspired by our sequence analysis we also show that that direct competition is just one of a multitude of factors which determine the global distribution of bacterial strains.

The use of *in silico* pharmacokinetic-pharmacodynamic (PKPD) models based on data from *in vitro* time-kill experiments can provide valuable information to guide dosing of antibiotics. Thus, the development a mechanism-based *in silico* model that can describe in vitro time-kill experiments of susceptible and resistant *E. coli* and the high correlation of killing kinetics with the experimentally determined MICs, suggest that the *in vitro* time-kill profile of a mutant strain is predictable by the MIC alone. The general applicability of this observation to other bacterial species and antibiotics is still unclear but it implies that research to identify optimal dosing regimens could be greatly simplified.

Our experimental work on fitness costs has generated some general implications of importance for understanding and predicting resistance development. Thus, almost without exception antibiotic resistance mechanisms (whether mutational or horizontally transferred) confer a fitness cost observed as a reduced growth rate/survival *in vitro* and/or *in vivo*. In general, we also observe that the fitness costs associated with various mutational resistance mechanisms are remarkably stable across different genetic contexts, bacterial species and clones, indicating that epistatic effects are weak for these particular chromosomal resistances. However, for other plasmid-borne transposons encoding antibiotic resistance only a limited number of hosts ensure their stable maintenance and here differences in fitness might explain the particular association of genetic elements (plasmids, transposons, resistance genes) with particular clones and species. One important implication is therefore that it is at present very difficult to predict the magnitude of the fitness effect of particular resistance mechanism in a particular genetic background.

In WP8, novel interventional strategies to tackle antimicrobial resistance were developed and thoroughly evaluated. Nowadays, it is well acknowledged that both new antibacterial therapies and out-of-the-box therapeutic strategies are needed to fight antimicrobial resistance. EvoTAR is contributing a lot to open new avenues for better cures and handling of patients with bacterial infections.

DAV132, developed partly in the context of EvoTAR by Da Volterra, is the first product with a clinically-demonstrated protection of intestinal microbiota from disruption during antibiotic treatments. This represents a tremendous achievement for all the teams involved in EvoTAR as it illustrates that DAV132, when administered in combination with antibiotic treatment, drastically minimizing the side-effects on the intestinal flora; both limiting the onsets of diseases such as *Clostridium difficile* infections and curbing the emergence and spread of resistant bacteria. DAV132 is a really unique innovation and its further development up to patients has strongly been boosted by the results generated in EvoTAR. The EvoTAR project has also been beneficial for animal health as several data of importance on antibiotic resistance in animals were gathered during the work on DAV133. DAV133 is the only product in the world developed to prevent the emergence and rise of resistant bacteria in companion animals when they

receive antibiotic treatments. It has also benefited a lot from the studies performed collaboratively by EvoTAR's partners and all the EvoTAR data will support the future registration and use of the product.

Future studies will be needed to confirm the efficiency of DAV132 in protecting the microbiome from different antibiotics in a clinical context, but there is a potential that the adjunction of the product becomes a standard of care in antibiotic treatment. Health benefits of that practice could be absolutely huge, given the links between the antibiotic treatments and both the short term adverse consequences, such as *C. difficile* infections, and potential long term consequences, linked to loss of gut microbial richness, associated to the risk of pathologies associated to obesity and the metabolic syndrome, such as type 2 diabetes, hepatic and cardiovascular complications and certain cancers.

Work done in WP8 by Instituto Biomar and its partners in Universidad de Cantabria has allowed characterizing of conjugation inhibitors, and the evaluation and demonstration of their potential to prevent the transfer of antibiotic resistance genes through different conjugation systems. These results could lead to the development of products based on the inhibition of conjugation to be administered concomitantly with antibiotic applications in different settings. This work has resulted in scientific publications, and if the challenges remaining in the route to create commercial products based on these properties are overcome, the Evotar project will have been essential in the development of these products, that will have a huge social and economic impact.

Project web-site: www.evotar.eu

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4.2 Use and dissemination of foreground

Section A (public)

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
1	Antibiotics and the resistant microbiome	Morten OA Sommer , Gautam Dantas	Current Opinion in Microbiology	Vol. 14/Issue 5	Elsevier Limited	United Kingdom	2011	556-563		Yes
2	Transfer of an Escherichia coli ST131 multiresistance cassette has created a Klebsiella pneumoniae-specific plasmid associated with a major nosocomial outbreak	L. Sandegren , M. Linkevicius , B. Lytsy , A. Melhus , D. I. Andersson	Journal of Antimicrobial Chemotherapy	Vol. 67/Issue 1	Oxford University Press	United Kingdom	2012	74-83		Yes
3	Optimizing future treatment of enterococcal infections: attacking the biofilm?	Fernanda L. Paganelli , Rob J. Willems , Helen L. Leavis	Trends in Microbiology	Vol. 20/Issue 1	Elsevier Limited	United Kingdom	2012	40-49	10.1016/j.tim.2011.11.001	Yes
4	Bottlenecks in the Transferability of Antibiotic Resistance from Natural Ecosystems	José L. Martínez	Frontiers in Microbiology	Vol. 2	Frontiers Research Foundation	Switzerland	2012	265		Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	to Human Bacterial Pathogens									
5	Natural Antibiotic Resistance and Contamination by Antibiotic Resistance Determinants: The Two Ages in the Evolution of Resistance to Antimicrobials	José L. Martínez	Frontiers in Microbiology	Vol. 3	Frontiers Research Foundation	Switzerland	2012	1		Yes
6	Hospital and Community Ampicillin-Resistant Enterococcus faecium Are Evolutionarily Closely Linked but Have Diversified through Niche Adaptation	Marieke J. A. de Regt , Willem van Schaik , Miranda van Luit-Asbroek , Huberta A. T. Dekker , Engeline van Duijkeren , Catherina J. M. Koning , Marc J. M. Bonten , Rob J. L. Willems	PLoS One	Vol. 7/Issue 2	Public Library of Science	United States	2012	e30319		Yes
7	Insight into antimicrobial susceptibility and population structure of contemporary	A. Kuch , R. J. L. Willems , G. Werner , T. M. Coque , A. M. Hammerum ,	Journal of Antimicrobial Chemotherapy	Vol. 67/Issue 3	Oxford University Press	United Kingdom	2012	551-558	10.1093/jac/dkr544	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	human Enterococcus faecalis isolates from Europe	A. Sundsfjord , I. Klare , P. Ruiz-Garbajosa , G. S. Simonsen , M. van Luit-Asbroek , W. Hryniewicz , E. Sadowy								
8	The antibiotic resistome: challenge and opportunity for therapeutic intervention	José L Martínez	Future Medicinal Chemistry	Vol. 4/Issue 3	Future Science	United Kingdom	2012	347-359	10.4155/fmc.12.2	Yes
9	Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in Escherichia coli: 20 years of surveillance in resource-limited settings from Latin America.	Bartoloni A, Pallecchi L, Riccobono E, Mantella A, Magnelli D, Di Maggio T, Villagran AL, Lara Y, Saavedra C, Strohmeyer M, Bartalesi F, Trigoso C, Rossolini GM	Clinical Microbiology and Infection	19(4)	Blackwell Publishing		2013	356-361	10.1111/j.1469-0691.2012.03807.x	Yes
10	Dynamics of ampicillin-resistant Enterococcus faecium clones colonizing	Maja Weisser , Evelien A Oostdijk , Rob JL Willems , Marc JM	BMC Infectious Diseases	Vol. 12/Issue 1	BioMed Central	United Kingdom	2012	68	10.1186/1471-2334-12-68	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	hospitalized patients: Data from a prospective observational study	Bonten , Reno Frei , Luigia Elzi , Jorg Halter , Andreas F Widmer , Janetta Top								
11	High-density fecal Enterococcus faecium colonization in hospitalized patients is associated with the presence of the polyclonal subcluster CC17	P. Ruiz-Garbajosa , M. Regt , M. Bonten , F. Baquero , T. M. Coque , R. Cantón , H. J. Harmsen , R. J. L. Willems	European Journal of Clinical Microbiology and Infectious Diseases	Vol. 31/Issue 4	Springer Verlag	Germany	2012	519-522	10.1007/s10096-011-1342-7	Yes
12	Microcolony Imaging of Aspergillus fumigatus Treated with Echinocandins Reveals Both Fungistatic and Fungicidal Activities	Colin J. Ingham , Peter M. Schneeberger	PLoS One	Vol. 7/Issue 4	Public Library of Science	United States	2012	e35478	10.1371/journal.pone.0035478	Yes
13	Fitness Cost and Interference of Arm/Rmt Aminoglycoside Resistance with the RsmF Housekeeping Methyltransferases	B. Gutierrez , J. A. Escudero , A. San Millan , L. Hidalgo , L. Carrilero , C. M. Ovejero , A. Santos-Lopez ,	Antimicrobial Agents and Chemotherapy	Vol. 56/Issue 5	American Society for Microbiology	United States	2012	2335-2341		Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		D. Thomas-Lopez , B. Gonzalez-Zorn								
14	Differential Epigenetic Compatibility of qnr Antibiotic Resistance Determinants with the Chromosome of Escherichia coli	María B. Sánchez , José L. Martínez	PLoS One	Vol. 7/Issue 5	Public Library of Science	United States	2012	e35149		Yes
15	Rapid detection of intestinal carriage of Klebsiella pneumoniae producing KPC carbapenemase during an outbreak	T. Giani , C. Tascini , F. Arena , I. Ciullo , V. Conte , A. Leonildi , F. Menichetti , G.M. Rossolini	Journal of Hospital Infection	Vol. 81/Issue 2	W.B. Saunders Ltd	United Kingdom	2012	119-122		Yes
16	Genome-Wide Identification of Ampicillin Resistance Determinants in Enterococcus faecium	Xinglin Zhang , Fernanda L. Paganelli , Damien Bierschenk , Annemarie Kuipers , Marc J. M. Bonten , Rob J. L. Willems , Willem van Schaik	PLoS Genetics	Vol. 8/Issue 6	Public Library of Science	United States	2012	e1002804		Yes
17	Vancomycin-	M.J. Bonten;	Nederlands	156:A5233	Bohn Stafleu van Loghum		2012	01-feb		Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	resistant enterococcus-chronicle of a fortold problem	R.J. Willems	Tijdschrift voor Geneeskunde							
18	Intelligibility in microbial complex systems: Wittgenstein and the score of life.	Baquero F, Moya A.	Front Cell Infect Microbiol.	2 Article 88			2012	01-aug		Yes
19	Metagenomics and antibiotics	L. Garmendia , A. Hernandez , M. B. Sanchez , J. L. Martinez	Clinical Microbiology and Infection	Vol. 18	Blackwell Publishing	United Kingdom	2012	27-31		Yes
20	The microbiome as a human organ	F. Baquero , C. Nombela	Clinical Microbiology and Infection	Vol. 18	Blackwell Publishing	United Kingdom	2012	02-apr		Yes
21	Metagenomic epidemiology: a public health need for the control of antimicrobial resistance	F. Baquero	Clinical Microbiology and Infection	Vol. 18	Blackwell Publishing	United Kingdom	2012	67-73		Yes
22	Whole-Genome Sequence of Stenotrophomonas maltophilia D457, a Clinical Isolate and a Model Strain	F. Lira , A. Hernandez , E. Belda , M. B. Sanchez , A. Moya , F. J. Silva , J. L. Martinez	Journal of Bacteriology	Vol. 194/Issue 13	American Society for Microbiology	United States	2012	3563-3564		Yes
23	A Degenerate Primer MOB Typing (DPMT)	Andrés Alvarado , M. Pilar Garcillán-	PLoS One	Vol. 7/Issue 7	Public Library of Science	United States	2012	e40438	10.1371/journal.pone.0040438	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Method to Classify Gamma-Proteobacterial Plasmids in Clinical and Environmental Settings	Barcia , Fernando de la Cruz								
24	Restricted Gene Flow among Hospital Subpopulations of Enterococcus faecium	R. J. L. Willems , J. Top , W. van Schaik , H. Leavis , M. Bonten , J. Siren , W. P. Hanage , J. Corander	MBio	Vol. 3/Issue 4	American Society for Microbiology	United States	2012	e00151-12-e00151-12		Yes
25	Bacterial pathogens: from natural ecosystems to human hosts	José L. Martínez	Environmental Microbiology	Vol. 15/Issue 2	Blackwell Publishing	United Kingdom	2013	325-333	10.1111/j.1462-2920.2012.02837.x	Yes
26	Overproduction of the multidrug efflux pump MexEF-OprN does not impair Pseudomonas aeruginosa fitness in competition tests, but produces specific changes in bacterial regulatory networks	Jorge Olivares , Carolina Alvarez-Ortega , Juan F. Linares , Fernando Rojo , Thilo Köhler , José Luis Martínez	Environmental Microbiology	Vol. 14/Issue 8	Blackwell Publishing	United Kingdom	2012	1968-1981		Yes
27	AsrR Is an Oxidative Stress	François Lebreton ,	PLoS Pathogens	Vol. 8/Issue 8	Public Library of Science	United States	2012	e1002834		Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Sensing Regulator Modulating Enterococcus faecium Opportunistic Traits, Antimicrobial Resistance, and Pathogenicity	Willem van Schaik , Maurizio Sanguinetti , Brunella Posteraro , Riccardo Torelli , Florian Le Bras , Nicolas Verneuil , Xinglin Zhang , Jean-Christophe Giard , Anne Dhalluin , Rob J. L. Willems , Roland Leclercq , Vincent Cattoir								
28	Quinolone Resistance in Absence of Selective Pressure: The Experience of a Very Remote Community in the Amazon Forest	Lucia Pallecchi , Alessandro Bartoloni , Eleonora Riccobono , Connie Fernandez , Antonia Mantella , Donata Magnelli , Dario Mannini ,	PLoS Neglected Tropical Diseases	Vol. 6/Issue 8	Public Library of Science	United States	2012	e1790		Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Marianne Strohmeyer , Filippo Bartalesi , Hugo Rodriguez , Eduardo Gotuzzo , Gian Maria Rossolini								
29	Collective navigation of cargo-carrying swarms	A. Shklarsh , A. Finkelshtein , G. Ariel , O. Kalisman , C. Ingham , E. Ben-Jacob	Journal of the Royal Society Interface	Vol. 2/Issue 6	Royal Society of London		2012	786-798	10.1098/rsfs.2012.0029	Yes
30	The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens	K. J. Forsberg , A. Reyes , B. Wang , E. M. Selleck , M. O. A. Sommer , G. Dantas	Science	Vol. 337/Issue 6098	American Association for the Advancement of Science	United States	2012	1107-1111		Yes
31	Association of Extended-Spectrum -Lactamase VEB-5 and 16S rRNA Methyltransferase ArmA in Salmonella enterica from the United Kingdom	L. Hidalgo , K. L. Hopkins , D. W. Wareham , B. Gutierrez , B. Gonzalez-Zorn	Antimicrobial Agents and Chemotherapy	Vol. 56/Issue 9	American Society for Microbiology	United States	2012	4985-4987		Yes
32	Ecology of antimicrobial resistance: humans,	Gonzalez-Zorn, B, and Escudero JA	International Microbiology	15			2012	101-109	10.2436/20.1501.01.163	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	animals, food and environment									
33	The Inactivation of Intrinsic Antibiotic Resistance Determinants Widens the Mutant Selection Window for Quinolones in Stenotrophomonas maltophilia.	García-León G, Sánchez MB, Martínez JL.	Antimicrob Agents Chemother	56 (12)			2012	6397-6399	10.1128/AAC.01558-12	Yes
34	Context matters — the complex interplay between resistome genotypes and resistance phenotypes	Gautam Dantas , Morten OA Sommer	Current Opinion in Microbiology	Vol. 15/Issue 5	Elsevier Limited	United Kingdom	2012	577-582	10.1016/j.mib.2012.07.004	Yes
35	Selection of resistance at lethal and non-lethal antibiotic concentrations	Diarmaid Hughes , Dan I Andersson	Current Opinion in Microbiology	Vol. 15/Issue 5	Elsevier Limited	United Kingdom	2012	555-560	10.1016/j.mib.2012.07.005	Yes
36	Characterization of the Polymyxin B Resistome of Pseudomonas aeruginosa	L. Fernandez , C. Alvarez-Ortega , I. Wiegand , J. Olivares , D. Kocincova , J. S. Lam , J. L. Martinez , R. E. W. Hancock	Antimicrobial Agents and Chemotherapy	Vol. 57/Issue 1	American Society for Microbiology	United States	2012	110-119	10.1128/AAC.01583-12	Yes
37	Different Genetic	Novais C,	Antimicrob	56 (11)			2012	6014-8	10.1128/A	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Supports for the tet(S) Gene in Enterococci.	Freitas AR, Silveira E, Baquero F, Peixe L, Roberts AP, Coque TM.	Agents Chemother						AC.00758-12	
38	A tet(S/M) hybrid from CTn6000 and CTn916 recombination.	Novais C, Freitas AR, Silveira E, Baquero F, Peixe L, Roberts AP, Coque TM.	Microbiology	158 (Pt 11)			2012	2710-11	10.1099/mic.0.062729-0	Yes
39	Evolutionary analyses of non-genealogical bonds produced by introgressive descent.	Bapteste E, Lopez P, Bouchard F, Baquero F, McInerney JO, Burian RM.	Proc Natl Acad Sci U S A	109 (45)			2012	18266-18272	10.1073/pnas.1206541109	Yes
40	The public health risk of enterobacterial isolates producing extended-spectrum beta-lactamases (ESBL) or AmpC beta-lactamases in food and food-producing animals: An EU perspective of epidemiology, analytical methods,	Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, Mevius D, Peixe L, Poirel L, Schuepbach-Regula G, Torneke K, Torren-Edo J, Torres C, Threlfall J.	Clin Infect Dis	56 (7)			2013	1030-7	10.1093/cid/cis1043	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	risk factors and control options.									
41	Influence of acquired -lactamases on the evolution of spontaneous carbapenem resistance in Escherichia coli	M. Adler , M. Anjum , D. I. Andersson , L. Sandegren	Journal of Antimicrobial Chemotherapy	Vol. 68/Issue 1	Oxford University Press	United Kingdom	2013	51-59	10.1093/jac/dks368	Yes
42	Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era.	Gilmore MS, Lebreton F, van Schaik W.	Current Opinion in Microbiology	16 (1)	Elsevier Limited		2013	10-jun	10.1016/j.mib.2013.01.006	Yes
43	Bloody coli: a Gene Cocktail in Escherichia coli O104:H4	F. Baquero , R. Tobes	MBio	Vol. 4/Issue 1	American Society for Microbiology	United States	2013	e00066-13-e00066-13	10.1128/mBio.00066-13	Yes
44	Antibiotics as selectors and accelerators of diversity in the mechanisms of resistance: from the resistome to genetic plasticity in the β-lactamases world	Juan-Carlos Galán , Fernando González-Candelas , Jean-Marc Rolain , Rafael Cantón	Frontiers in Microbiology	Vol. 4	Frontiers Research Foundation	Switzerland	2013	jan-17	10.3389/fmicb.2013.00009	Yes
45	Evolution of Conjugation and	Guglielmini J, de la Cruz F,	Mol Biol Evol	30 (2)			2012	315-31	10.1093/molbev/mss22	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Type IV Secretion Systems.	Rocha EP							1	
46	RND multidrug efflux pumps: what are they good for?	Carolina Alvarez-Ortega*, Jorge Olivares and José L. Martínez*	Frontiers in Microbiology	4	Frontiers Research Foundation		2013	01-nov	10.3389/fmicb.2013.00007	Yes
47	Identification of a genetic determinant in clinical Enterococcus faecium strains that contributes to intestinal colonization during antibiotic treatment.	Zhang X, Top J, de Been M, Bierschenk D, Rogers M, Leendertse M, Bonten MJ, van der Poll T, Willems RJ, van Schaik W.	Journal of Infectious Diseases	207 (11)	Oxford University Press		2013	1780-6	10.1093/infdis/jit076	Yes
48	Antibiotic resistance shaping multi-level population biology of bacteria	Fernando Baquero , Ana P. Tedim , Teresa M. Coque	Frontiers in Microbiology	Vol. 4	Frontiers Research Foundation	Switzerland	2013	jan-15	10.3389/fmicb.2013.00015	Yes
49	Microevolutionary events involving narrow host plasmids influences local fixation of vancomycin-resistance in Enterococcus populations.	Freitas AR, Novais C, Tedim AP, Francia MV, Baquero F, Peixe L, Coque TM.	PLoS One	8 (3)	Public Library of Science		2013	01-nov	10.1371/journal.pone.0060589	Yes
50	CTX-M-type β-	Marco Maria	International	Vol.	Urban und Fischer	Germany	2013	305-317	10.1016/j.ij	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	lactamases: A successful story of antibiotic resistance	D'Andrea , Fabio Arena , Lucia Pallecchi , Gian Maria Rossolini	Journal of Medical Microbiology	303/Issue 6-7	Verlag GmbH und Co. KG				mm.2013.02.008	
51	Enterococcus faecium Biofilm Formation: Identification of Major Autolysin AtlAEfm, Associated Acm Surface Localization, and AtlAEfm-Independent Extracellular DNA Release	F. L. Paganelli , R. J. L. Willems , P. Jansen , A. Hendrickx , X. Zhang , M. J. M. Bonten , H. L. Leavis	MBio	Vol. 4/Issue 2	American Society for Microbiology	United States	2013	e00154-13-e00154-13	10.1128/mBio.00154-13	Yes
52	β-lactam antibiotics promote bacterial mutagenesis via an RpoS-mediated reduction in replication fidelity	A. Gutierrez , L. Laureti , S. Crussard , H. Abida , A. Rodríguez-Rojas , J. Blázquez , Z. Baharoglu , D. Mazel , F. Darfeuille , J. Vogel , I. Matic	Nature Communications	Vol. 4	Nature Publishing Group	United Kingdom	2013	1610	10.1038/ncomms2607	Yes
53	Phenotypic Resistance to Antibiotics	Fernando Corona , Jose Martinez	Antibiotics	Vol. 2/Issue 2	MDPI AG, Basel, Switzerland	Switzerland	2013	237-255	10.3390/antibiotics2020237	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
54	The intrinsic resistome of bacterial pathogens	Jorge Olivares , Alejandra Bernardini , Guillermo Garcia-Leon , Fernando Corona , Maria B. Sanchez , Jose L. Martinez	Frontiers in Microbiology	Vol. 4	Frontiers Research Foundation	Switzerland	2013	jan-15	10.3389/fmicb.2013.00103	Yes
55	Functional genomic analysis of bile salt resistance in Enterococcus faecium.	Zhang X, Bierschenk D, Top J, Anastasiou I, Bonten MJ, Willems RJ, van Schaik W	BMC Genomics	14	BioMed Central		2013	299	10.1186/1471-2164-14-299	Yes
56	Epidemic diffusion of KPC carbapenemase-producing Klebsiella pneumoniae in Italy: results of the first countrywide survey, 15 May to 30 June 2011.	Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R; AMCLI-CRE Survey Participants, Pantosti A, Pagani L, Luzzaro F, Rossolini GM.	Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin	Vol. 0	Centre Europeen pour la Surveillance Epidemiologique du SIDA		2013	01-sep		Yes
57	The Enterococcus faecium Enterococcal Biofilm Regulator,	Janetta Top , Fernanda L. Paganelli , Xinglin Zhang ,	PLoS One	Vol. 8/Issue 5	Public Library of Science	United States	2013	e65224	10.1371/journal.pone.0065224	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	EbrB, Regulates the esp Operon and Is Implicated in Biofilm Formation and Intestinal Colonization	Willem van Schaik , Helen L. Leavis , Miranda van Luit-Asbroek , Tom van der Poll , Masja Leendertse , Marc J. M. Bonten , Rob J. L. Willems								
58	Characterization of the phd-doc and ccd Toxin-Antitoxin Cassettes from Vibrio Superintegrons	A.-M. Guerout , N. Iqbal , N. Mine , M. Ducos-Galand , L. Van Melderen , D. Mazel	Journal of Bacteriology	Vol. 195/Issue 10	American Society for Microbiology	United States	2013	2270-2283	10.1128/JB.01389-12	Yes
59	Diversity and biofilm-production ability among isolates of Escherichia coli phylogroup D belonging to ST69, ST393 and ST405 clonal groups.	Novais Â, Vuotto C, Pires J, Montenegro C, Donelli G, Coque TM, Peixe L	BMC Microbiology	13 (144)	BioMed Central		2013	01-sep	10.1186/1471-2180-13-144	Yes
60	Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-	Ruiz-Garbajosa P, Curiao T, Tato M, Gijón D, Pintado V, Valverde A,	Journal of Antimicrobial Chemotherapy	Epub ahead of print	Oxford University Press		2013	01-jun	10.1093/jac/dkt237	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	producing Klebsiella pneumoniae clones in Madrid, Spain.	Baquero F, Morosini MI, Coque TM, Cantón R.								
61	Identification and characterization of a highly motile and antibiotic refractory subpopulation involved in the expansion of swarming colonies of	Dalit Roth , Alin Finkelshtein , Colin Ingham , Yael Helman , Alexandra Sirota-Madi , Leonid Brodsky , Eshel Ben-Jacob	Environmental Microbiology	Vol. 15/Issue 9	Blackwell Publishing	United Kingdom	2013	2532-2544	10.1111/1462-2920.12160	Yes
62	Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in Enterobacteriaceae isolated in India and the UK	Laura Hidalgo, Katie L. Hopkins, Belen Gutierrez, Cristina M. Ovejero1, Suruchi Shukla, Stephen Douthwaite, Kashi N. Prasad, Neil Woodford and Bruno Gonzalez-Zorn	Journal of Antimicrobial Chemotherapy	Volume 68, Issue 7	Oxford University Press		2013	1543-1550	10.1093/jac/dkt078	Yes
63	Spread of multidrug-resistant Enterococcus to animals and	C. Novais , A. R. Freitas , E. Silveira , P. Antunes , R.	Journal of Antimicrobial Chemotherapy	Vol. 68/Issue 12	Oxford University Press	United Kingdom	2013	2746-2754	10.1093/jac/dkt289	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	humans: an underestimated role for the pig farm environment	Silva , T. M. Coque , L. Peixe								
64	Shared reservoir of ccrB gene sequences between coagulase-negative staphylococci and methicillin-resistant Staphylococcus aureus	A. C. Fluit , N. Carpaij , E. A. M. Majoor , M. J. M. Bonten , R. J. L. Willems	Journal of Antimicrobial Chemotherapy	Vol. 68/Issue 8	Oxford University Press	United Kingdom	2013	1707-1713	10.1093/jac/dkt121	Yes
65	Antibiotic resistant enterococci-Tales of a drug resistance gene trafficker.	Werner G, Coque TM, Franz CM, Grohmann E, Hegstad K, Jensen L, van Schaik W, Weaver K	International Journal of Medical Microbiology	303 (6-7)	Urban und Fischer Verlag GmbH und Co. KG		2013	360-79	10.1016/j.ijmm.2013.03.001	Yes
66	The cell wall architecture of Enterococcus faecium: from resistance to pathogenesis	Antoni PA Hendrickx, Willem van Schaik & Rob JL Willems	Future Microbiology	Vol. 8, No. 8	Future Medicine Ltd.		2013	993-1010	10.2217/fmb.13.66	Yes
67	Recent Recombination Events in the Core Genome Are Associated with Adaptive Evolution	M. de Been , W. van Schaik , L. Cheng , J. Corander , R. J. Willems	Genome Biology and Evolution	Vol. 5/Issue 8	Oxford University Press	United Kingdom	2013	1524-1535	10.1093/gbe/evt111	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	in Enterococcus faecium									
68	Emergence of Epidemic Multidrug-Resistant Enterococcus faecium from Animal and Commensal Strains	F. Lebreton , W. van Schaik , A. Manson McGuire , P. Godfrey , A. Griggs , V. Mazumdar , J. Corander , L. Cheng , S. Saif , S. Young , Q. Zeng , J. Wortman , B. Birren , R. J. L. Willems , A. M. Earl , M. S. Gilmore	MBio	Vol. 4/Issue 4	American Society for Microbiology	United States	2013	e00534-13-e00534-13	10.1128/mBio.00534-13	Yes
69	A LacI-Family Regulator Activates Maltodextrin Metabolism of Enterococcus faecium	Xinglin Zhang , Malbert Rogers , Damien Bierschenk , Marc J. M. Bonten , Rob J. L. Willems , Willem van Schaik	PLoS One	Vol. 8/Issue 8	Public Library of Science	United States	2013	e72285	10.1371/journal.pone.0072285	Yes
70	Characterization of plasmid pAX22, encoding VIM-1 metallo- -lactamase, reveals a new	V. Di Pilato , S. Pollini , G. M. Rossolini	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 1	Oxford University Press	United Kingdom	2013	67-71	10.1093/jac/dkt311	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	putative mechanism of In70 integron mobilization									
71	In Vivo Emergence of Colistin Resistance in Klebsiella pneumoniae Producing KPC-Type Carbapenemases Mediated by Insertional Inactivation of the PhoQ/PhoP mgrB Regulator	A. Cannatelli , M. M. D'Andrea , T. Giani , V. Di Pilato , F. Arena , S. Ambretti , P. Gaibani , G. M. Rossolini	Antimicrobial Agents and Chemotherapy	Vol. 57/Issue 11	American Society for Microbiology	United States	2013	5521-5526	10.1128/AAC.01480-13	Yes
72	Epigenetics, epistasis and epidemics	F. Baquero	Evolution, Medicine, and Public Health	Vol. 2013/Issue 1	Oxford University Press	United Kingdom	2013	86-88	10.1093/emph/eot009	Yes
73	Klebsiella pneumoniae Sequence Type 11 from Companion Animals Bearing ArmA Methyltransferase, DHA-1 -Lactamase, and QnrB4	L. Hidalgo , B. Gutierrez , C. M. Ovejero , L. Carrilero , S. Matrat , C. K. S. Saba , A. Santos-Lopez , D. Thomas-Lopez , A. Hoefer , M. Suarez , G. Santurde , C. Martin-Espada ,	Antimicrobial Agents and Chemotherapy	Vol. 57/Issue 9	American Society for Microbiology	United States	2013	4532-4534	10.1128/AAC.00491-13	Yes

