

Other significant outcomes of the project included the development of the following three new microarrays for the detection of antibiotic resistance genes:

(i) A new DNA microarray for antibiotic resistance genotyping in multidrug-resistant *Acinetobacter baumannii* isolates. The results obtained using this microarray showed 100% concordance with the results of the national reference center for Gram-negative isolates in a blinded evaluation study. The administration of the four antibiotics to healthy volunteers outside the hospital environment did not appear to create selection pressure for the emergence of pathogens of major clinical importance.

(ii) A new DNA labelling and amplification system allowing rapid microarray-based detection of 116 antibiotic resistance genes in Gram-positive bacteria. Microarray results revealed that healthy people may harbour in the nose and skin coagulase-negative staphylococci with an extended assortment of antibiotic resistance genes. The number of bacteria containing the tetracycline-minocycline resistance gene *tet(M)* increased in volunteers who received minocycline. An increase in *erm* genes, which confer resistance to macrolide-lincosamide-streptogramin B antibiotics, was observed in streptococci from volunteers who received clindamycin. An increase in resistance genes not directly associated with the antibiotic treatment (macrolide and tetracycline resistance genes) was observed in staphylococci isolated from volunteers treated with minocycline or ciprofloxacin indicating co-selection of resistance.

ANTIRESDEV project partners:

- University College London (Coordinator, UK)
- Karolinska Institutet (SE)
- Università di Siena (IT)
- University of Zurich (CH)
- University of Berne (CH)
- Ruhr-Universität Bochum (DE)
- Animal Health and Veterinary Laboratories Agency (UK)
- Academic Centre for Dentistry Amsterdam (NL)
- Helperby Therapeutics Limited (UK)
- TNO (NL)

(iii) An expanded microarray able to detect over ninety different antimicrobial resistance genes in Gram-negative bacteria. The results showed that many of the strains of *Escherichia coli* in the human intestine are naturally resistant to a number of antibiotics. These resistance genes may be present on mobile elements such as plasmids and conjugative transposons that can be transferred to other bacteria. The elimination of some of the plasmids from selected strains affected their ability to grow in the presence of antibiotics and stress modulators indicating that the plasmids may be associated with fitness and adaptation of strains to different environments. Chick colonisation models showed these *E. coli* isolates were able to colonise and survive well in the chick intestinal tract. Furthermore, there was some indication of the possible transfer of the resistance genes from the isolates to members of the gut microbiota.

Staphylococcus aureus rapidly acquires antibiotic resistance and adapts to changing environmental conditions by modulating resistance, virulence and fitness, as seen in community-acquired methicillin-resistant *S. aureus*. Those volunteers colonized with *S. aureus* were usually found to carry one single strain type, with two exceptions. The strain-types were representative of the clones usually found in Europe. Most isolates were resistant to amoxicillin, but none to methicillin. The administration of minocycline or amoxicillin had no measurable effect on the resistance or fitness of the *S. aureus* found in healthy volunteers.

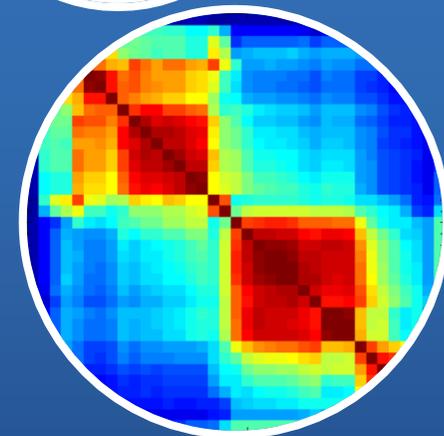
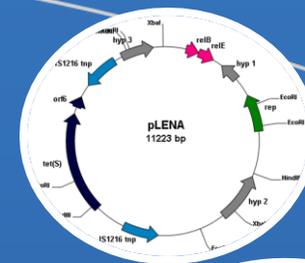
Most of the minocycline-resistant strains isolated in the study contained mobile genetic elements of the Tn916 family. These elements have complex regulatory circuits that can sense tetracycline and other environmental stresses. These are currently under investigation. It is important to understand how elements that stimulate the spread of antibiotic resistance are regulated so that healthcare providers can avoid creating environments in which the transfer of resistance is promoted.

A novel genetic element on which antibiotic and antiseptic resistance genes are linked was found during the study. This shows that exposure to one type of antimicrobial agent could also promote the spread of resistance to a completely different type of antimicrobial agent.