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		B. Gonzalez-Zorn								
74	Sources of Antimicrobial Resistance	M. E. J. Woolhouse , M. J. Ward	Science	Vol. 341/Issue 6153	American Association for the Advancement of Science	United States	2013	1460-1461	10.1126/science.1243444	Yes
75	RpoS Plays a Central Role in the SOS Induction by Sub-Lethal Aminoglycoside Concentrations in Vibrio cholerae	Zeynep Baharoglu , Evelyne Krin , Didier Mazel	PLoS Genetics	Vol. 9/Issue 4	Public Library of Science	United States	2013	e1003421	10.1371/journal.pgen.1003421	Yes
76	Normal Mutation Rate Variants Arise in a Mutator (Mut S) Escherichia coli Population	María-Carmen Turrientes , Fernando Baquero , Bruce R. Levin , José-Luis Martínez , Aida Ripoll , José-María González-Alba , Raquel Tobes , Marina Manrique , Maria-Rosario Baquero , Mario-José Rodríguez-Domínguez , Rafael Cantón ,	PLoS One	Vol. 8/Issue 9	Public Library of Science	United States	2013	e72963	10.1371/journal.pone.0072963	Yes

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		Juan-Carlos Galán								
77	Indigenous and acquired modifications in the aminoglycoside binding sites of Pseudomonas aeruginosa rRNAs	Belen Gutierrez , Stephen Douthwaite , Bruno Gonzalez-Zorn	RNA Biology	Vol. 10/Issue 8	Landes Bioscience	United States	2013	1324-1332	10.4161/rna.25984	Yes
78	Lon protease inactivation, or translocation of the lon gene, potentiate bacterial evolution to antibiotic resistance	Hervé Nicoloff and Dan I. Andersson	Molecular Microbiology	Volume 90, Issue 6	Blackwell Publishing		2013	1233–1248	10.1111/mmi.12429	Yes
79	From organized internal traffic to collective navigation of bacterial swarms	Gil Ariel , Adi Shklarsh , Oren Kalisman , Colin Ingham , Eshel Ben-Jacob	New Journal of Physics	Vol. 15/Issue 12	Institute of Physics Publishing	United Kingdom	2013	125019	10.1088/1367-2630/15/12/125019	Yes
80	Characterization of Fecal Extended-Spectrum- -Lactamase-Producing Escherichia coli in a Remote Community during a Long Time Period	P.-L. Woerther , C. Angebault , H. Jacquier , O. Clermont , A. El Mniai , B. Moreau , F. Djossou , G. Peroz , F. Catzeflis , E.	Antimicrobial Agents and Chemotherapy	Vol. 57/Issue 10	American Society for Microbiology	United States	2013	5060-5066	10.1128/AAC.00848-13	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Denamur , A. Andremont								
81	Co-transfer of resistance to high concentrations of copper and first-line antibiotics among Enterococcus from different origins (humans, animals, the environment and foods) and clonal lineages	E. Silveira , A. R. Freitas , P. Antunes , M. Barros , J. Campos , T. M. Coque , L. Peixe , C. Novais	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 4	Oxford University Press	United Kingdom	2014	899-906	10.1093/jac/dkt479	Yes
82	Experimental Approaches for Defining Functional Roles of Microbes in the Human Gut	Gautam Dantas , Morten O.A. Sommer , Patrick H. Degnan , Andrew L. Goodman	Annual Review of Microbiology	Vol. 67/Issue 1	Annual Reviews Inc.	United States	2013	459-475	10.1146/annurev-micro-092412-155642	Yes
83	Effects of selective digestive decontamination (SDD) on the gut resistome	E. Buelow , T. B. Gonzalez , D. Versluis , E. A. N. Oostdijk , L. A. Ogilvie , M. S. M. van Mourik , E. Oosterink , M. W. J. van Passel , H. Smidt , M. M. D'Andrea , M.	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 8	Oxford University Press	United Kingdom	2014	2215-2223	10.1093/jac/dku092	Yes

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		de Been , B. V. Jones , R. J. L. Willems , M. J. M. Bonten , W. van Schaik								
84	Microbiology: Barriers to the spread of resistance	Morten O. A. Sommer	Nature	Vol. 509/Issue 7502	Nature Publishing Group	United Kingdom	2014	567-568	10.1038/nature13342	Yes
85	Human Intestinal Cells Modulate Conjugational Transfer of Multidrug Resistance Plasmids between Clinical Escherichia coli Isolates	Ana Manuel Dantas Machado , Morten O. A. Sommer	PLoS One	Vol. 9/Issue 6	Public Library of Science	United States	2014	e100739	10.1371/journal.pone.0100739	Yes
86	Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria	Elizabeth A. Rettedal , Heidi Gumpert , Morten O.A. Sommer	Nature Communications	Vol. 5	Nature Publishing Group	United Kingdom	2014	4714	10.1038/ncomms5714	Yes
87	CTX-M-1 - lactamase expression in Escherichia coli is dependent on cefotaxime concentration,	T. S. B. Kjeldsen , M. Overgaard , S. S. Nielsen , V. Bortolaia , L. Jelsbak , M. Sommer , L.	Journal of Antimicrobial Chemotherapy	Vol. 69	Oxford University Press	United Kingdom	2014	01-sep	10.1093/jac/dku332	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	growth phase and gene location	Guardabassi , J. E. Olsen								
88	Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic Resistance Dynamics of Staphylococcus aureus Clonal Complex 398	M. J. Ward , C. L. Gibbons , P. R. McAdam , B. A. D. van Bunnik , E. K. Girvan , G. F. Edwards , J. R. Fitzgerald , M. E. J. Woolhouse	Applied and Environmental Microbiology	Vol. 80/Issue 23	American Society for Microbiology	United States	2014	7275-7282	10.1128/AEM.01777-14	Yes
89	Efficient surveillance for healthcare-associated infections spreading between hospitals	M. Ciccolini , T. Donker , H. Grundmann , M. J. M. Bonten , M. E. J. Woolhouse	Proceedings of the National Academy of Sciences of the United States	Vol. 111/Issue 6	National Academy of Sciences	United States	2014	2271-2276	10.1073/pnas.1308062111	Yes
90	Policy: An intergovernmental panel on antimicrobial resistance	Mark Woolhouse , Jeremy Farrar	Nature	Vol. 509/Issue 7502	Nature Publishing Group	United Kingdom	2014	555-557	10.1038/509555a	Yes
91	High Fitness Costs and Instability of Gene Duplications Reduce Rates of Evolution of New Genes by Duplication-Divergence Mechanisms	M. Adler , M. Anjum , O. G. Berg , D. I. Andersson , L. Sandegren	Molecular Biology and Evolution	Vol. 31/Issue 6	Oxford University Press	United Kingdom	2014	1526-1535	10.1093/molbev/msu111	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
92	Microbiological effects of sublethal levels of antibiotics	Dan I. Andersson , Diarmaid Hughes	Nature Reviews Microbiology	Vol. 12/Issue 7	Nature Publishing Group	United Kingdom	2014	465-478	10.1038/nrmicro3270	Yes
93	Selection of a Multidrug Resistance Plasmid by Sublethal Levels of Antibiotics and Heavy Metals	E. Gullberg , L. M. Albrecht , C. Karlsson , L. Sandegren , D. I. Andersson	MBio	Vol. 5/Issue 5	American Society for Microbiology	United States	2014	e01918-14-e01918-14	10.1128/mBio.01918-14	Yes
94	Emergence and spread of antibiotic resistance: setting a parameter space	José Luis Martínez , Fernando Baquero	Upsala Journal of Medical Sciences	Vol. 119/Issue 2	Informa Healthcare	United Kingdom	2014	68-77	10.3109/03009734.2014.901444	Yes
95	Counteracting antibiotic resistance: breaking barriers among antibacterial strategies	Fernando Baquero , Teresa M Coque , Rafael Cantón	Expert Opinion on Therapeutic Targets	Vol. 18/Issue 8	Informa Healthcare	United Kingdom	2014	851-861	10.1517/14728222.2014.925881	Yes
96	A model-guided analysis and perspective on the evolution and epidemiology of antibiotic resistance and its future	Bruce R Levin , Fernando Baquero , Pål J Johnsen	Current Opinion in Microbiology	Vol. 19	Elsevier Limited	United Kingdom	2014	83-89	10.1016/j.mib.2014.06.004	Yes
97	Targeted adsorption of molecules in the colon with the novel adsorbent-based Medicinal Product, DAV132: A proof	Jean de Gunzburg , Annie Ducher , Christiane Modess , Danilo Wegner	Journal of Clinical Pharmacology	vol. 0	SAGE Publications Inc.	United States	2014	n/a-n/a	10.1002/jcph.359	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	of concept study in healthy subjects	, Stefan Oswald , Jennifer Dressman , Violaine Augustin , Céline Feger , Antoine Andremont , Werner Weitschies , Werner Siegmund								
98	MgrB Inactivation Is a Common Mechanism of Colistin Resistance in KPC-Producing Klebsiella pneumoniae of Clinical Origin	A. Cannatelli , T. Giani , M. M. D'Andrea , V. Di Pilato , F. Arena , V. Conte , K. Tryfinopoulou , A. Vatopoulos , G. M. Rossolini	Antimicrobial Agents and Chemotherapy	Vol. 58/Issue 10	American Society for Microbiology	United States	2014	5696-5703	10.1128/AAC.03110-14	Yes
99	Characterization of pFOX-7a, a conjugative IncL/M plasmid encoding the FOX-7 AmpC-type -lactamase, involved in a large outbreak in a neonatal intensive care unit	V. Di Pilato , F. Arena , T. Giani , V. Conte , S. Cresti , G. M. Rossolini	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 10	Oxford University Press	United Kingdom	2014	2620-2624	10.1093/jac/dku216	Yes
100	In Vivo Evolution to	A. Cannatelli ,	Antimicrobial	Vol.	American Society	United	2014	4399-	10.1128/A	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Colistin Resistance by PmrB Sensor Kinase Mutation in KPC-Producing Klebsiella pneumoniae Is Associated with Low-Dosage Colistin Treatment	V. Di Pilato , T. Giani , F. Arena , S. Ambretti , P. Gaibani , M. M. D'Andrea , G. M. Rossolini	Agents and Chemotherapy	58/Issue 8	for Microbiology	States		4403	AC.02555-14	
101	Colistin resistance superimposed to endemic carbapenem-resistant Klebsiella pneumoniae: a rapidly evolving problem in Italy, November 2013 to April 2014	Monaco M, Giani T, Raffone M, Arena F, Garcia-Fernandez A, Pollini S, Network EuSCAPE-Italy C, Grundmann H, Pantosti A, Rossolini G.	Euro surveillance : bulletin europeen sur les maladies transmissibles = European communicable disease bulletin	Vol. 19/Issue 42	Centre Europeen pour la Surveillance Epidemiologique du SIDA		2014	01-mei		Yes
102	Emergence of Escherichia coli ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy	M. Accogli , T. Giani , M. Monaco , M. Giufre , A. Garcia-Fernandez , V. Conte , F. D'Ancona , A. Pantosti , G. M. Rossolini , M. Cerquetti	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 8	Oxford University Press	United Kingdom	2014	2293-2296	10.1093/jac/dku132	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
103	Epidemic Diffusion of OXA-23-Producing Acinetobacter baumannii Isolates in Italy: Results of the First Cross-Sectional Countrywide Survey	L. Principe , A. Piazza , T. Giani , S. Bracco , M. S. Caltagirone , F. Arena , E. Nucleo , F. Tammaro , G. M. Rossolini , L. Pagani , F. Luzzaro	Journal of Clinical Microbiology	Vol. 52/Issue 8	American Society for Microbiology	United States	2014	3004-3010	10.1128/JCM.00291-14	Yes
104	Cross-Infection of Solid Organ Transplant Recipients by a Multidrug-Resistant Klebsiella pneumoniae Isolate Producing the OXA-48 Carbapenemase, Likely Derived from a Multiorgan Donor	T. Giani , V. Conte , S. Mandala , M. M. D'Andrea , F. Luzzaro , P. G. Conaldi , P. Grossi , G. M. Rossolini	Journal of Clinical Microbiology	Vol. 52/Issue 7	American Society for Microbiology	United States	2014	2702-2705	10.1128/JCM.00511-14	Yes
105	Rapid Resistome Fingerprinting and Clonal Lineage Profiling of Carbapenem-Resistant Klebsiella pneumoniae Isolates by Targeted Next-Generation Sequencing	F. Arena , P. A. Rolfe , G. Doran , V. Conte , S. Gruszka , T. Clarke , Y. Adesokan , T. Giani , G. M. Rossolini	Journal of Clinical Microbiology	Vol. 52/Issue 3	American Society for Microbiology	United States	2014	987-990	10.1128/JCM.03247-13	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
106	Breakthrough Bacteremia by Linezolid-Susceptible Enterococcus faecalis under Linezolid Treatment in a Severe Polytrauma Patient	F. Arena , T. Giani , A. Galano , M. Pasculli , V. Peccianti , M. I. Cassetta , A. Novelli , G. M. Rossolini	Antimicrobial Agents and Chemotherapy	Vol. 57/Issue 12	American Society for Microbiology	United States	2013	6411-6412	10.1128/AAC.01112-13	Yes
107	Diversity of Capsular Polysaccharide Gene Clusters in Kpc-Producing Klebsiella pneumoniae Clinical Isolates of Sequence Type 258 Involved in the Italian Epidemic	Marco Maria D'Andrea , Francesco Amisano , Tommaso Giani , Viola Conte , Nagaia Ciacci , Simone Ambretti , Luisa Santoriello , Gian Maria Rossolini	PLoS One	Vol. 9/Issue 5	Public Library of Science	United States	2014	e96827	10.1371/journal.pone.0096827	Yes
108	Carbapenemase-producing Enterobacteriaceae during 2011-12 in the Bolzano area (Northern Italy): increasing diversity in a low-endemicity setting	Richard Aschbacher , Tommaso Giani , Daniele Corda , Viola Conte , Fabio Arena , Valentina Pasquetto , Katia Scalzo ,	Diagnostic Microbiology and Infectious Disease	Vol. 77/Issue 4	Elsevier Inc.	United States	2013	354-356	10.1016/j.diagmicrobio.2013.08.029	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Maira Nicoletti , Gian Maria Rossolini , Elisabetta Pagani								
109	Large Oligoclonal Outbreak Due to Klebsiella pneumoniae ST14 and ST26 Producing the FOX-7 AmpC - Lactamase in a Neonatal Intensive Care Unit	F. Arena , T. Giani , E. Becucci , V. Conte , G. Zanelli , M. M. D'Andrea , G. Buonocore , F. Bagnoli , A. Zanchi , F. Montagnani , G. M. Rossolini	Journal of Clinical Microbiology	Vol. 51/Issue 12	American Society for Microbiology	United States	2013	4067-4072	10.1128/JCM.01982-13	Yes
110	General principles of antibiotic resistance in bacteria	Jose L. Martinez	Drug Discovery Today: Technologies	Vol. 11	Elsevier Limited	United Kingdom	2014	33-39	10.1016/j.ddtec.2014.02.001	Yes
111	Interplay between intrinsic and acquired resistance to quinolones in	Guillermo García-León , Fabiola Salgado , Juan Carlos Oliveros , María Blanca Sánchez , José Luis Martínez	Environmental Microbiology	Vol. 16/Issue 5	Blackwell Publishing	United Kingdom	2014	1282-1296	10.1111/1462-2920.12408	Yes
112	Short-sighted evolution of bacterial opportunistic	JosÃ© L. MartÃ-nez	Frontiers in Microbiology	Vol. 5	Frontiers Research Foundation	Switzerland	2014	01-apr	10.3389/fmicb.2014.00239	Yes

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	pathogens with an environmental origin									
113	Metabolic Compensation of Fitness Costs Associated with Overexpression of the Multidrug Efflux Pump MexEF-OprN in Pseudomonas aeruginosa	J. Olivares , C. Alvarez-Ortega , J. L. Martinez	Antimicrobial Agents and Chemotherapy	Vol. 58/Issue 7	American Society for Microbiology	United States	2014	3904-3913	10.1128/AAC.00121-14	Yes
114	Characterization of a novel Zn2+-dependent intrinsic imipenemase from Pseudomonas aeruginosa	A. Fajardo , S. Hernando-Amado , A. Oliver , G. Ball , A. Filloux , J. L. Martinez	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 11	Oxford University Press	United Kingdom	2014	2972-2978	10.1093/jac/dku267	Yes
115	A Function of SmeDEF, the Major Quinolone Resistance Determinant of Stenotrophomonas maltophilia, Is the Colonization of Plant Roots	G. Garcia-Leon , A. Hernandez , S. Hernando-Amado , P. Alavi , G. Berg , J. L. Martinez	Applied and Environmental Microbiology	Vol. 80/Issue 15	American Society for Microbiology	United States	2014	4559-4565	10.1128/AEM.01058-14	Yes
116	Recombination Blurs Phylogenetic Groups Routine Assignment in Escherichia coli:	María-Carmen Turrientes , José-María González-Alba , Rosa del	PLoS One	Vol. 9/Issue 8	Public Library of Science	United States	2014	e105395	10.1371/journal.pone.0105395	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Setting the Record Straight	Campo , María-Rosario Baquero , Rafael Cantón , Fernando Baquero , Juan Carlos Galán								
117	Rapid Detection of -Lactamase-Hydrolyzing Extended-Spectrum Cephalosporins in Enterobacteriaceae by Use of the New Chromogenic Lacta Test	M. I. Morosini , M. Garcia-Castillo , M. Tato , D. Gijon , A. Valverde , P. Ruiz-Garbajosa , R. Canton	Journal of Clinical Microbiology	Vol. 52/Issue 5	American Society for Microbiology	United States	2014	1741-1744	10.1128/JCM.03614-13	Yes
118	Individual variability in finger-to-finger transmission efficiency of	Rosa del Campo , Ana María Sánchez-Díaz , Javier Zamora , Carmen Torres , Luis María Cintas , Elvira Franco , Rafael Cantón , Fernando Baquero	MicrobiologyOpen	Vol. 3/Issue 1	Wiley-Blackwell	United Kingdom	2014	128-132	10.1002/mbo3.156	Yes
119	MALDI-TOF mass spectrometry as a tool for the discrimination of	Â. Novais , C. Sousa , J. de Dios Caballero , A. Fernandez-	European Journal of Clinical Microbiology and Infectious	Vol. 33/Issue 8	Springer Verlag	Germany	2014	1391-1399	10.1007/s10096-014-2071-5	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	high-risk Escherichia coli clones from phylogenetic groups B2 (ST131) and D (ST69, ST405, ST393)	Olmos , J. Lopes , H. Ramos , T. M. Coque , R. Cantón , L. Peixe	Diseases							
120	Structural independence of conjugative coupling protein TrwB from its Type IV secretion machinery	Delfina Larrea , Héctor D. de Paz , Ignacio Arechaga , Fernando de la Cruz , Matxalen Llosa	Plasmid	Vol. 70/Issue 1	Academic Press Inc.	United States	2014	146-153	10.1016/j.plasmid.2013.03.006	Yes
121	Functional Interactions of VirB11 Traffic ATPases with VirB4 and VirD4 Molecular Motors in Type IV Secretion Systems	J. Ripoll-Rozada , S. Zunzunegui , F. de la Cruz , I. Arechaga , E. Cabezon	Journal of Bacteriology	Vol. 195/Issue 18	American Society for Microbiology	United States	2014	4195-4201	10.1128/JB.00437-13	Yes
122	Molecular Characterization and Genetic Diversity of ESBL-Producing	John Báez , Marta Hernández-García , Constanza Guamparito , Sofía Díaz , Abdon Olave , Katherine Guerrero ,	Microbial Drug Resistance	Vol.00	Mary Ann Liebert Inc.	United States	2014	1,41014E+14	10.1089/mdr.2014.0158	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Rafael Cantón , Fernando Baquero , Joselyne Gahona , Nicomedes Valenzuela , Rosa del Campo , Juan Silva								
123	Complete sequence of pV404, a novel IncI1 plasmid harbouring blaCTX-M-14 in an original genetic context	Eleonora Riccobono , Vincenzo Di Pilato , Ana Liz Villagran , Alessandro Bartoloni , Gian Maria Rossolini , Lucia Pallecchi	International Journal of Antimicrobial Agents	Vol. 44/Issue 4	Elsevier	Netherlands	2014	374-376	10.1016/j.ijantimicag.2014.06.019	Yes
124	Negative Feedback and Transcriptional Overshooting in a Regulatory Network for Horizontal Gene Transfer	Raul Fernandez-Lopez , Irene del Campo , Carlos Revilla , Ana Cuevas , Fernando de la Cruz	PLoS Genetics	Vol. 10/Issue 2	Public Library of Science	United States	2014	e1004171	10.1371/journal.pgen.1004171	Yes
125	Key components of the eight classes of type IV secretion systems involved in	J. Guglielmini , B. Neron , S. S. Abby , M. P. Garcillan-	Nucleic Acids Research	Vol. 42/Issue 9	Oxford University Press	United Kingdom	2014	5715-5727	10.1093/nar/gku194	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	bacterial conjugation or protein secretion	Barcia , F. d. la Cruz , E. P. C. Rocha								
126	Ordering the bestiary of genetic elements transmissible by conjugation	Maria Pilar Garcillán-Barcia , Fernando de la Cruz	Mobile Genetic Elements	Vol. 3/Issue 1	Landes Bioscience	United States	2013	e24263	10.4161/mge.24263	Yes
127	Plasmid Conjugation from Proteobacteria as Evidence for the Origin of Xenologous Genes in Cyanobacteria	D. Encinas , M. P. Garcillan-Barcia , M. Santos-Merino , L. Delaye , A. Moya , F. de la Cruz	Journal of Bacteriology	Vol. 196/Issue 8	American Society for Microbiology	United States	2014	1551-1559	10.1128/JB.01464-13	Yes
128	PipX, the coactivator of NtcA, is a global regulator in cyanobacteria	J. Espinosa , F. Rodriguez-Mateos , P. Salinas , V. F. Lanza , R. Dixon , F. de la Cruz , A. Contreras	Proceedings of the National Academy of Sciences of the United States	Vol. 111/Issue 23	National Academy of Sciences	United States	2014	E2423-E2430	10.1073/pnas.1404097111	Yes
129	A high security double lock and key mechanism in HUH relaxases controls oriT-processing for plasmid conjugation	J. D. Carballeira , B. Gonzalez-Perez , G. Moncalian , F. d. la Cruz	Nucleic Acids Research	Vol. 42/Issue 16	Oxford University Press	United Kingdom	2014	10632-10643	10.1093/nar/gku741	Yes
130	Towards an integrated model of bacterial	Elena Cabezón , Jorge Ripoll-Rozada ,	FEMS Microbiology Reviews	Vol. 38/Issue 6	Blackwell Publishing	United Kingdom	2014	n/a-n/a	10.1111/1574-6976.12085	Yes

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	conjugation	Alejandro Peña , Fernando de la Cruz , Ignacio Arechaga								
131	High diversity of plasmids harbouring blaCMY-2 among clinical Escherichia coli isolates from humans and companion animals in the upper Midwestern USA	V. Bortolaia , K. H. Hansen , C. A. Nielsen , T. R. Fritsche , L. Guardabassi	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 6	Oxford University Press	United Kingdom	2014	1492-1496	10.1093/jac/dku011	Yes
132	Strain Diversity of CTX-M-Producing Enterobacteriaceae in Individual Pigs: Insights into the Dynamics of Shedding during the Production Cycle	K. H. Hansen , V. Bortolaia , P. Damborg , L. Guardabassi	Applied and Environmental Microbiology	Vol. 80/Issue 21	American Society for Microbiology	United States	2014	6620-6626	10.1128/AEM.01730-14	Yes
133	Complete sequences of IncHI1 plasmids carrying blaCTX-M-1 and qnrS1 in equine Escherichia coli provide new insights into plasmid evolution	M. Dolejska , L. Villa , M. Minoia , L. Guardabassi , A. Carattoli	Journal of Antimicrobial Chemotherapy	Vol. 69/Issue 9	Oxford University Press	United Kingdom	2014	2388-2393	10.1093/jac/dku172	Yes
134	Enterococcus faecalis Prophage Dynamics and	Renata C. Matos , Nicolas Lapaque ,	PLoS Genetics	Vol. 9/Issue 6	Public Library of Science	United States	2014	e1003539	10.1371/journal.pgen.1003539	Yes

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	Contributions to Pathogenic Traits	Lionel Rigottier-Gois , Laurent Debarbieux , Thierry Meylheuc , Bruno Gonzalez-Zorn , Francis Repoila , Maria de Fatima Lopes , Pascale Serror								
135	Culturable aerobic and facultative bacteria from the gut of the polyphagic dung beetle	Noemi Hernández , José A. Escudero , Álvaro San Millán , Bruno González-Zorn , Jorge M. Lobo , José R. Verdú , Mónica Suárez	Insect Science	Vol. 21/Issue 5	Blackwell Publishing	United Kingdom	2014	n/a-n/a	10.1111/1744-7917.12094	Yes
136	Prevalence and molecular characterisation of New Delhi metallo-β-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant	Mohibur Rahman , Sanket Kumar Shukla , Kashi Nath Prasad , Cristina M. Ovejero , Binod Kumar Pati ,	International Journal of Antimicrobial Agents	Vol. 44/Issue 1	Elsevier	Netherlands	2014	30-37	10.1016/j.ijantimicag.2014.03.003	Yes

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	Enterobacteriaceae from India	Aparna Tripathi , Avinash Singh , Ashwini K. Srivastava , Bruno Gonzalez-Zorn								
137	Multiple Pathways of Genome Plasticity Leading to Development of Antibiotic Resistance	Zeynep Baharoglu , Geneviève Garriss , Didier Mazel	Antibiotics	Vol. 2/Issue 2	MDPI AG, Basel, Switzerland	Switzerland	2013	288-315	10.3390/antibiotics2020288	Yes
138	Identification of genes involved in low aminoglycoside-induced SOS response in Vibrio cholerae: a role for transcription stalling and Mfd helicase	Z. Baharoglu , A. Babosan , D. Mazel	Nucleic Acids Research	Vol. 42/Issue 4	Oxford University Press	United Kingdom	2014	2366-2379	10.1093/nar/gkt1259	Yes
139	The Integron Integrase Efficiently Prevents the Melting Effect of Escherichia coli Single-Stranded DNA-Binding Protein on Folded attC Sites	C. Loot , V. Parissi , J. A. Escudero , J. Amarir-Bouhram , D. Bikard , D. Mazel	Journal of Bacteriology	Vol. 196/Issue 4	American Society for Microbiology	United States	2014	762-771	10.1128/JB.01109-13	Yes
140	vanO, a New Glycopeptide	D. D. Gudeta , A. Moodley ,	Antimicrobial Agents and	Vol. 58/Issue 3	American Society for Microbiology	United States	2014	1768-1770	10.1128/AAC.01880-	Yes

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	Resistance Operon in Environmental Rhodococcus equi Isolates	V. Bortolaia , L. Guardabassi	Chemotherapy						13	
141	Collective navigation of cargo-carrying swarms	A. Shklarsh , A. Finkelshtein , G. Ariel , O. Kalisman , C. Ingham , E. Ben-Jacob	Interface Focus	Vol. 2/Issue 6	The Royal Society	United Kingdom	2012	786-798	10.1098/rsfs.2012.0029	Yes
142	Gene flow in environmental Legionella pneumophila leads to genetic and pathogenic heterogeneity within a Legionnaires' disease outbreak	Paul R McAdam , Charles W Vander Broek , Diane Lindsay , Melissa J Ward , Mary F Hanson , Michael Gillies , Mick Watson , Joanne M Stevens , Giles F Edwards , J Fitzgerald	Genome Biology	Vol. 15/Issue 11	BioMed Central		2014	504	10.1186/s13059-014-0504-1	Yes
143	Antimicrobial resistance in humans, livestock and the wider environment	M. Woolhouse , M. Ward , B. van Bunnik , J. Farrar	Philosophical Transactions of the Royal Society B: Biological Sciences	Vol. 370/Issue 1670	Royal Society of London	United Kingdom	2015	20140083 - 20140083	10.1098/rstb.2014.0083	Yes
144	A single natural nucleotide mutation alters bacterial	David Viana , María Comos , Paul R	Nature Genetics	Vol. 47/Issue 4	Nature Publishing Group	United Kingdom	2015	361-366	10.1038/ng.3219	Yes

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	pathogen host tropism	McAdam , Melissa J Ward , Laura Selva , Caitriona M Guinane , Beatriz M González-Muñoz , Anne Tristan , Simon J Foster , J Ross Fitzgerald , José R Penadés								
145	Plasmid Flux in Escherichia coli ST131 Sublineages, Analyzed by Plasmid Constellation Network (PLACNET), a New Method for Plasmid Reconstruction from Whole Genome Sequences	Val F. Lanza , María de Toro , M. Pilar Garcillán-Barcia , Azucena Mora , Jorge Blanco , Teresa M. Coque , Fernando de la Cruz	PLoS Genetics	Vol. 10/Issue 12	Public Library of Science	United States	2014	e1004766	10.1371/journal.pgen.1004766	
146	Widening the Spaces of Selection: Evolution along Sublethal Antimicrobial Gradients: FIG 1	Fernando Baquero , Teresa M. Coque	MBio	Vol. 5/Issue 6	American Society for Microbiology	United States	2014	e02270-14	10.1128/mBio.02270-14	Yes
147	What is a resistance	José L.	Nature Reviews	Vol.	Nature Publishing	United	2014	116-123	10.1038/nr	Yes