Contact: [Professor Michael Wilson](#)

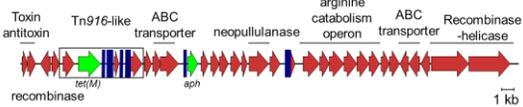
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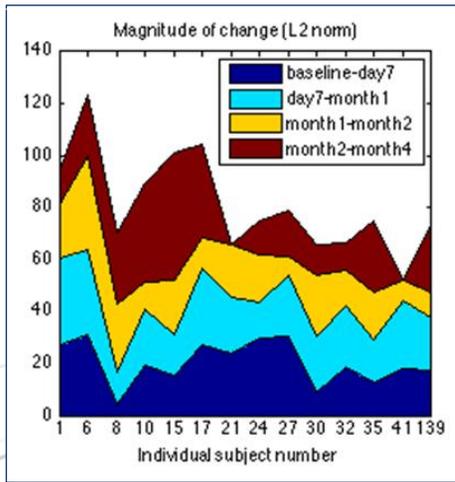


ANTIRESDEV Project Main Outcomes

The effects of antibiotic administration on the emergence and persistence of antibiotic-resistant bacteria in humans and on the composition of the indigenous microbiotas at various body sites



- ANTIRESEDEV was a €5.4 million European Union-funded research project in the “Health” theme of the Seventh Framework Programme.
- The objectives of ANTIRESEDEV were to use a multidisciplinary approach to study the impact of different antibiotics in selecting resistance among pathogenic and commensal members of the indigenous microbiota of humans and to determine their effects on the composition of indigenous microbial communities using culture-dependent and culture-independent techniques.

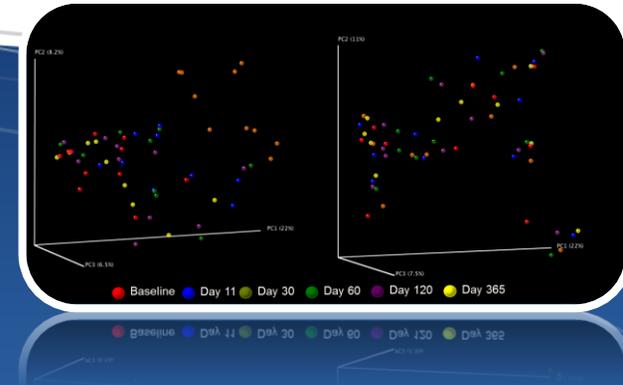


Main project outcomes:

Minocycline administration resulted in a statistically-significant increase in the proportion of minocycline-resistant bacteria isolated from the anterior nares and the oral cavity. However, no such increase was found in the intestinal tract or on the skin. One month after minocycline administration, the proportions of minocycline-resistant bacteria isolated from the four body sites in the placebo and test groups were not significantly different.

Administration of amoxicillin appeared to have little effect on the composition of the cultivable microbiota of the four body sites with the exception of the oral cavity where it induced a reduction in the proportion of *Streptococcus salivarius*. However, one month after amoxicillin administration, the proportions of *S. salivarius* isolated from the oral cavity in the placebo and test groups were not significantly different. Administration of amoxicillin did not result in a statistically-significant increase in the proportion of amoxicillin-resistant bacteria isolated from any of the body sites.

Administration of ciprofloxacin had no effect on the composition of the cultivable microbiota of the skin, nose or oral cavity. However, in the intestinal microbiota, the proportions of *Escherichia coli* and bifidobacteria decreased but these returned to baseline after one month. There was no increase in the proportion of ciprofloxacin-resistant bacteria in the nasal or skin microbiotas following ciprofloxacin administration. However, there was an increase in the proportions of ciprofloxacin-resistant bifidobacteria and *Escherichia coli* in the intestinal tract as well as increased



proportions of ciprofloxacin-resistant *Prevotella* spp. and *Veillonella* spp. in the oral cavity.

Administration of clindamycin had no effect on the composition of the cultivable microbiota of the skin or nose. However, in the intestinal microbiota, the proportions of bacteroides, bifidobacteria and lactobacilli decreased although all returned to baseline after one year. In the oral microbiota only the proportion of *Leptotrichia* spp. was affected – this decreased but returned to baseline after four months. Following clindamycin administration the proportions of clindamycin-resistant bacteria increased at all body sites except the skin. Hence, there were increases in the proportions of: (a) clindamycin-resistant coagulase-negative staphylococci in the nasal microbiota, (b) clindamycin-resistant *Bacteroides* spp. in the intestinal microbiota, (c) clindamycin-resistant *Veillonella* spp., *Streptococcus salivarius*, fusobacteria, *Prevotella* spp. and alpha-haemolytic streptococci in the oral cavity.

Continued overleaf

Culture-independent analysis of the oral and intestinal microbiotas of volunteers who did not receive antibiotics showed that these microbiomes exhibited high compositional stability over a one year period.

Administration of minocycline, clindamycin and ciprofloxacin, but not amoxicillin, had a profound effect on the oral microbiome. However, after one month, its composition returned to that which existed prior to antibiotic administration.

The composition of the intestinal microbiome was severely affected by all four antibiotics. However, one month after the administration of amoxicillin or minocycline, the intestinal microbiome was similar to that found prior to antibiotic administration. In contrast, a return to pre-administration values took 4 months in the case of ciprofloxacin and between 4 months and one year for clindamycin.

Administration of minocycline to healthy volunteers appeared to have little effect on the composition of the cultivable microbiota of the skin, anterior nares or intestinal tract. In contrast, it did affect the oral microbiota where it increased the proportion of alpha-haemolytic streptococci. However, one month after administration of the antibiotic, the proportions of alpha-haemolytic streptococci isolated from the oral cavity in the placebo and test groups were not significantly different.