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	gene? Ranking risk in resistomes	Martínez , Teresa M. Coque , Fernando Baquero	Microbiology	13/Issue 2	Group	Kingdom			micro3399	
148	Causes and interventions: need of a multiparametric analysis of microbial ecobiology	Fernando Baquero	Environmental Microbiology Reports	Vol. 7/Issue 1	Wiley-Blackwell	United States	2015	13-14	10.1111/1758-2229.12242	Yes
149	Amdinocillin (Mecillinam) Resistance Mutations in Clinical Isolates and Laboratory-Selected Mutants of Escherichia coli	Elisabeth Thulin , Martin Sundqvist , Dan I. Andersson	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 3	American Society for Microbiology	United States	2015	1718-1727	10.1128/AAC.04819-14	Yes
150	Public health evolutionary biology of antimicrobial resistance: priorities for intervention	Fernando Baquero , Val F. Lanza , Rafael Cantón , Teresa M. Coque	Evolutionary Applications	Vol. 8/Issue 3	Wiley-Blackwell		2015	223-239	10.1111/eva.12235	Yes
151	Population Biology of Intestinal Enterococcus Isolates from Hospitalized and Nonhospitalized Individuals in	Ana P. Tedim , Patricia Ruiz-Garbajosa , Jukka Corander , Concepción M. Rodríguez , Rafael Cantón ,	Applied and Environmental Microbiology	Vol. 81/Issue 5	American Society for Microbiology	United States	2015	1820-1831	10.1128/AEM.03661-14	

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Different Age Groups	Rob J. Willems , Fernando Baquero , Teresa M. Coque								
152	Investigating the mobilome in clinically important lineages of Enterococcus faecium and Enterococcus faecalis	Theresa Mikalsen , Torunn Pedersen , Rob Willems , Teresa M Coque , Guido Werner , Ewa Sadowy , Willem van Schaik , Lars Bogø Jensen , Arnfinn Sundsfjord , Kristin Hegstad	BMC Genomics	Vol. 16/Issue 1	BioMed Central	United Kingdom	2015	282	10.1186/s12864-015-1407-6	Yes
153	Tackling antibiotic resistance: the environmental framework	Thomas U. Berendonk,Célia M. Manaia,Christophe Merlin,Despo Fatta-Kassinos,Eddie Cytryn,Fiona Walsh,Helmut Bürgmann,Henning	Nature Reviews Microbiology	13	Nature Publishing Group		2015	310-317	10.1038/nrmicro3439	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Sørum,Madelaine Norström,Marie-Noëlle Pons,Norbert Kreuzinger,Pentti Huovinen,Stefania Stefani,Thomas Schwartz,Veljo Kisand, Fernando Baquero& José Luis Martinez								
154	Antibiotic-Resistant Klebsiella pneumoniae and Escherichia coli High-Risk Clones and an IncFII k Mosaic Plasmid Hosting Tn 1 (bla TEM-4) in Isolates from 1990 to 2004	Irene Rodríguez , Ângela Novais , Felipe Lira , Aránzazu Valverde , Tânia Curião , José Luis Martínez , Fernando Baquero , Rafael Cantón , Teresa M. Coque	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 5	American Society for Microbiology	United States	2015	2904-2908	10.1128/AAC.00296-15	Yes
155	Prioritizing risks of antibiotic resistance genes in all	José L. Martínez , Teresa M.	Nature Reviews Microbiology	Vol. 13/Issue 6	Nature Publishing Group	United Kingdom	2015	396-396	10.1038/nrmicro3399-c2	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	metagenomes	Coque , Fernando Baquero								
156	Antimicrobial resistance in humans, livestock and the wider environment	M. Woolhouse , M. Ward , B. van Bunnik , J. Farrar	Philosophical Transactions of the Royal Society B: Biological Sciences	Vol. 370/Issue 1670	Royal Society of London	United Kingdom	2015	20140083 - 20140083	10.1098/rstb.2014.0083	Yes
157	Polymorphic Variation in Susceptibility and Metabolism of Triclosan-Resistant Mutants of Escherichia coli and Klebsiella pneumoniae Clinical Strains Obtained after Exposure to Biocides and Antibiotics	Tânia Curiao , Emmanuela Marchi , Carlo Viti , Marco R. Oggioni , Fernando Baquero , José Luis Martinez , Teresa M. Coque	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 6	American Society for Microbiology	United States	2015	3413-3423	10.1128/AAC.00187-15	Yes
158	Efficient national surveillance for health-care-associated infections	B. A. D. van Bunnik , M. Ciccolini , C. L. Gibbons , G. Edwards , R. Fitzgerald , P. R. McAdam , M. J. Ward , I. F. Laurenson , M. E. J. Woolhouse	BMC Public Health	Vol. 15/Issue 1	BioMed Central	United Kingdom	2015	832	10.1186/s12889-015-2172-9	Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
159	A membrane computing simulator of trans-hierarchical antibiotic resistance evolution dynamics in nested ecological compartments (ARES)	Marcelino Campos , Carlos Llorens , José M. Sempere , Ricardo Futami , Irene Rodriguez , Purificación Carrasco , Rafael Capilla , Amparo Latorre , Teresa M. Coque , Andres Moya , Fernando Baquero	Biology Direct	Vol. 10/Issue 1	BioMed Central	United Kingdom	2015	41	10.1186/s13062-015-0070-9	Yes
160	Utility of Whole-Genome Sequencing of Escherichia coli O157 for Outbreak Detection and Epidemiological Surveillance: FIG 1	Anne Holmes , Lesley Allison , Melissa Ward , Timothy J. Dallman , Richard Clark , Angie Fawkes , Lee Murphy , Mary Hanson	Journal of Clinical Microbiology	Vol. 53/Issue 11	American Society for Microbiology	United States	2015	3565-3573	10.1128/JCM.01066-15	Yes
161	The inactivation of RNase G reduces the Stenotrophomonas maltophilia susceptibility to	Alejandra Bernardini , Fernando Corona , Ricardo Dias , Maria B.	Frontiers in Microbiology	Vol. 6	Frontiers Research Foundation	Switzerland	2015	1-	10.3389/fmicb.2015.01068	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	quinolones by triggering the heat shock response	Sánchez , Jose L. Martínez								
162	Draft Genome Sequence of the First Hypermucoviscous Klebsiella quasipneumoniae subsp. quasipneumoniae Isolate from a Bloodstream Infection	Fabio Arena , Lucia Henrici De Angelis , Filippo Pieralli , Vincenzo Di Pilato , Tommaso Giani , Francesca Torricelli , Marco Maria D'Andrea , Gian Maria Rossolini	Genome Announcement	Vol. 3/Issue 5	American Society for Microbiology	United States	2015	e00952-15	10.1128/genomeA.00952-15	Yes
163	Polymyxin Resistance Caused by mgrB Inactivation Is Not Associated with Significant Biological Cost in Klebsiella pneumoniae	Antonio Cannatelli , Alfonso Santos-Lopez , Tommaso Giani , Bruno Gonzalez-Zorn , Gian Maria Rossolini	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 5	American Society for Microbiology	United States	2015	2898-2900	10.1128/AAC.04998-14	Yes
164	MgrB Inactivation Is a Common Mechanism of Colistin Resistance in KPC-Producing Klebsiella	A. Cannatelli , T. Giani , M. M. D'Andrea , V. Di Pilato , F. Arena , V. Conte , K.	Antimicrobial Agents and Chemotherapy	Vol. 58/Issue 10	American Society for Microbiology	United States	2014	5696-5703	10.1128/AAC.03110-14	Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	pneumoniae of Clinical Origin	Tryfinopoulou , A. Vatopoulos , G. M. Rossolini								
165	A core genome MLST scheme for high-resolution typing of Enterococcus faecium	Mark de Been , Mette Pinholt , Janetta Top , Stefan Bletz , Alexander Mellmann , Willem van Schaik , Ellen Brouwer , Malbert Rogers , Yvette Kraat , Marc Bonten , Jukka Corander , Henrik Westh , Dag Harmsen , Rob J. L. Willems	Journal of Clinical Microbiology	53	American Society for Microbiology	United States	2015	3788-3797	10.1128/JCM.01946-15	Yes
166	Antibiotic-Driven Dysbiosis Mediates Intraluminal Agglutination and Alternative Segregation of Enterococcus faecium from the Intestinal Epithelium	A. P. A. Hendrickx , J. Top , J. R. Bayjanov , H. Kemperman , M. R. C. Rogers , F. L. Paganelli , M. J. M. Bonten , R. J. L. Willems	MBio	Vol. 6/Issue 6	American Society for Microbiology	United States	2015	e01346-15-e01346-15	10.1128/mBio.01346-15	Yes
167	Dissemination of	Mark de Been ,	PLoS Genetics	Vol.	Public Library of	United	2014	e1004776	10.1371/jou	Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Cephalosporin Resistance Genes between Escherichia coli Strains from Farm Animals and Humans by Specific Plasmid Lineages	Val F. Lanza , María de Toro , Jelle Scharringa , Wietske Dohmen , Yu Du , Juan Hu , Ying Lei , Ning Li , Ave Tooming-Klunderud , Dick J. J. Heederik , Ad C. Fluit , Marc J. M. Bonten , Rob J. L. Willems , Fernando de la Cruz , Willem van Schaik		10/Issue 12	Science	States			rnal.pgen.1004776	
168	Deletions in a ribosomal protein-coding gene are associated with tigecycline resistance in Enterococcus faecium	Marc Niebel , Joshua Quick , Ana Maria Guzman Prieto , Robert L.R. Hill , Rachel Pike , Damon Huber , Miruna David , Michael Hornsey , David Wareham ,	International Journal of Antimicrobial Agents	Vol. 46/Issue 5	Elsevier	Netherlands	2015	572-575	10.1016/j.ijantimicag.2015.07.009	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Beryl Oppenheim , Neil Woodford , Willem van Schaik , Nicholas Loman								
169	Regulation of Sm qnr expression by Sm qnrR is strain-specific in Stenotrophomonas maltophilia : Table 1.	María Blanca Sánchez , José Luis Martínez	Journal of Antimicrobial Chemotherapy	Vol. 70/Issue 10	Oxford University Press	United Kingdom	2015	2913-2914	10.1093/jac/dkv196	Yes
170	The Efflux Pump SmeDEF Contributes to Trimethoprim-Sulfamethoxazole Resistance in Stenotrophomonas maltophilia: TABLE 1	María Blanca Sánchez , José Luis Martínez	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 7	American Society for Microbiology	United States	2015	4347-4348	10.1128/AAC.00714-15	Yes
171	High-level quinolone resistance is associated with the overexpression of smeVWX in Stenotrophomonas maltophilia clinical isolates	G. García-León , C. Ruiz de Alegría Puig , C. García de la Fuente , L. Martínez-Martínez , J.L. Martínez , M.B. Sánchez	Clinical Microbiology and Infection	Vol. 21/Issue 5	Blackwell Publishing	United Kingdom	2015	464-467	10.1016/j.cmi.2015.01.007	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
172	Amelioration of the Fitness Costs of Antibiotic Resistance Due To Reduced Outer Membrane Permeability by Upregulation of Alternative Porins	Michael Knopp , Dan I. Andersson	Molecular Biology and Evolution	32	Oxford University Press	United Kingdom	2015	3252-3263	10.1093/molbev/msv195	Yes
173	Accuracy of different methods for susceptibility testing of gentamicin with KPC carbapenemase-producing Klebsiella pneumoniae	Fabio Arena , Tommaso Giani , Guendalina Vaggelli , Giovanni Terenzi , Patrizia Pecile , Gian Maria Rossolini	Diagnostic Microbiology and Infectious Disease	Vol. 81/Issue 2	Elsevier Inc.	United States	2015	132-134	10.1016/j.diagmicrobio.2014.10.011	Yes
174	Limited dissemination of the wastewater treatment plant core resistome	Christian Munck , Mads Albertsen , Amar Telke , Mostafa Ellabaan , Per Halkjær Nielsen , Morten O. A. Sommer	Nature Communications	Vol. 6	Nature Publishing Group	United Kingdom	2015	8452	10.1038/ncomms9452	Yes
175	Mining microbial metatranscriptomes for expression of	Dennis Versluis , Marco Maria D'Andrea ,	Scientific Reports	Vol. 5	Nature Publishing Group	United Kingdom	2015	11981	10.1038/srep11981	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	antibiotic resistance genes under natural conditions	Javier Ramiro Garcia , Milkha M. Leimena , Floor Hugenholtz , Jing Zhang , Başak Öztürk , Lotta Nylund , Detmer Sipkema , Willem van Schaik , Willem M. de Vos , Michiel Kleerebezem , Hauke Smidt , Mark W.J. van Passel								
176	Transcription factor-based biosensors enlightened by the analyte	Raul Fernandez-López , Raul Ruiz , Fernando de la Cruz , Gabriel Moncalián	Frontiers in Microbiology	Vol. 6	Frontiers Research Foundation	Switzerland	2015	1-	10.3389/fmicb.2015.00648	
177	The human gut resistome	W. van Schaik	Philosophical Transactions of the Royal Society B: Biological Sciences	Vol. 370/Issue 1670	Royal Society of London	United Kingdom	2015	20140087 - 20140087	10.1098/rstb.2014.0087	Yes
178	Bacterial Swarms Recruit Cargo	Alin Finkelshtein ,	MBio	Vol. 6/Issue 3	American Society for Microbiology	United States	2015	e00074-15	10.1128/mBio.00074-	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Bacteria To Pave the Way in Toxic Environments	Dalit Roth , Eshel Ben Jacob , Colin J. Ingham							15	
179	Extended spectrum β-lactamase-producing Escherichia coli forms filaments as an initial response to cefotaxime treatment	Thea Kjeldsen , Morten Sommer , John E Olsen	BMC Microbiology	Vol. 15/Issue 1	BioMed Central	United Kingdom	2015	63	10.1186/s12866-015-0399-3	Yes
180	Novel bla ROB-1 - Bearing Plasmid Conferring Resistance to β-Lactams in Haemophilus parasuis Isolates from Healthy Weaning Pigs	Javier Moleres , Alfonso Santos-López , Isidro Lázaro , Javier Labairu , Cristina Prat , Carmen Ardanuy , Bruno González-Zorn , Virginia Aragon , Junkal Garmendia	Applied and Environmental Microbiology	Vol. 81/Issue 9	American Society for Microbiology	United States	2015	3255-3267	10.1128/AEM.03865-14	Yes
181	Persistence of Vancomycin Resistance in Multiple Clones of Enterococcus faecium Isolated from Danish	Valeria Bortolaia , Manuela Mander , Lars B. Jensen , John E. Olsen , Luca	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 5	American Society for Microbiology	United States	2015	2926-2929	10.1128/AAC.05072-14	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Broilers 15 Years after the Ban of Avoparcin	Guardabassi								
182	Streptococcus gallolyticus subsp. gallolyticus from Human and Animal Origins: Genetic Diversity, Antimicrobial Susceptibility, and Characterization of a Vancomycin-Resistant Calf Isolate Carrying a vanA -Tn 1546 - Like Element	Beatriz Romero-Hernández , Ana P. Tedim , José Francisco Sánchez-Herrero , Pablo Librado , Julio Rozas , Gloria Muñoz , Fernando Baquero , Rafael Cantón , Rosa del Campo	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 4	American Society for Microbiology	United States	2015	2006-2015	10.1128/AAC.04083-14	Yes
183	Molecular characterization and antibiotic resistance of Enterococcus species from gut microbiota of Chilean Altiplano camelids	Katheryne Guerrero-Olmos , John Báez , Nicomédes Valenzuela , Joselyne Gahona , Rosa del Campo , Juan Silva	Infection Ecology & Epidemiology	Vol. 4/Issue 0	Co-Action Publishing	Sweden	2014	1-	10.3402/iee.v4.24714	
184	Distinct SagA from Hospital-Associated Clade A1 Enterococcus	F. L. Paganelli , M. de Been , J. C. Braat , T. Hoogenboezem	Applied and Environmental Microbiology	Vol. 81/Issue 19	American Society for Microbiology	United States	2015	6873-6882	10.1128/AEM.01716-15	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	faecium Strains Contributes to Biofilm Formation	, C. Vink , J. Bayjanov , M. R. C. Rogers , J. Huebner , M. J. M. Bonten , R. J. L. Willems , H. L. Leavis								
185	Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms	Diarmaid Hughes , Dan I. Andersson	Nature Reviews Genetics	Vol. 16/Issue 8	Nature Publishing Group	United Kingdom	2015	459-471	10.1038/nrg3922	Yes
186	Fitness of Salmonella mutants resistant to antimicrobial peptides	H. Lofton , N. Anwar , M. Rhen , D. I. Andersson	Journal of Antimicrobial Chemotherapy	Vol. 70/Issue 2	Oxford University Press	United Kingdom	2015	432-440	10.1093/jac/dku423	Yes
187	Indirect resistance to several classes of antibiotics in cocultures with resistant bacteria expressing antibiotic-modifying or -degrading enzymes	Hervé Nicoloff , Dan I. Andersson	Journal of Antimicrobial Chemotherapy	12	Oxford University Press	United Kingdom	2015	dkv312	10.1093/jac/dkv312	Yes
188	Advancing gut microbiome research using	Morten OA Sommer	Current Opinion in Microbiology	Vol. 27	Elsevier Limited	United Kingdom	2015	127-132	10.1016/j.mib.2015.08.004	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	cultivation									
189	Collateral Resistance and Sensitivity Modulate Evolution of High-Level Resistance to Drug Combination Treatment in Staphylococcus aureus	M. Rodriguez de Evgrafov , H. Gumpert , C. Munck , T. T. Thomsen , M. O. A. Sommer	Molecular Biology and Evolution	Vol. 32/Issue 5	Oxford University Press	United Kingdom	2015	1175-1185	10.1093/molbev/msv006	Yes
190	Prediction of resistance development against drug combinations by collateral responses to component drugs	C. Munck , H. K. Gumpert , A. I. N. Wallin , H. H. Wang , M. O. A. Sommer	Science Translational Medicine	Vol. 6/Issue 262	American Association for the Advancement of Science	United States	2014	262ra156 - 262ra156	10.1126/scitranslmed.3009940	Yes
191	Application of the Human Intestinal Tract Chip to the non-human primate gut microbiota	T.D.J. Bello González , M.W.J. van Passel , S. Tims , S. Fuentes , W.M. De Vos , H. Smidt , C. Belzer	Beneficial Microbes	Vol. 6/Issue 3	Wageningen Academic Publishers	Netherlands	2015	271-276	10.3920/BM2014.0087	Yes
192	Lack of dissemination of acquired resistance to β-lactams in small wild mammals around an isolated village in the	Nathalie Grall,Olivier Barraud,Ingrid Wieder,Anna Hua,Marion Perrier,Ana Babosan,Marga	Environmental Microbiology Reports	7	Wiley-Blackwell		2015	698-708	10.1111/1758-2229.12289	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Amazonian forest	ux Gaschet,Olivier Clermont,Erick Denamur,François Catzeflis,Dominique Decré,Marie-Cécile Ploy,Antoine Andremont								
193	Synthetic Fatty Acids Prevent Plasmid-Mediated Horizontal Gene Transfer	María Getinoa, David J. Sanabria-Ríosb, Raúl Fernández-Lópeza, Javier Campos-Gómeza*, José M. Sánchez-Lópezc, Antonio Fernándezc, Néstor M. Carballeirad, Fernando de la Cruza	MBio	6	American Society for Microbiology		2015	e01032-15	10.1128/mBio.01032-15	Yes
194	Comprehensive Functional Analysis of the 18 Vibrio cholerae N16961 Toxin-Antitoxin	Naeem Iqbal , Anne-Marie Guérout , Evelyne Krin , Frédérique Le	Journal of Bacteriology	Vol. 197/Issue 13	American Society for Microbiology	United States	2015	2150-2159	10.1128/JB.00108-15	Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Systems Substantiates Their Role in Stabilizing the Superintegron	Roux , Didier Mazel								
195	Small-Plasmid-Mediated Antibiotic Resistance Is Enhanced by Increases in Plasmid Copy Number and Bacterial Fitness	Alvaro San Millan , Alfonso Santos-Lopez , Rafael Ortega-Huedo , Cristina Bernabe-Balas , Sean P. Kennedy , Bruno Gonzalez-Zorn	Antimicrobial Agents and Chemotherapy	Vol. 59/Issue 6	American Society for Microbiology	United States	2015	3335-3341	10.1128/AAC.00235-15	Yes
196	Molecular epidemiology and virulence of Escherichia coli O16:H5-ST131: Comparison with H30 and H30-Rx subclones of O25b:H4-ST131	Ghizlane Dahbi , Azucena Mora , Rosalia Mamani , Cecilia López , María Pilar Alonso , Juan Marzoa , Miguel Blanco , Alexandra Herrera , Susana Viso , Fernando García-Garrote , Veronika Tchesnokova ,	International Journal of Medical Microbiology	Vol. 304/Issue 8	Urban und Fischer Verlag GmbH und Co. KG	Germany	2014	1247-1257	10.1016/j.ijmm.2014.10.002	Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Mariya Billig , Fernando de la Cruz , María de Toro , Juan José González-López , Guillermo Prats , Fernando Chaves , Luis Martínez-Martínez , Lorena López-Cerezo , Erick Denamur , Jorge Blanco								
197	Degenerate primer MOB typing of multiresistant clinical isolates of E. coli uncovers new plasmid backbones	M. Pilar Garcillán-Barcia , Belén Ruiz del Castillo , Andrés Alvarado , Fernando de la Cruz , Luis Martínez-Martínez	Plasmid	Vol. 77	Academic Press Inc.	United States	2015	17-27	10.1016/j.plasmid.2014.11.003	Yes
198	Bacterial computing with engineered populations	Martyn Amos , Ilka Maria Axmann , Nils Blüthgen , Fernando de la Cruz , Alfonso	Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering	Vol. 373/Issue 2046	Royal Society of London	United Kingdom	2015	20140218	10.1098/rsta.2014.0218	Yes

A1: List of scientific (peer reviewed) publications, starting with the most important ones										
No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
		Jaramillo , Alfonso Rodriguez-Paton , Friedrich Simmel	Sciences							
199	Rebooting the genome: The role of negative feedback in horizontal gene transfer	Raul Fernandez-Lopez , Fernando de la Cruz	Mobile Genetic Elements	Vol. 4/Issue 6	Landes Bioscience	United States	2014	42156	10.4161/21595256X.2014.988069	Yes
200	The Soil Microbiota Harbors a Diversity of Carbapenem-Hydrolyzing ß-Lactamases of Potential Clinical Relevance	Dereje Dadi Gudeta , Valeria Bortolaia , Greg Amos , Elizabeth M. H. Wellington , Kristian K. Brandt , Laurent Poirel , Jesper Boye Nielsen , Henrik Westh , Luca Guardabassi	Antimicrobial Agents and Chemotherapy	in press	American Society for Microbiology	United States	2015	AAC.01424-15	10.1128/AAC.01424-15	Yes
201	Enterococcus Diversity, Origins in Nature, and Gut Colonization	Francois Lebreton, Rob J. L. Willems, Michael S. Gilmore	Enterococci: From Commensals to Leading Causes of Drug Resistant Infection			United States	2014	3-44		Yes
202	Enterococcal	Kelli L. Palmer,	Enterococci: From Commensals to Leading Causes			United	2014	188-224		Yes

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No	Title	Author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year	Relevant pages	Permanent identifiers	Is/will open access provided
	Genomics	Willem van Schaik, Rob J. L. Willems, Michael S. Gilmore	of Drug Resistant Infection			States				
203	Plasmid Diversity and Adaptation Analyzed by Massive Sequencing of Escherichia coli Plasmids	María de Toro , Fernando de la Cruz , M. Pilar Garcillán-Barcia	Plasmids: Biology and Impact in Biotechnology and Discovery		American Society of Microbiology	United States	2015	219	10.1128/microbiolspec.PLAS-0031-2014	Yes
204	The Integron: Adaptation On Demand	José Antonio Escudero* , Aleksandra Nivina , Céline Loot* , Didier Mazel	Plasmids: Biology and Impact in Biotechnology and Discovery		American Society of Microbiology	United States	2015	139	10.1128/microbiolspec.MDNA3-0019-2014	Yes
205	The Plasmidome of Firmicutes: Impact on the Emergence and the Spread of Resistance to Antimicrobials	Val Fernández Lanza , Fernando Baquero , Teresa M. Coque , Ana P. Tedim , José Luís Martínez	Plasmids: Biology and Impact in Biotechnology and Discovery		American Society of Microbiology	United States	2015	381	10.1128/microbiolspec.PLAS-0039-2014	Yes

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
1	Activity stand	Woolhouse, M	European Researchers' Night Edinburgh, Explorathon, meet the experts (http://www.explorathon.co.uk/)	Sep-15	Edinburgh, UK	Companies, Research, Lay Public	Unknown	Scotland
2	Business plan	Ingham, C	Diagnostics related business plans/commercial documents	Apr-12	Utrecht, The Netherlands	Private Investors and Governmental Bodies	3-7 key decision makers	Europe
3	Computer Simulations	Baquero, F	Computing Simulator of Trans-Hierarchical Antibiotic Resistance Evolution Dynamics in Nested Ecological Compartments (ARES). http://gydb.uv.es/ares/public/index.php/auth	Sep-14	Madrid, Spain	Researchers	Worldwide	Worldwide
4	Investor meeting	Ingham, C	Investor meeting/funding pitch	May-12	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands
5	Investor meeting	Ingham, C	Investor meeting/funding pitch	Jun-12	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands
6	Investor meeting	Ingham, C	Investor to investor meeting	Mar-13	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands
7	Investor meeting	Ingham, C	Investors on progress Biodiscovery including EVOTAR strain collection.	Nov-13	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands
8	Investor meeting	Ingham, C	Investors on progress Biodiscovery including EVOTAR strain collection.	Dec-13	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
9	Investor meeting	Ingham, C	regional subsidy providers (Friesland) on antibiotic resistance related project.	Sep-14	Utrecht, The Netherlands	Private Investors and Governmental Bodies	Unknown	Netherlands
10	Invited Expert Panel	Coque TM	. Use of Whole Genome Sequencing (WGS) of food-borne pathogens for public health protection (EFSA SCIENTIFIC COLLOQUIUM 20). 16-17 June 2014	Jun-14	Parma, Italy	Researchers	100	Europe
11	Invited speaker	R.J.L. Willems	London Dental Institute	Oct-12	London, UK	Researchers	30	UK
12	Invited speaker	Baquero F.	Bacterial population biology: a model for analyzing the structure of a complex system. Seminarios de Investigación IRYCIS,	Jan-12	Madrid, Spain	CME (Continuing Medical Education)	100	Spain
13	Invited speaker	Martinez	Annual meeting American Association for the Advancement of Science	Feb-12	Vancouver, Canada	Research & industry	10000	Worldwide
14	Invited speaker	J.L. Martinez	Speaker. British Columbia University	Feb-12	Vancouver, Canada	Researchers	50	Canada
15	Invited speaker	Sommer	the Royal Academy of Sciences in Denmark	Feb-12	Copenhagen, Denmark	Researchers	200	Denmark
16	Invited speaker	R.J.L. Willems	at the Polar Bear Symposium on Antimicrobial Resistance	Mar-12	Longyearbyen, Svalbard	Researchers	50	Scandinavia
17	Invited speaker	Canton	at Educational Workshop: organized by Merckk-Sharp-Domme. Infección por Micrrorganismos Resistentes. Talleres de Infecciosas en Medicina Interna. MSD	Mar-12	Madrid, Spain	CME (Continuing Medical Education)	100	Spain
18	Invited speaker	Sommer	at the Annual meeting of the Amercian Chemical Association	Mar-12	San Diego, USA	Researchers	200	USA
19	Invited speaker	Sommer	at the UCLA evolutionary biology seminar	Mar-12	Los Angeles, USA	Researchers	50	USA

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
20	Invited speaker	Baquero F	Does mathematical modelling help prediction of outbreaks? European Congress for Clinical Microbiology and Infectious Diseases (ECCMID)	Mar-12	London, UK	Researchers	4000	UK
21	Invited speaker	Cantón R	Chairperson.Non-MBL carbapenemases in Gram-negative bacilli. 22nd ESCMID Conference	Mar-12	London, UK	Researchers	~4000	London
22	Invited speaker	Baquero F	Sustainability of antibiotic susceptibility: from containment to restoration approaches. Scientific Spring Meeting, Netherlands Society for Microbiology	Apr-12	Aachen, Germany	Researchers	Unknown	Netherlands
23	Invited speaker	Cantón R	Expert rules in susceptibility testing-rationale, advantages and disadvantages. Educational Workshops. 22nd ESCMID	Apr-12	London, UK	CME (Continuing Medical Education)	4000	UK
24	Invited speaker	Cantón R	Challenges in ESKAPE infections. ICASIS 2012. International Course on Antimicrobial Strategies in Sepsis	Apr-12	Sitges. Barcelona, Spain	CME (Continuing Medical Education)	200	Spain
25	Invited speaker	Cantón R	Detecting, Controlling, and Treating Carbapenemase-Producing Enterobacteriaceae. 52nd ICAAC 2012. 9-12 Septiembre 201	Apr-12	San Francisco, USA	Researchers	~6000	USA
26	Invited speaker	Cantón R	Evolution and spread of antimicrobial resistance. En: Antimicrobial resistance as a Public Health problem. 1st biomarkers and antimicrobial resistance meeting. 28 de Abril de 2012	Apr-12	Madrid	CME (Continuing Medical Education)	100	Spain
27	Invited speaker	Baquero F	Ecogenetics of antibiotic resistance. 35th International Congress of the Society for Microbial Ecology and Disease (SOMED)	May-12	Valencia, Spain	Researchers	600	Spain
28	Invited speaker	R.J.L. Willems	at the Workshop on the mathematical modelling of antibiotic resistance	May-12	Paris, France	Researchers	100	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
29	Invited speaker	Coque TM	LGT en la diversificación clonal de microorganismos de interés biomédico: Nuevas estrategias ECOEVO para el diagnóstico de bacterias multiresistentes. En: Panel de los elementos de transmisión genética horizontal en la resistencia antimicrobianos. Soceidad Enfermedades Infecciosas y Medicina Clínica (SEIMC-GEMARA)	Jun-12	Madrid, Spain	CME (Continuing Medical Education)	100	Spain
30	Invited speaker	Sommer	at the PhD School of Biophysics in Denmark	Jun-12	Denamrk	Researchers	100	Denmark
31	Invited speaker	Martinez	112th General Meeting of the American Society for Microbiology	Jun-12	San Francisco, USA	Research & industry	10000	Worldwide
32	Invited speaker	Baquero	3rd ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans and the Environment	Jun-12	Aix-en-Provence, France	Research & industry	160	Worldwide
33	Invited speaker	Cantón	Microorganismos emergentes en Fibrosis Quística. II congreso Argentino de Fibrosis Quístico. Organiza: Sociedad argentina de pediatría-Hosp. De Niño de Córdoba-Legislatura Unicameral Provincia de Córdoba. 9-11 de Agosto de 2012	Aug-12	Córdoba, Argentina	Researchers	1000	Argentina
34	Invited speaker	Baquero F	Epidemiología metagenómica en las enfermedades del microbioma. Symposium Fronteras Actuales en Microbiogía. Universidad Internacional de Andalucía	Sep-12	Sevilla. Spain	CME (Continuing Medical Education)	500	Spain
35	Invited speaker	Cantón R.	EUCAST expert rules. ESCMID Postgraduate Technical Workshop "Antimicrobial Susceptibility Testing and Surveillance: from Laboratory to Clinic - the EUCAST, ESGARS and EPASG Perspective". 25-28 September 2012	Sep-12	Madrid, Spain.	CME (Continuing Medical Education)	200	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
36	Invited speaker	Baqueo F.	The challenge of predicting evolutionary trajectories in antibiotic resistance. Central European Symposium on Antibiotic Resistance (CESAR)	Sep-12	Primostan, Croacia	Researchers	600	Croacia
37	Invited speaker	Baquero F.	New Technologies: Towards Global Surveillance and Prediction? 52st ICAAC,	Sep-12	San Francisco (USA).	Researchers	6000	USA
38	Invited speaker	Cantón R.	Antimicrobial susceptibility testing: automatic systems. ESCMID Postgraduate Technical Workshop "Antimicrobial Susceptibility Testing and Surveillance: from Laboratory to Clinic - the EUCAST, ESGARS and EPASG Perspective". 25-28 September 2012,	Sep-12	Madrid, Spain	CME (Continuing Medical Education)	200	Spain
39	Invited speaker	de la Cruz, F.	"Diversity in genetic design of conjugative transfer systems" Bilbao Advanced Courses on Biophysics 2012.	Sep-12	Bilbao, Spain	Researchers	100	Spain
40	Invited speaker	de la Cruz, F	"Plasmids: diversity, structure and design" 2012 Skirball Symposium, "Mighty Microbes: From Menace to Marvel",	Sep-12	New York, USA	Researchers	200	USA
41	Invited speaker	Baquero F.	Mechanisms and Evolution of Antibiotic Resistance at the Local and Global Scale Fondation Mediterranée-Infection, Université de Marseille,	Oct-12	Gordes, France	Researchers	100	France
42	Invited speaker	R.J.L. Willems	at Symposium "VRE wat moet je ermee?	Oct-12	Nijmegen, The Netherlands	Clinical Microbiologist, infectious diseases, infection control teams	200	Netherlands
43	Invited speaker	Baquero F.	Avances en Genética y Ecología Microbiana. Meeting de: Programación de circuitos microbianos en medicina protectiva y terapéutica (PROMPT).	Nov-12	Madrid, Spain	Researchers	100	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			Meeting, Facultad de Farmacia, Universidad Complutense de Madrid					
44	Invited speaker	Baquero F.	Preventing mechanisms and evolution of antibiotic resistance at the local and global scales. Meeting "In joint battle against infectious disease and antibiotic resistance". Uppsala University	Nov-12	Uppsala, Sweden	Researchers	Unknown	Sweden
45	Invited speaker	Martinez, J.L.	at the International Conference on Antimicrobial Resistance ICAR 2012	Nov-12	Lisboa, Portugal	Researchers	500	Worldwide
46	Invited speaker	Cantón R.	Carbapenem breakpoint: The EUCAST approach. XXXVI National Conference of Indian Association of Medical Microbiologists. 22-25 November 2012.	Nov-12	New Delhi, India.	Researchers	1000	India
47	Invited speaker	Cantón R.	Educational Workshop organized by Novartis. Epidemiología de las infecciones por gram positivos en pacientes críticos. Simposium Novartis-29 Nov). IV Reunión GTIPO-SEDAR – X Reunión Sepsis. 29,30 y 01 de Diciembre de 2012.	Dec-12	Valladolid, Spain	CME (Continuing Medical Education)	350	Spain
48	Invited speaker	R.J.L. Willems	at Symposium "Herken Nationale Uitbraken,	Jan-13	Utrecht, The Netherlands	Clinical Microbiologist, infectious diseases, infection control teams	200	Netherlands
49	Invited speaker	Baquero F.	Cupid and Psyche: the wedding of Robustness and Evolvability. Closing Lecture, Colloquium on Robustness and Evolvability. Centro Nacional de Biotecnología, CNB-CSIC, Febrero 2013, Tres Cantos,	Feb-13	Madrid, Spain	Researchers	100	Spain
50	Invited speaker	Coque TM.	Transferencia de elementos genéticos que confieren resistencia a los antimicrobianos. En: Bases moleculares de la patogenia y	Mar-13	Madrid, Spain	CME (Continuing Medical	100	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			terapia antimicrobiana y antiparasitaria. Master en Microbiología y Parasitología. Facultad de Medicina. UCM. 14-23 de Marzo de 2013.			Education)		
51	Invited speaker	Baquero F.	El microbioma intestinal: un nuevo órgano afectado por la fibrosis quística. Reunión Nacional "Sira Carrasco" de Fibrosis Quística. Marzo 2013,	Mar-13	Madrid, Spain	Companies, Research, Lay Public	500	Spain
52	Invited speaker	W. van Schaik,	at symposium Antibiotic resistance: an ecological perspective	Mar-13	Amsterdam, The Netherlands	Researchers	250	Netherlands
53	Invited speaker	R.J.L. Willems	at minisymposium Enterococci: once the good guys, now the bad guys, Werkgroep Algemene Medische Microbiologie,	Mar-13	Nieuwegein, The Netherlands	Clinical Microbiologist, infectious diseases, infection control teams	100	Netherlands
54	Invited speaker	Sommer	Science & Cocktails – Invited key note speaker	Mar-13	Copenhagen, Denmark	Companies, Research, Lay Public	300	Denmark
55	Invited speaker	Baquero F.	Metagenómica de la microbiota intestinal y resistencias bacterianas a los antibióticos. Conferencia inaugural de la Jornada sobre Avances en Genética y Farmacogenética de la Infección. Aspectos Regulatorios y Optimización de Antimicrobianos. Hospital Universitario 12 de Octubre.	Apr-13	Madrid, Spain	Companies, Research, Lay Public	200	Spain
56	Invited speaker	Baquero F.	Breaking barriers among antibacterial interventions: the patient, the group, and the environment. Symposium: New ways of thinking of new antibacterial drugs. 23rd Congress of the European Society of Clinical Microbiology and Infectious	Apr-13	Berlin, Germany	Researchers	4000	Berlin

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			Diseases, Abril 2013.					
57	Invited speaker	Coque TM	Successful clones: Epidemiological and Clinical Implications: Klebsiella pneumoniae ST258. 23rd ESCMID	Apr-13	Berlin, Germany	Researchers	~4000	Berlin
58	Invited speaker	Baquero F.	Invitational Conference on Antibiotic Resistance. Joint Programming Initiative-Antibiotic Resistance. European Commission, May 2013,	May-13	Geneva, Switserland	Researchers	100	Switzerland
59	Invited speaker	RJL Willems	at the NABATIVI meeting,	May-13	Camogli, Italy	Researchers	30	Europe
60	Invited speaker	Baquero F.	Multidimensional Darwinism and the Genetic Hypercode. III Darwin World Summit on Evolution: Why does Evolution Matter? Junio 2013,	Jun-13	San Cristóbal, Islas Galápagos, Ecuador	Researchers	Unknown	Ecuador
61	Invited speaker	Baquero F.	¿Están los antibióticos acelerando la evolución de las poblaciones bacterianas? Universidad de San Francisco, Mayo 2013	Jun-13	Quito, Ecuador	Researchers	Unknown	Ecuador
62	Invited speaker	Baquero F.	Evaluation du Laboratoire d'Excellence. Chairman "Integrative Biology of Emerging Infectious Diseases", 1st Scientific Meeting. Institut Pasteur, Junio 2013,	Jun-13	París, France	Researchers	100	France
63	Invited speaker	R.J.L. Willems	2nd International Conference on Prevention & Infection Control,	Jun-13	Geneve, Switzerland	Researchers	1500	Worldwide
64	Invited speaker	R.J.L.Willems	at the NABATIVI Summer School 2013	Jul-13	Milan, Italy.	Researchers	50	Europe
65	Invited speaker	Coque TM.	WG meeting on Carbapenem resistance in food animal ecosystems (EFSA-Q-2013-00010).	Jul-13	Brussels, Belgium	Researchers	6000	Europe
66	Invited speaker	Sommer	at Exeter University	Jul-13	Exeter, UK	Researchers	50	UK
67	Invited	Baquero F.	" ECO-EVO DRUGS. "En:	Jul-13	Leizpig,	Researchers	2000	Germany

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
	speaker		Unconventional antiinfectiva: Eco-evo drugs, viurulence blockers, and new targets. 5th Congress of European microbiologist, FEMS 2013.		Germany.			
68	Invited speaker	Baquero F.	Eco-Evo Drugs. 5th Congress of the Federation of European Microbiological Societies (FEMS), July 2013,	Jul-13	Leipzig, Germany	Researchers	2000	Germany
69	Invited speaker	Baquero F.	The Scientific Program of the Joint Programming Initiative on Antibiotic Resistance. JPI Invitational Meeting, EU Commission. 5th Congress of the Federation of European Microbiological Societies (FEMS), Julio 2013	Jul-13	Leipzig, Germany	Opinion Leaders; Policy makers; Researchers; General Public	50	Germany
70	Invited speaker	Sommer	Microbes Conference	Jul-13	Wuhan, China	Researchers	500	Worldwide
71	Invited speaker	Sommer	at Chinese Academy of Science	Aug-13	Beijing, China	Researchers	100	China
72	Invited speaker	Woolhouse, M	Seminar Talk Oxford University	Aug-13	Oxford, Uk	Researchers	20	UK
73	Invited speaker	Coque TM.	Effects of Antibiotics on Microbiomes and Bacterial Pathogens. 65th Annual Meeting of the German Society for Hygiene and Microbiology September 2013.	Sep-13	Rostock, Germany.	Researchers	600	Germany
74	Invited speaker	Baquero F.	OPENING KEYNOTE SESSION. 005 - A Vision of Antimicrobial Therapy for the Future. 53rd ICAAC,	Sep-13	Denver, Colorado, USA.	Researchers	6000	USA
75	Invited speaker	Baquero, F.	The twilight of simplicity in antimicrobial therapy. M2M Seminar of the Department of Epidemiology, Rollins School of Public Health, September 2013,	Sep-13	Atlanta, USA.	CME (Continuing Medical Education)	50	USA
76	Invited speaker	Sommer	at ICSB 2013	Sep-13	Copenhagen, Denmark	Researchers	500	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
77	Invited speaker	Cantón R.	Meet with Experts. Interactive Colloquium. The End of Beta-Lactams as We Know Them! C2-962 - Molecular Epidemiology: Successful Clones, Plasmids, Mobile Elements. 53rd ICAAC,	Sep-13	Denver, Colorado, USA.	CME (Continuing Medical Education)	~6000	USA
78	Invited speaker	Canton R	Meet with Experts. Interactive Colloquium. New Insights in the Epidemiology of Carbapenemases. 53rd ICAAC 2013.	Sep-13	Denver, Colorado, USA	CME (Continuing Medical Education)	~6000	USA
79	Invited speaker	Martinez JL	at the International Congress on Antimicrobial Agents and Chemotherapy. ICAAC2013	Sep-13	Denver USA	Researchers	10000	Worldwide
80	Invited speaker	Martinez JL	at the V International Conference on Environmental, Industrial and Applied Microbiology. BioMicroWorld 2013.	Oct-13	Madrid, Spain	Researchers	500	Worldwide
81	Invited speaker	Baquero F.	A tale of multiple replicons –bacterial diseases. Plenary Lecture of the 20th Anniversary of the Belgian Society of Clinical Microbiology and Infectious Diseases, Solvay Auditorium, October 2013.	Oct-13	Brussels, Belgium	Researchers	600	Belgium
82	Invited speaker	Ricardo León-Sampedro	Diversidad de Tn5801 entre Firmicutes. Spanish Network for the Study of Plasmids and Extrachromosomal Elements (REDEEX) for funding cooperation among Spanish microbiologists working on the biology of MGEs (Spanish Ministry of Science and Innovation BFU2011-14145-E)	Oct-13	Madrid, Spain	Researchers		Spain
83	Invited speaker	Ana Tedim	Transferencia horizontal y diversificación clonal en Enterococcus faecium. Spanish Network for the Study of Plasmids and Extrachromosomal Elements (REDEEX) for funding cooperation among Spanish	Oct-13	Madrid, Spain	Researchers		Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			microbiologists working on the biology of MGEs (Spanish Ministry of Science and Innovation BFU2011-14145-E)					
84	Invited speaker	Baquero F.	Plasticity of the Escherichia coli genome: can we define specific pathotypes? Closing Lecture in The Global threat of antibiotic resistance: science for intervention. ESCMID Conference on Escherichia coli: An Old Friend with New Tidings. International Center for Scientific Debate,	Nov-13	Barcelona, Spain.	Researchers	4000	Worldwide
85	Invited speaker	Baquero F.	Antibiotic resistance as an environmental disease: the possibility of eco-evo interventions. Andalusian Society for Microbiology and Infectious Diseases,	Nov-13	Cadiz, Spain.	Researchers	500	Spain
86	Invited speaker	Baquero F.	Children's Infections: yesterday and today. 18th Meeting of Madrid-La Mancha Pediatrics Society,	Nov-13	Madrid, Spain.	Researchers	150	Spain
87	Invited speaker	Baquero F.	Conceptual changes in research on antibiotic therapy. Seville Institute of Research (ITRIBIS), Seville University (UNIA),	Nov-13	Sevilla, Spain.	Researchers	100	Spain
88	Invited speaker	De la Cruz,	F. Inhibition of plasmid conjugation I. Inter American University Bayamón Campus	Nov-13	Puerto Rico	Researchers	100	Puerto Rico
89	Invited speaker	De la Cruz, F.	Inhibition of plasmid conjugation. II UNIVERSITY OF PUERTO RICO at Rio Piedras	Nov-13	Puerto Rico	Researchers	100	Puerto Rico
90	Invited speaker	De la Cruz, F.	Inhibition of plasmid conjugation. III Universidad Metropolitana.	Nov-13	Puerto Rico	Researchers	100	Puerto Rico
91	Invited speaker	De la Cruz, F.	Inhibition of plasmid conjugation. IV Inter American University.	Nov-13	Puerto Rico.	Researchers	100	Puerto Rico
92	Invited speaker	Baquero F.	Replicators: modes and codes. Annual Meeting of the Systems Biology Program, Molecular Environmental Microbiology	Jan-14	Salamanca, Spain.	Researchers	40	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			Laboratory, National Center for Biotechnology CSIC,					
93	Invited speaker	Baquero F.	Epistemological thoughts about Cochrane's evidence-based actions. Ramón y Cajal Cochrane's Unit, IRYCIS,	Jan-14	Madrid, Spain.	Researchers	80	Spain
94	Invited speaker	Baquero F.	How to prevent antibiotic resistance? 48th Annual Course on Antibiotic Therapy, Nuestra señora del Mar Hospital,	Jan-14	Barcelone, Spain.	Clinical Microbiologist, infectious diseases, infection control teams	200	Spain
95	Invited speaker	Van Schaik,	Seminar Veterinary Public Health	Feb-14	Hannover, Germany	Researchers	~150	Germany
96	Invited speaker	Martínez, J. L.	Workshop organized by the European Academies Science Advisory Council (EASAC) "Antimicrobial drug discovery, greater steps ahead" What is the function of antibiotics and antibiotic resistance genes in nature?.	Mar-14	Hannover, Germany	Opinion Leaders; Policy makers; Researchers; General Public	50	Europe
97	Invited speaker	van Schaik,	4th ASM Conference on Enterococci,	Mar-14	Cartagena, Colombia	Researchers	~250	Worldwide
98	Invited speaker	Willems	4th ASM Conference on Enterococci,	Mar-14	Cartagena, Colombia	Researchers	~250	Worldwide
99	Invited speaker	RJL: Willems	at the Workshop 'RAPID NGS for Clinical & Public Health Microbiology' March 12-14, 2014	Mar-14	Münster, Germany	Researchers	100	Worldwide
100	Invited speaker	Martinez JL	Dutch Society of Microbiology	Apr-14	Papendal, The Netherlands	Researchers	500	Netherlands
101	Invited speaker	Martínez J. L.	Seminar at the Health Techonological Park.	May-14	Granada, Spain	Researchers	50	Spain
102	Invited speaker	Baquero F.	Knowledge and social norms shaping the discovery, use, and resistance trends of	May-14	Barcelone, Spain.	Researchers	4000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			antimicrobial agents. Key-Note Plenary Lecture at the European Congress for Clinical Microbiology and Infectious Diseases,					
103	Invited speaker	Baquero F.	Antibiotic therapy and bacterial spread. External Invited Speaker, SATURN Annual Meeting, 7th Framework Programme,	May-14	Warsaw, Poland.	Researchers	30	Poland
104	Invited speaker	Baquero F.	Session: Knowledge and social norms shaping the discovery, use and resistance trends on antimicrobial agents. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
105	Invited speaker	Baquero F.	Perspectives on microbial evolution and antibiotic resistance evolution. MBI 8001 Course on Molecular and clinical aspects of Infection, Inflammation and Immunity, Department of Pharmacy, UiT, The Arctic Univeristy,	Jun-14	Tromsø, Sweden.	Researchers	200	Sweden
106	Invited speaker	Baquero F.	Infection, antibiotic resistance, and evolution. Closing Lecture of the Master in Microbiology, Faculty of Pharmacy, Complutensis University,	Jul-14	Madrid, Spain.	Researchers	100	Spain
107	Invited speaker	Baquero F.	Phylogenetic epidemiology of bloodstream bacterial infections. Plenary Lecture, UNIPATH South African Meeting,	Sep-14	Pretoria, South Africa.	Researchers	500	Worldwide
108	Invited speaker	Baquero F.	Screening criteria for detecting carbapenem resistances in Enterobacteriaceae. UNIPATH South African Meeting,	Sep-14	Pretoria, South Africa.	Researchers	500	Worldwide
109	Invited speaker	Ingham C	German Microbiology Society Meeting,	Oct-14	Dresden, Germany	Researchers	50	Europe
110	Invited speaker	Martínez J.L.	Swiss National Science Foundation.	Nov-14	Zurich, Switserland	Opinion Leaders;	10	Europe

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
						Policy makers; Researchers; General Public		
111	Invited speaker	Martínez J. L.	ISF Stepehen F. Lowry Colloquim.	Dec-14	Paris, France	Researchers	700	Worldwide
112	Invited speaker	Baquero, F.	ISF Stepehen F. Lowry Colloquim.	Dec-14	Paris, France	Researchers	700	Worldwide
113	Invited speaker	Baquero, F.	Endogenous bacterial infections and the intestinal interphase: transmission, colonization and infection. 18th National Polish Congress of Microbiology and Infectious Diseases,	Dec-14	Warsaw, Poland	Researchers	350	Poland
114	Invited speaker	Willems, RJL	Transition of Enterococcus faecium from commensal to nosocomial pathogen. University of Cologne,	Jan-15	Cologne, Germany	Clinical Microbiologist, infectious diseases, infection control teams, researchers	30	Cologne
115	Invited speaker	Coque T.	Conocimiento, crecimiento económico y sociedad: Retos de la investigación hospitalaria competitiva en el s XXI" IRYCIS Seminars,	Feb-15	Madrid, Spain	Companies, Research, Lay Public	50	Spain
116	Invited speaker	Coque, T.	Impact of Lateral Genetic Transfer on the Spread of Antibiotic Resistance. Master UCM,	Mar-15	Madrid, Spain	Researchers	<100	Spain
117	Invited speaker	van Schaik, W	VRE typing at the Spring Meeting of the Royal Dutch Society for Microbiology at the Spring Meeting of the Royal Dutch Society for Microbiology	Apr-15	Arnhem, The Netherlands	Researchers, Clinical Microbiologists	50	The Netherlands
118	Invited speaker	Willems, RJL	Gram-positive pathogens: Staphylococcus aureus and Enterococcus spp high-risk	Apr-15	Copenhagen, Denmark.	Researchers, Clinical	150	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			clones. 25th European Congress of Clinical Microbriology and Infectious Diseases.			Microbiologists		
119	Invited speaker	Baquero F.	Antibiotics at sub-inhibitory concentrations. 3rd International Symposium on the Environmental Dimension of Antibiotic Resistance,	May-15	Wernigerode, Germany	Researchers	300	Worldwide
120	Invited speaker	Baquero F.	Chairman Plenary Session: Experimental and theoretical models in transmission. International Symposium: Microbiology: Transmission. Areces Foundation Invited Speaker.	May-15	Madrid, Spain	Researchers	500	Worldwide
121	Invited speaker	Coque, T.	Food to Human Transmission. International Symposium: Microbiology: Transmission. Areces Foundation.	May-15	Madrid, Spain	Researchers	500	Worldwide
122	Invited speaker	Baquero, F.	Transmission, Introgression, and Evolution. International Symposium: Microbiology: Transmission. Areces Foundation.	May-15	Madrid, Spain	Researchers	500	Worldwide
123	Invited speaker	Coque, T.	Escenarios de transmisión de plásmidos en microorganismos de interés en biomedicina. Interactivity of plasmid modules and the genomes of bacterial pathogens (INTERMODS). Centro de Investigaciones Biológicas. CSIC,	May-15	Madrid, Spain	Researchers	100	Worldwide
124	Invited speaker	F. Baquero,	Transmission: a basic process in microbiology. Lwoff Award Plenary Lecture. FEMS 6th Congress of European Microbiologists.	Jun-15	Maastricht, The Netherlands	Research & industry	1200	Worldwide
125	Invited speaker	M. Sommer.	ICETAR 2015 meeting, 24-26 June,	Jun-15	Amsterdam, The Netherlands	Researchers	130	Worldwide
126	Invited speaker	Willems, RJL	Challenges in whole genome sequence-based molecular epidemiology of bacterial	Jun-15	Maastricht, The	Researchers	150	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			pathogens. 6th Congress of European Microbiologists FEMS 2015,		Netherlands			
127	Invited speaker	Willems, RJL	Why and how to study transmission of Antibiotic Resistance, Kennisnetwerk Zoönosen Midden-Nederland	Jun-15	Utrecht, The Netherlands	Companies, Research, Lay Public	75	The Netherlands
128	Invited speaker	Willems, RJL	Changes in the mammalian intestinal architecture during antibiotic-induced perturbation of the microbiota. ICETAR2015,	Jun-15	Amsterdam, The Netherlands	Researchers	150	Worldwide
129	Invited speaker	M. Sommer.	UCSF Dept. Seminar,	Jul-15	San Francisco, USA	Researchers	100	USA
130	Invited speaker	M. Sommer.	Berkely Dept. Seminar	Jul-15	Berkely, USA	Researchers	150	USA
131	Invited speaker	M. Sommer.	Stanford Dept. Seminar	Jul-15	San Francisco, USA	Researchers	100	USA
132	Invited speaker	Baquero F.	Clones de alto riesgo, mito o realudad? Antibiotics Meeting, Menéndez-Pelayo International University, Santander,	Sep-15	Santander, Spain	Researchers	200	Spain
133	Invited speaker	Baquero F.	The controversy about bactericidal-bacteriostatic antibiotics and its influence on antimicrobial resistance. Central European Symposium on Antibiotics and Antibiotic Resistance,	Sep-15	Sybenic, Croatia	Researchers	150	Worldwide
134	Invited speaker	Coque T.	The evolving evolution of vancomycin-resistance in E. faecium: vanA to van. Vancomycin resistant E. faecium: Its more than just about vanA!" Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)/International Congress on Chemotherapy (ICC) 2015,	Sep-15	San Diego, USA	Researchers	1000	Worldwide
135	Invited speaker	Coque T,	Desarrollo de una plataforma de captura de secuencia NimbleGen y aplicaciones en	Sep-15	Madrid, Spain	Companies, Research, Lay	200	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			salud pública. Jornada sobre Captura de Secuencia Roche NimbleGen.			Public		
136	Media briefing	Willems, RJL	For magazine 'Medicines'.	Feb-12	Utrecht, The Netherlands	Research & industry	Unknown	Netherlands
137	Media briefing	Van Schaik	in magazine 'Libelle'	Feb-14	Utrecht, The Netherlands	Companies, Research, Lay Public	100000	Netherlands
138	Media briefing	Andersson D	Discussion meeting: EASAC Brain stoming meeting	Mar-14	Budapest, Hunagry	Opinion Leaders; Policy makers; Researchers; General Public	50	Europe
139	Media briefing	Woolhouse, M	Discussion meeting plus magazine article: New Stateman and Wellcome Trust Anti-Microbial Resistance Roundtable	Jun-14	London, UK	Opinion Leaders; Policy makers; Researchers; General Public	25	UK
140	Oral presentation	Willems, R.J.L	at the Spring Meeting of the Royal Dutch Society for Microbiology	Apr-12	Papendal, Netherlands	Researchers	~100	Netherlands
141	Oral presentation	Martinez, JL	Speaker. Instituto de Investigaciones Biomédicas-CSIC,	Apr-12	Madrid, Spain	Researchers	50	Spain
142	Oral presentation	Baquero F	Symposium at Society for Microbial Ecology and Disease(SOMED)	May-12	Valencia, Spain	Researchers	400	Europe
143	Oral presentation	Cantón R,	Repertoir of clones in the emergence and spread of carbapenemases producing Enterobacteriaceae in Europe. Conference: Multidrug Reistnace High Risk Clones (MDR-HiRiC): Features, Epidemiology and Detection. 5-6 de Junio de 2012.	Jun-12	Barcelona, Spain	CME (Continuing Medical Education)	200	Barcelona

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
144	Oral presentation	Gonzalez-Zorn, B	at ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans and the Environment	Jun-12	Aix-en-Provence, France	Researchers	300	Worldwide
145	Oral presentation	van Schaik	Seminar at Boston College,	Aug-12	Bosten, USA	Researchers	30	USA
146	Oral presentation	van Schaik	Seminar at Boston Colleg	Aug-12	Boston, USA	Researchers	30	USA
147	Oral presentation	van Schaik	Seminar at Tufts University	Aug-12	Medford, USA	Researchers	30	USA
148	Oral presentation	Tedim AR.	Ecogenetics of antibiotic resistance plasmids of Enterococcus faecium (Efm) and Enterococcus faecalis (Efc). Tedim AP, Tobes R, Manrique M, Freitas AR, Peixe L, Baquero F, Coque TM. IPBC, 12th-15th September 2012,	Sep-12	Santander, Spain	Researchers	300	Spain
149	Oral presentation	Coque TM.	IncF(k) plasmids from Klebsiella pneumoniae in the global spread of extended-spectrum beta-lactam resistance among Enterobacteriaceae. IPBC, 12th-15th September 2012,	Sep-12	Santander, Spain	Researchers	300	Spain
150	Oral presentation	Andersson, D	at Interscience Conference on Antimicrobial Agents and Chemotherapy,	Sep-12	San Francisco, USA	Researchers	250	Worldwide
151	Oral presentation	Rossolini, G	at ICAAC	Sep-12	San Francisco, USA	Research & industry	300	Worldwide
152	Oral presentation	Andersson, D	at Interscience Conference on Antimicrobial Agents and Chemotherapy,	Sep-12	San Francisco, USA	Researchers	250	Worldwide
153	Oral presentation	Coque TM	International Conference in Plasmid Biology	Sep-12	Santander, Spain	Researchers	200	Worldwide
154	Oral presentation	Gonzalez-Zorn, B	International Conference in Plasmid Biology	Sep-12	Santander, Spain	Researchers	200	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
155	Oral presentation	Gonzalez-Zorn, B	at Bilbao Advanced Courses on Biophysics Workshop on Biophysics aspects of Type IV Secretion	Sep-12	Bilbao, Spain	Researchers	50	Worldwide
156	Oral presentation	Andersson, D	Seminar at Emory University	Sep-12	Atlanta, USA	Researchers	70	USA
157	Oral presentation	Baquero, F	at the Central European Symposium on Antimicrobial Resistance, 2012,	Sep-12	Primošten, Croatia	Researchers	120	Worldwide
158	Oral presentation	Martinez, JL	at the Central European Symposium on Antimicrobial Resistance, 2012,	Sep-12	Primošten, Croatia	Researchers	120	Worldwide
159	Oral presentation	Guardabassi, L	at the Central European Symposium on Antimicrobial Resistance, 2012,	Sep-12	Primošten, Croatia	Researchers	120	Worldwide
160	Oral presentation	van Schaik, W	at Platform Molecular Genetics	Oct-12	Lunteren, The Netherlands	Researchers	80	Netherlands
161	Oral presentation	van Schaik, W	at 14th Gut Day	Nov-12	Leuven, Belgium	Researchers	150	BeNeLux
162	Oral presentation	Smidt, H	at NBIC metagenomics course	Feb-13	Nijmegen, The Netherlands	Researchers	40	Worldwide
163	Oral presentation	Smidt, H	at the Scientific Spring Meeting KNVM & NVMM 2013	Apr-13	Papendal, The Netherlands	Researchers	40	Worldwide
164	Oral presentation	Andersson, D	at 4th Genome Maintenance Meeting in	Sep-13	Oslo, Norway	Researchers	100	Worldwide
165	Oral presentation	Merino I	Alta prevalencia de CTX-M-15 B2-Escherichia coli ST 131 en bacteriemias de origen urinario en España. Estudio multicéntrico ITU-BRAS. XVII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC).	May-13	Zaragoza, Spain	Researchers	500	Spain
166	Oral presentation	Turrientes MC,	Identificación de un nuevo sublinaje en la estructura poblacional de Escherichia coli.	May-13	Zaragoza, Spain	Researchers	500	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			XVII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC).					
167	Oral presentation	Coque TM.	Avances en Genética y Ecología Microbiana. Meeting de: Programación de circuitos microbianos en medicina protectiva y terapéutica (PROMPT). Meeting, Facultad de Farmacia, Universidad Complutense de Madrid, Noviembre 2012 http://www.prompt.es/pdfs/Jornada_PROMPT_2013.pdf	Jun-13	Madrid, Spain	Researchers	100	Spain
168	Oral presentation	de la Cruz, F	Mobilization of RSF1010 between Anabaena strains illustrates conjugation among cyanobacteria 14th INTERNACIONAL SYMPOSIUM ON PHOTOTROPHIC PROKARYOTES	Aug-13	Portugal	Researchers	250	Portugal
169	Oral presentation	RJL Willems	at the 10th IMMEM,	Oct-13	Paris, France	Researchers	250	Worldwide
170	Oral presentation	Santos-López, A.,	"Adaptación plásmido pequeño/bacteria hospedadora en el modelo Haemophilus influenzae/pB1000" REDEEX-2.	Nov-13	Almagro. Spain	Researchers	Unknown	Spain
171	Oral presentation	Guardabassi, L	Different epidemiology of blaCMY-2 plasmids among clinical Escherichia coli from companion animals in Portugal and Denmark. ESCMID conference on Escherichia coli.	Nov-13	Barcelona, Spain	Researchers	200	Worldwide
172	Oral presentation	Andersson, D	Seminar at San Diego State University	Jan-14	San Diego, USA	Researchers	80	USA
173	Oral presentation	Andersson, D	Seminar at Brigham Young University	Jan-14	Provo, USA	Researchers	70	USA
174	Oral presentation	Andersson, D	Conference European academy of microbiology	Mar-14	Paris, France	Researchers	80	Europe

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
175	Oral presentation	Andersson, D	Conference Dutch Society for Microbiology	Apr-14	Papendal, The Netherlands	Researchers	200	Worldwide
176	Oral presentation	Smidt, H	Scientific Spring Meeting KNVM & NVMM 2014	Apr-14	Papendal, The Netherlands	Researchers	50	Worldwide
177	Oral presentation	Smidt, H	Scientific Spring Meeting KNVM & NVMM 2014	Apr-14	Papendal, The Netherlands	Researchers	300	Worldwide
178	Oral presentation	Woolhouse, M	Centre for Immunity, Infection and Evolution Workshop	May-14	Pitlochry, UK	Researchers	50	Pitlochry, UK
179	Oral presentation	RJL Willems	at the 24th ECCMID,	May-14	Barcelona, Spain	Researchers	4000	Worldwide
180	Oral presentation	Woolhouse, M	At meeting of the Royal Society of London on Anti-Microbial Resistance	May-14	London, UK	Opinion Leaders; Policy makers; Researchers; General Public	200	Worldwide
181	Oral presentation	Santos-López A.	”El comportamiento de plásmidos ColE1 durante la cohabitación”. X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	Unknown	Spain
182	Oral presentation	Carrilero L.	“Un mutante espontáneo de Enterococcus faecalis sensible a cefalosporinas: en el camino de la identificación de nuevas vías de resistencia”. X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain
183	Oral presentation	Andersson, D	Conference and course on antibiotic resistance at Kavli Institute	Aug-14	Delft, The Netherlands	Researchers	70	Worldwide
184	Oral presentation	Martinez, JL	ICCE-ANQUE-BIOTEC Congress	Jul-14	Madrid, Spain	Researchers	400	Worldwide

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
185	Oral presentation	Woolhouse, M	Daniel Wilson Lab Away Day	Aug-14	Oxford, UK	Researchers	50	Oxford, UK
186	Oral presentation	Andersson, D	at European Society for Evolution Biannual meeting,	Aug-13	Lisbon, Portugal	Researchers	300	Worldwide
187	Oral presentation	Andersson, D	Swiss Meeting for infectious disease dynamics	Sep-14	Bern, Switserland	Researchers	60	Europe
188	Oral presentation	Woolhouse, M	At ICAR 2014 meeting	Oct-14	Madrid, Spain	Researchers	500	Worldwide
189	Oral presentation	Baquero F.	Inside the bacterial species: the structure of collective adaptation. Meeting Institute Mediérranèe-Infection.	Oct-14	Port de Bannes, France	Researchers	50	Worldwide
190	Oral presentation	Martinez, JL	Instituto de Agrobiotecnolog'a Navarro	Jan-15	Navarro, Spain	Researchers	50	Spain
191	Oral presentation	Martinez, JL	Keystone Symposium - Gram Negative Resistance (D1)	Mar-15	Tahoe city, USA	Researchers	300	Worldwide
192	Oral presentation	Martinez, JL	Universidad Internacional MenŽndez Pelayo (2015),	Mar-15	Madrid, Spain	Researchers	30	Spain
193	Oral presentation	van Schaik, W	Annual Meeting Society for General Microbiology,	Mar-15	Birmingham UK	Researchers	350	Worldwide
194	Oral presentation	Coque T.	Terapia global para enfermedades multicausales y mejora de la expectativa de vida en infecciones terminales Foro PROMPT, Regional Government of Madrid,	Apr-15	Madrid, Spain	Companies, Research, Lay Public	100	Spain
195	Oral presentation	Lanza VF,	Genome diversity and accessory gene flow of Escherichia coli O25b-ST131 population causing bacteriemia in a single tertiary Spanish hospital (1996-2012). 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Europe
196	Oral presentation	De Gunzburg, J.	DaVolterra: Novel and promising products in development for human use. 25th European Congress of Clinical	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Europe

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			Microbriology and Infectious Diseases.					
197	Oral presentation	León-Sampedro R	Resistencia a mercurio (merA) en Enterococcus de niños expuestos a contaminación medioambiental y análisis de diversidad y evolvabilidad de elementos mercuriales en Gram positivos. XIX Congreso SEIMC - O-162.	May-15	Sevilla, Spain	Clinical Microbiologist, infectious diseases, infection control teams	300	Spain
198	Oral presentation	González JM,	Revisión de la historia evolutiva de Escherichia coli y de los modelos para la asignación de los grupos filogenéticos XIX Congreso SEIMC - O-162.	May-15	Sevilla, Spain	Clinical Microbiologist, infectious diseases, infection control teams	300	Spain
199	Oral presentation	Rodríguez I,	Bacteriemias causadas por Escherichia coli: estudio longitudinal de 17 años (1996-2012) en el Hospital Universitario Ramón y Cajal. XIX Congreso SEIMC - O-162.	May-15	Sevilla, Spain	Clinical Microbiologist, infectious diseases, infection control teams	300	Spain
200	Oral presentation	Tedim AP,	Análisis del genoma de Enterococcus faecium ST117, un clon globalmente diseminado y endémico en hospitales españoles, utilizando combinación de técnicas genómicas y bioinformáticas de última generación. XIX Congreso SEIMC - O-162.	May-15	Sevilla, Spain	Clinical Microbiologist, infectious diseases, infection control teams	300	Spain
201	Oral presentation	Tedim AP,	Estructura poblacional y evolución de los aislados de Enterococcus faecium responsables de bacteriemias en un hospital terciario español en los últimos 18 años (1995-2012). XIX Congreso SEIMC - O-162.	May-15	Sevilla, Spain	Clinical Microbiologist, infectious diseases, infection control teams	300	Spain
202	Oral	Martínez-García	Un Modelo para la Evaluación de la	May-15	Sevilla,	Clinical	300	Spain

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
	presentation	L,	Transmisión Mano-Mano de Clones Bacterianos. XIX Congreso SEIMC - O-162.		Spain	Microbiologist, infectious diseases, infection control teams		
203	Oral presentation	Martinez, JL	Ramon Areces Foundation International Symposium "Microbiology: Transmission"	May-15	Madrid, Spain	Researchers	150	Worldwide
204	Oral presentation	De Gunzburg	DAV132 was presented in an oral session during the ICETAR conference	Jun-15	Amsterdam, The Netherlands	Researchers	>100	Worldwide
205	Oral presentation	Gudeta	Hunting carbapenemases in the soil microbiota: International Conference on the Evolution and Transfer of Antibiotic Resistance ICETAR Meeting	Jun-15	Amsterdam, The Netherlands	Researchers	>100	Worldwide
206	Oral presentation	Andersson, D	Advanced Bacterial Genetics Course, Cold Spring Harbor Laboratories	Jun-15	New York, USA	Researchers	25	Worldwide
207	Oral presentation	Martinez, JL	6th Congress of European Microbiologists FEMS 2015,	Jun-15	Maastricht, The Netherlands	Researchers	3000	Worldwide
208	Oral presentation	van Schaik, W	6th Congress of European Microbiologists FEMS 2015,	Jun-15	Maastricht, The Netherlands	Researchers	3000	Worldwide
209	Oral presentation	Andersson, D	Latsis Symposium on Evolution of Resistance,	Jul-15	Zurich Switzerland	Researchers	100	Worldwide
210	Oral presentation	Andersson, D	Forecasting Evolution Conference,	Jul-15	Lisbon Portugal,	Researchers	60	Worldwide
211	Oral presentation	Andersson, D	Stanford Course in Bacterial Evolution and Ecology,	Jul-15	Monterey, USA,	Researchers	20	USA
212	Oral presentation	Andersson, D	32nd Annual Meeting of Nordic Societies of Clinical Microbiology and Infectious Diseases,	Sep-15	UmeŒ Sweden,	Researchers	150	Scandinavia
213	Oral	Andersson, D	12th Annual European Initiative for Basic	Sep-15	Stockholm	Researchers	70	EU

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
	presentation		Research in Infectious Diseases,		Sweden,			
214	Oral presentation	Martinez, JL	3rd Florence Conference on Phenotype MicroArray Analysis of Microbial and Mammalian Cells	Sep-15	Florence, Italy	Researchers	150	Worldwide
215	Oral presentation	Gudeta	Chromobacterium sp. Harbor New Ambler Class A β-Lactamases with High Similarity to Klebsiella pneumoniae carbapemenase (KPC). ICAAC/ICC 2015, Joint 55th Interscience Conference on Antimicrobial Agents and Chemotherapy and 28th International Congress of Chemotherapy Meeting. 7 - 21 September 2015	Sep-15	San Diego, USA	Researchers	4000	Worldwide
216	Participant	Andersson, D	6th National Infection Biology Meeting,	May-14	Marstrand, Sweden	Researchers	200	Sweden
217	Poster presentation	van Schaik, W	at the International Human Microbiome Conference	Mar-12	Paris, France	Researchers	~500	Worldwide
218	Poster presentation	Sinnige, J	et al., 22nd ESCMID.	Mar-12	London, UK	Researchers	4000	London
219	Poster presentation	Freitas AR,	Global dissemination of vancomycin-resistant VanB Enterococcus faecium causing outbreaks in different countries is mainly associated with chromosomal Tn1549/5382-like platforms. 22nd ESCMID.	Mar-12	London, UK	Researchers	4000	London
220	Poster presentation	Silveira E,	Spread of large conjugative plasmids carrying antibiotic, copper and mercury resistance genes among Enterococcus from different sources. 22nd ESCMID.	Mar-12	London, UK	Researchers	4000	London
221	Poster presentation	Tedim AP,	Growth dynamics revealed inter- and intra-clonal fitness differences among major Enterococcus faecalis clonal complexes. 22nd ESCMID.	Mar-12	London, UK	Researchers	4000	London

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
222	Poster presentation	Tedim AP	Growth dynamics of CC17 and non-CC17 (CC9, CC22, CC94, ST96, ST172) Enterococcus faecium revealed inter-and intra-clonal differences in fitness. 22nd ESCMID.	Mar-12	London, UK	Researchers	4000	London
223	Poster presentation	Coque TM	European Conference on Clinical Microbiology and Infectious Diseases, ECCMID	Mar-12	London, UK	Researchers	300	Worldwide
224	Poster presentation	Stephanie Matrat	”Identification of the Enterococcus faecalis SOS regulon”. ECCMID.	Apr-12	L ondon, UK.	Researchers	Worldwide	Worldwide
225	Poster presentation	Hidalgo L	“Association of extended spectrum beta-lactamase VEB-5 and 16S rRNA methyltransferase ArmA in Salmonella enterica from United Kingdom”. ECCMID.	Apr-12	London UK	Researchers	Worldwide	Worldwide
226	Poster presentation	Hidalgo, L	“Discovery of a new 16S rRNA methylase, RmtF, conferring high-level aminoglycoside resistance in human Enterobacteriaceae isolates from India”. ECCMID.	Apr-12	London, UK	Researchers	Worldwide	Worldwide
227	Poster presentation	C.S. Saba	“Genetic relationship and antimicrobial susceptibility among Escherichia coli isolates from humans, poultry and street foods in Ghana”. 3rd ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans, and the Environment.	Jun-12	Aix-en-Provence, France.	Researchers	Worldwide	Worldwide
228	Poster presentation	B.Gutiérrez	Characterization of human and animal clinical isolates from Serbia carrying 16S ribosomal RNA aminoglycoside resistance methyltransferases. 3rd ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans, and the Environment.	Jun-12	Aix-en-Provence, France.	Researchers	Worldwide	Worldwide

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
229	Poster presentation	Top	at the 112th Meeting of the American Society of Microbiology	Jun-12	San Francisco, USA	Researchers	~10000	Worldwide
230	Poster presentation	De Gunzburg,	at ICAAC 2012	Sep-12	San Francisco, USA	Clinical Microbiologist, infectious diseases, infection control teams	>1,000	Worldwide
231	Poster presentation	Guardabassi, L	at the Central European Symposium on Antimicrobial Resistance, 2012,	Sep-12	Primošten, Croatia	Researchers	120	Worldwide
232	Poster presentation	de la Cruz, F	Structural studies of the protein machinery for DNA processing and translocation in bacterial conjugation. 22nd IUBMB Congress/37th FEBS Congress	Sep-12	Sevilla, Spain	Researchers	500	Spain
233	Poster presentation	de la Cruz	Relaxing relaxases: deregulation of the nic-cleavage reaction for biotechnological applications Plasmid Biology 2012	Sep-12	Sevilla, Spain	Researchers	200	Spain
234	Poster presentation	de la Cruz	Relaxing relaxases: deregulation of the nic-cleavage reaction for biotechnological applications 22nd IUBMB Congress/37th FEBS Congress	Sep-12	Sevilla, Spain	Researchers	500	Spain
235	Poster presentation	van Schaik, W	Poster at Gut Day	Nov-12	Leuven, Belgium	Researchers	150	BeNeLux
236	Poster presentation	Pallecchi,L	at the XLI Congresso Nazionale AMCLI	Nov-12	Rimini, Italy	Researchers	~1000	Italy
237	Poster presentation	Guardabassi, L	Diversity of blaCMY-2-positive plasmids in clinical Escherichia coli from humans and companion animals in USA. 9th ISAAR,	Feb-13	Kuala Lumpur, Malaysia	Researchers	>1000	Worldwide
238	Poster presentation	Guardabassi, L	Effect of cefotaxime on conjugation of IncN and IncI1 plasmids encoding beta-lactamases. 9th ISAAR,	Feb-13	Kuala Lumpur, Malaysia	Researchers	>1000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
239	Poster presentation	Freitas AR	Microevolutionary Events Involving Narrow Host Plasmids Influences Local Fixation of Vancomycin-Resistance in Enterococcus. ECCMID	Apr-13	Berlin, Germany	Researchers	4000	Berlin
240	Poster presentation	Willems, RJL	at Spring Meeting KNVM/NVMM (	Apr-13	Arnhem, The Netherlands	Researchers	300	Netherlands
241	Poster presentation	van Schaik, W	at ECCMID (23rd European Congress of Clinical Microbiology and Infectious Diseases)	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
242	Poster presentation	Coque, TM	at ECCMID (23rd European Congress of Clinical Microbiology and Infectious Diseases)	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
243	Poster presentation	Martinez, JL	at ECCMID (23rd European Congress of Clinical Microbiology and Infectious Diseases)	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
244	Poster presentation	Pallecchi,L	at ECCMID (23rd European Congress of Clinical Microbiology and Infectious Diseases)	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
245	Poster presentation	Mansfeld	et al., ECCMID	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
246	Poster presentation	Sinnige	et al., ECCMID	Apr-13	Berlin, Germany	Researchers	~4000	Worldwide
247	Poster presentation	Laura Hidalgo	“Association of RmtB methyltransferase and NDM among clinical Escherichia coli isolates from India and the UK”. ESCMID.	Apr-13	Berlin. Germany	Researchers	Worldwide	Worldwide
248	Poster presentation	Cristina M. Ovejero	“Tigecycline resistant Klebsiella pneumoniae from companion animals”. ESCMID.	Apr-13	Berlin. Germany	Researchers	Worldwide	Worldwide
249	Poster presentation	Ingham		Apr-13		Research & industry	Unknown	Europe
250	Poster presentation	Valverde A	La diseminación de la carbapenemasa KPC-2en Enterobacterias en el ámbito	May-13	Zaragoza, Spain	Researchers	500	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			hospitalario y en efluentes urbanos no tratados en Madrid se asocia al plásmido pMAD-2. XVII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC).					
251	Poster presentation	Buelow	Effects of selective digestive decontamination (SDD) on the gut resistome	Jun-13	Paris, France	Researchers	~500	Worldwide
252	Poster presentation	van Schaik, W	7th International Conference on Gram-positive Microorganisms	Jun-13	Montecatini di Terme, Italy	Researchers	300	Worldwide
253	Poster presentation	Grall	4th World forum on Healthcare-Associated Infections and Antimicrobial Resistance.	Jun-13	Annecy, France	Researchers	70	Worldwide
254	Poster presentation	Gral	2nd International Conference on Prevention & Infection Control,	Jun-13	Geneva, Switserland	Researchers	1500	Worldwide
255	Poster presentation	Cristina M	"Human adapted Klebsiella pneumoniae ST11 and ST147 resistant to tigecycline from pet animals". MED-VET-NET.	Jun-13	Copenhague .Denmark	Researchers	Worldwide	Worldwide
256	Poster presentation	Martinez, JL	FEMS2013, 5th Congress of European Microbiologists	Jul-13	Leipzig, Germany	Researchers	2300	Worldwide
257	Poster presentation	Smidt, H	at FEMS 2013 Congress	Jul-13	Leipzig, Germany	Researchers	>1000	Worldwide
258	Poster presentation	Woolhouse, M	at the StaphGBI	Aug-13	Dublin, Ireland	Researchers	120	Worldwide
259	Poster presentation	Martinez, JL	at the 14th International Conference on Pseudomonas	Sep-13	Lausanne, Switzerland	Researchers	600	Worldwide
260	Poster presentation	Guardabassi, L	at ICAAC 2013	Sep-13	Denver, Co, USA	Researchers	>1,000	Worldwide
261	Poster presentation	Willems	Molecular epidemiology of vancomycin-resistant Enterococcus faecium in hospitals in the Netherland Epidemics4 Conference	Nov-13	Amsterdam, The Netherlands	Researchers	400	Worldwide
262	Poster	Woolhouse, M	at the Epidemics4 Conference 2013	Nov-13	Amsterdam,	Researchers	400	Worldwide

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No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
	presentation				The Netherlands			
263	Poster presentation	Woolhouse, M	at the Epidemics4 conference 2013	Nov-13	Amsterdam, The Netherlands	Researchers	400	Worldwide
264	Poster presentation	Guzman Prieto	, 4th ASM Conference on Enterococci,	Mar-14	Cartagena Colombia	Researchers	~250	Worldwide
265	Poster presentation	Tedim AP	Intestinal colonization by Enterococcus in hospitalized and ambulatory patients of different age groups. 4th ASM Conference on Enterococci.	Mar-14	Cartagena Colombia	Researchers	~250	Worldwide
266	Poster presentation	Tedim AP	A new burst of bacteremia caused by BAPS 2.1 (ST117, ST80, ST203) Enterococcus faecium that are superimposed (2006-2012) but do not replace those caused by BAPS 3.3 (ST18, ST17 and ST16) (1995-2012). 4th ASM Conference on Enterococci.	Mar-14	Cartagena Colombia	Researchers	~250	Worldwide
267	Poster presentation	Tedim AP	Fitness cost of plasmids carrying Tn1546-vanA in diverse Enterococcus faecium clonal contexts. 4th ASM Conference on Enterococci.	Mar-14	Cartagena Colombia	Researchers	~250	Worldwide
268	Poster presentation	León-Sampedro R	Population structure of Enterococcus faecalis from wild and migratory birds 4th ASM Conference on Enterococci.	Mar-14	Cartagena, Colombia.	Researchers	~250	Worldwide
269	Poster presentation	León-Sampedro R	Spread of CTn5801 among enterococcal species from different origins. 4th ASM Conference on Enterococci.	Mar-14	Cartagena, Colombia.	Researchers	~250	Worldwide
270	Poster presentation	Turrientes MC	Diferentes frecuencias de recombinación y patrones de resistencia antibiótica en diferentes grupos filogenéticos de Escherichia coli XVIII Congreso SEIMC 2014.	Apr-14	Valencia Spain	Researchers	500	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
271	Poster presentation	Turrientes MC	Diferentes frecuencias de recombinación y mutación en cepas patógenas y no patógenas de Escherichia coli. XVIII SEIMC 2014.	Apr-14	Valencia, Spain	Researchers	500	Spain
272	Poster presentation	Ducher	DAV132, developed to prevent side effects of antibiotics in the gut flora: Results of a pilot cross-over study in healthy volunteers. ECCMID 2014 - P0248 poster	Apr-14	Barvelona, Spain	Researchers	4000	Worldwide
273	Poster presentation	Miossec	DAV131, an oral adsorbent-based product, exerts a dose-dependent protection of hamsters against moxifloxacin-induced Clostridium difficile lethal infection. ECCMID 2014 - P0804 poster.	Apr-14	Barcelona, Spain	Researchers	4000	Worldwide
274	Poster presentation	Conte V	ECCMID, 10-13 May 2014,	May-14	Barcelona, Spain	Researchers	>1000	Worldwide
275	Poster presentation	Buelow	Effects of selective digestive decontamination (SDD) on the gut resistome	May-14	Barcelona, Spain	Researchers	Worldwide	Worldwide
276	Poster presentation	Guardabassi, L	Therapeutic concentrations of cefotaxime increase the conjugation frequency of a widespread IncI1 plasmid encoding CMY-2 β-lactamase in Escherichia coli. 24th ECCMID,	May-14	Barcelona, Spain	Researchers	4000	Worldwide
277	Poster presentation	Guardabassi, L	Exploring transmission of Escherichia coli plasmids encoding CMY-2 β-lactamase between animals and humans in Denmark. 24th ECCMID,	May-14	Barcelona, Spain	Researchers	4000	Worldwide
278	Poster presentation	Rodríguez	Intraclonal diversity of ST131 Escherichia coli causing bacteremia in a Spanish hospital along 15 years (1996-2012). 24th European Congress of Clinical Microbiology and Infectious Diseases.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
279	Poster presentation	Tobes R	Sequencing, assembly and comparative genomics analysis of six Enterococcus	May-14	Barcelona, Spain	Researchers	4000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			faecium (EFM) ST117, emergent multiresistant clone responsible for an increase of EFM bacteraemia and faecal carriage in Spain. 24th ESCMID.					
280	Poster presentation	Tobes R	Deciphering evolutionary events and presence of resistance genes by full de novo genome sequencing of clonally related multidrug-resistant OXA-48 Klebsiella pneumoniae ST11 isolates. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
281	Poster presentation	Tedim AP	Evolution of healthcare bacteraemic episodes of Enterococcus faecium (1995-2012) reflects changes in phylogenomic groups with internal clonal epidemic waves. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
282	Poster presentation	Turrientes MC	Different recombination frequencies and antibiotic resistance patterns among Escherichia coli phylogenetic groups. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
283	Poster presentation	Turrientes M	Differences in recombination events and mutation frequencies among pathogenic and commensal and environmental Escherichia coli strains. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
284	Poster presentation	Freitas AR	Extended virulence profile of Enterococcus faecium from swine (Europe/USA, 1995-2008). 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
285	Poster presentation	Gijón D	Multiclonal spread of OXA-48-producers involving CG258 (ST11) Klebsiella pneumoniae and other Enterobacteriaceae in a VIM and KPC carbapenemase-endemic hospital and emergence in non-hospitalised patients. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
286	Poster presentation	León R	Plasmid diversity among Enterococcus faecalis of different origins, including	May-14	Barcelona, Spain	Researchers	4000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			migratory birds, farm animals and humans. 24th ESCMID.					
287	Poster presentation	Rodrigues C	Discrimination of antibiotic resistant Klebsiella pneumoniae clones by Fourier Transform Infrared Spectroscopy (FTIR). 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
288	Poster presentation	Sánchez-Valenzuela A	Copper-resistance in Enterobacteriaceae and other proteobacteria from childrens' intestines. 24th ESCMID.	May-14	Barcelona Spain	Researchers	4000	Worldwide
289	Poster presentation	Curiao T	. Common transcriptomic changes in the adaptation of Salmonella enterica Typhimurium to biocides and antibiotics. 24th ESCMID.	May-14	Barcelona, Spain	Researchers	4000	Worldwide
290	Poster presentation	Santos-López	"Universal method for detecting and capturing ColE1 plasmids reveals high prevalence of small plasmids in multiresistant clinical Enterobacteriaceae and Pasteurellaceae". 24th ECCMID.	May-14	Barcelona, Spain	Researchers	Worldwide	Worldwide
291	Poster presentation	Ovejero, C. M.	"Association of 16S rRNA Methyltransferases and NDM carbapenamase in India". 24th ECCMID.	May-14	Barcelona, Spain	Researchers	Worldwide	Worldwide
292	Poster presentation	Gutiérrez B.	"Emergence of 16S rRNA methylase-producing Enterobacteriaceae and P. aeruginosa human clinical isolates and Aeromonas hydrophila isolated from a fish in Serbia". 24th ECCMID.	May-14	Barcelona, Spain	Researchers	Worldwide	Worldwide
293	Poster presentation	Carrilero L.	"The SOS regulon of Enterococcus faecalis is not involved in virulence traits". 24th ECCMID.	May-14	Barcelona, Spain	Researchers	Worldwide	Worldwide
294	Poster presentation	Van Schaik	114th General Meeting of the American Society for Microbiology,	May-14	Boston, USA	Researchers	~5000	Worldwide
295	Poster presentation	Top	114th General Meeting of the American Society for Microbiology,	May-14	Boston, USA	Researchers	~5000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
296	Poster presentation	De Been	EMBO Conference on Microbiology after the genomics revolution: Genomes 2014	May-14	Paris, France	Researchers	~500	Worldwide
297	Poster presentation	Guzman Prieto	EMBO Conference on Microbiology after the genomics revolution: Genomes 2014	May-14	Paris, France	Researchers	~500	Worldwide
298	Poster presentation	de Toro M	A method for plasmid reconstruction from next generation sequence data: plasmid constellation network (PLACNET). Genomes 2014: EMBO Conference on Microbiology after the genomics revolution.	Jun-14	Paris, France	Researchers	500	Worldwide
299	Poster presentation	Thomas-López D.	"Secuenciación y análisis de Enterococcus faecalis JH2-2: diferencias con la cepa clínica V583 en los determinantes de virulencia y de resistencia a antibióticos". X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain
300	Poster presentation	Hoefer, A.	"Phenotypic changes caused by the truncation of the 5' UTR suggest a stringent translational regulation of the aminoglycoside resistance methyltransferase ArmA". X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain
301	Poster presentation	Bernabé-Balas C.	"Bases de la adaptación de un plásmido ColE1 a su hospedador en un ensayo de evolución experimental". X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain
302	Poster presentation	Gutiérrez B.	"Adquisición de metiltransferasas del ARNr 16S, interferencia con metilaciones intrínsecas y evolución hacia un ribosoma resistente a aminoglucósidos". X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
303	Poster presentation	Ovejero, C. M.	”Caracterización de replicones prevalentes en clones multirresistentes en India”. X Reunión del Grupo Especializado de la SEM de Microbiología Molecular.	Jun-14	Segovia. Spain.	Researchers	300	Spain
304	Poster presentation	Guradabassi, L	ISME16 . Abstract book, abstract number 299B.	Aug-14	Seoul, South Korea	Researchers	>1000	Worldwide
305	Poster presentation	Gudeta	ISME16 . Abstract book, abstract number 299B.	Aug-14	Seoul, South Korea	Researchers	>1000	Worldwide
306	Poster presentation	Luzzaro F	54th ICAAC, 5-9 September 2014,	Sep-14	Washington, USA	Researchers	>1000	Worldwide
307	Poster presentation	Sennati S	Congresso Nazionale della Società Italiana di Microbiologia, 28 Sept – 1 Oct 2014,	Sep-14	Torino, Italy	Researchers	500	Italy
308	Poster presentation	Woolhouse, M	Public Health Science: A National Conference Dedicated to New Research in UK	Nov-14	Glascow, UK	Researchers	400	Worldwide
309	Poster presentation	De Gunzburg	Novel and promising products in development for human use. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen , Denmark.	Researchers	>1000	Worldwide
310	Poster presentation	Curiao T	Characterization of the plasmidome of B1-ST359 Escherichia coli, an ExPEC with zoonotic potential. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen , Denmark.	Researchers	>1000	Worldwide
311	Poster presentation	Martínez García L	Hand’s transmission efficiency of nosocomialclinical isolates: a finger-to-finger transmission model. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen , Denmark.	Researchers	>1000	Worldwide
312	Poster presentation	Ripoll A	Mercury resistance among Escherichia coli faecal isolates from a children population exposed to metal contamination. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen , Denmark.	Researchers	>1000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
313	Poster presentation	Rodríguez Fernandez I.	Diversity of B2-non-ST131 extraintestinal pathogenic Escherichia coli in a Spanish University Hospital over 17 years. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Worldwide
314	Poster presentation	Sánchez-Valenzuela A	Mercury resistance (merA) among Enterococcus from children various levels of exposure to heavy metals in Spain. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Worldwide
315	Poster presentation	Sinnige, J	Detection of the first two VanD-type vancomycin-resistant Enterococcus faecium in the Netherlands. 25th European Congress of Clinical Microbriology and Infectious Diseases.	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Worldwide
316	Poster presentation	Gudeta	CSP-1, a new subclass B3 metallo-ß-lactamase in Chryseobacterium isolated from soil. The 25th European Congress of Clinical Microbriology and Infectious Diseases (ECCMID 2015).	Apr-15	Copenhagen, Denmark.	Researchers	>1000	Worldwide
317	Poster presentation	Hendrickx, A	Inhibition of lipoteichoic acid synthesis of multi-drug resistant Enterococcus faecium isolates as potential novel treatment for enterococcal infections. ASM General Meeting.	May-15	New Orleans, USA	Researchers	>1000	Worldwide
318	Poster presentation	Hendrickx, A	Antibiotics driven dysbiosis generates an alternative segregation of Enterococcus faecium from the intestine. ASM General Meeting.	May-15	New Orleans, USA	Researchers	>1000	Worldwide
319	Poster presentation	León-Sampedro R	Detección de Tn5801 (tetM) en Enterococcus y análisis de su historia evolutiva a partir de otros patógenos oportunistas Gram positivos. XIX Congreso SEIMC,	May-15	Sevilla, Spain.	Clinical Microbiologist, infectious diseases, infection	300	Spain

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
						control teams		
320	Poster presentation	Lanza VF	The Deep Resistome Discovering the resistome of minority populations. ICETAR (EVOTAR) 2015.	Jun-15	Amsterdam, The Netherlands	Research & industry	300	Worldwide
321	Poster presentation	Lanza VF	Identification and dynamics of the accessory genes in the Escherichia coli O25b-ST131 clonal group causing blood stream infections in a Spanish University Hospital. ICETAR (EVOTAR) 2015.	Jun-15	Amsterdam, The Netherlands	Research & industry	300	Worldwide
322	Poster presentation	Tedim AP	Fitness cost of plasmids carrying Tn1546-vanA in diverse Enterococcus faecium clonal contexts. ICETAR (EVOTAR) 2015.	Jun-15	Amsterdam, The Netherlands	Research & industry	300	Worldwide
323	Poster presentation	Tedim AP	Comparative genomics of worldwide spread Enterococcus faecium ST117 clone. ICETAR (EVOTAR) 2015.	Jun-15	Amsterdam, The Netherlands	Research & industry	300	Worldwide
324	Poster presentation	van Schaik, W	6th Congress of European Microbiologists FEMS 2015, 3 poster presentations	Jun-15	Maastricht, The Netherlands	Researchers	3000	Worldwide
325	Poster presentation	Top, J	Functional Characterization of an Inositol Metabolism Encoding Gene Cluster contained in ICEEfm1 of Enterococcus faecium. 6th Congress of European Microbiologists FEMS 2015,	Jun-15	Maastricht, The Netherlands,	Researchers	3000	Worldwide
326	Poster presentation	Rogers, M	Comparative genomic analysis of the first two vanD-type vancomycin-resistant Enterococcus faecium in The Netherlands. ASM Conference on rapid Next-Generation Sequencing and Bioinformatics Pipelines for Enhanced Molecular Epidemiologic Investigations of Pathogens.	Sep-15	Washington, USA	Researchers	200	Worldwide
327	Poster	De Gunzburg	Results from the clinical study DAV132-	Sep-15	San Diego,	Researchers	>2000	Worldwide

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
	presentation		CL-1002 were. Presented at the IDWeek conference in		USA			
328	Poster presentation	Gudeta	A novel glycopeptide resistance operon in environmental Rhodococcus equi : The 53rd Interscience Conference on Antimicrobial Agent and chemotherapy (ICAAC 2013).	Sep-15	San Diego, USA	Researchers	>2000	Worldwide
329	Poster presentation	De Gunzburg	Analytical methods developed in the context of the EvoTAR program have been presented at the European Bioanalysis Forum (EBF) in	Nov-15	Barcelona, Spain	Researchers	>100	Europe
330	Press release	Willems, RJL	24 miljoen voor onderzoek antibioticaresistentie	Oct-11	Utrecht, The Netherlands	Companies, Research, Lay Public	Unknown	Netherlands
331	Press release	Willems, RJL	UMC Utrecht to lead research on antibiotic resistance	Oct-11	Utrecht, The Netherlands	Companies, Research, Lay Public	Worldwide	Worldwide
332	Press release	Willems, RJL	Start of EvoTAR project	Nov-11	Utrecht, The Netherlands	Companies, Research, Lay Public	Unknown	France
333	Press release	Willems, RJL	"VRE-epidemie breidt zich uit in Nederland"	Jul-12	Utrecht, Netherlands	Companies, Research, Lay Public	Unknown	Netherlands
334	Press release	Turrientes MC	A DISEASED ECOSYSTEM TACKLING ANTIBIOTIC RESISTANCE FROM A GLOBAL PERSPECTIVE, MÈTODE, 78 (2013): 00-00. University of Valencia DOI: 10.7203/metode.78.2627 ISSN: 2174-3487. http://metode.cat/en/Issues/Monographs/The-Light-of-Evolution/Un-ecosistema-malalt	Jul-13	Madrid, Spain	Researchers	Unknown	Unknown
335	Press release	Turrientes MC	UN ECOSISTEMA ENFERMO LA LUCHA CONTRA LA RESISTENCIA A	Jul-13	Madrid, Spain	Researchers	Unknown	Unknown

A2: List of dissemination activities								
No	Type of activity	Author	Title	Dates	Place	Type of audience	Size of audience	Countries addressed
			ANTIBIÓTICOS DESDE UNA PERSPECTIVA GLOBAL, (Ejemplar dedicado a: La luz de la evolución).MÈTODE, 78 (2013): 60-65. Universitat de València DOI: 10.7203/metode.78.2627 ISSN: 2171-911X. http://metode.cat/es/Revistas/Monografics/La-luz-de-la-evolucion/Un-ecosistema-malalt					
336	Press release	Turrientes MC	Un ecosistema malalt: la lluita contra la resistčncia a antibiōtics des d'una perspectiva global. Mčtode: Revista de difusió de la investigació de la Universitat de Valencia 78 (2013): 68-73. ISSN 1133-3987. ISSN: 2171-911X	Jul-13	Madrid, Spain	Researchers	Unknown	Unknown
337	Symposium/ Workshop organisation	Andersson, D	at ICAAC (San Francisco, USA)	Sep-12	San Francisco, Spain	Researchers	300	Worldwide
338	Symposium/ Workshop organisation	de la Cruz, F	The International Conference in Plasmid Biology	Sep-12	Santander, Spain	Researchers	200	Worldwide
339	Symposium/ Workshop organisation	Baquero, F	Microbiology: Transmission *. Organized by CSIC, Fundación Ramón Areces and Lilly	May-15	Madrid, Spain	Researchers	500	Worldwide
340	Video	De Gunzburg	A novel video of presentation of DAV132 was published on Da Volterra's website to explain the interest of protecting the microbiota and illustrate the mechanism of action of DAV132	Sep-15	Paris, France	Wide	Unknown	Worldwide
341	Website	van Schaik, W	Launch of EvoTAR-website at www.evotar.eu	Oct-11	Utrecht, The Netherlands	Companies, Research, Lay Public	Worldwide	Worldwide

Section B (Confidential or public: confidential information to be marked clearly)

B1: List of applications for patents, trademarks, registered designs, ETC.					
Type of IP Rights	Confidential YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on application)

Part B2

Type of Exploitable Foreground	**Description of exploitable foreground**	**Confidential YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**