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Antibiotics and the post-genome revolution

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The emergence of pathogenic bacteria resistant to multiple antimicrobial agents is turning into a major crisis in human and veterinary medicine. This necessitates a serious re-evaluation of our approaches toward antibacterial drug discovery and use. Concurrent advances in genomics including whole-genome sequencing, genotyping, and gene expression profiling have the potential to transform our basic understanding of antimicrobial pathways and lead to the discovery of novel targets and therapeutics.

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Introduction

The golden era of antibiotic discovery was from the 1940s to the late 1960s, where many diverse classes of antibiotics were discovered [1]. Development of second-generation, third-generation, and fourth-generation antibiotics further improved upon the activity and efficacy of existing compounds [2••,3]. The success of that diverse arsenal to combat infectious diseases made many pharmaceutical companies abandon their antibiotic development programs [2••]. With the emergence of multi-drug resistant pathogens, antibiotic discovery efforts were reinitiated and surged, but there was a long gap until drugs like linezolid and daptomycin [2••,4••,5] with novel modes of action were approved. However, just a few years past their introduction into the market, clinical strains resistant to those antibiotics were reported [6,7,8]. These observations imply that the traditional approach of antibiotic discovery, though highly successful in the introduction of numerous drugs, cannot sustain the high demand for development of novel compounds [9]. Therefore, a more efficient antibiotic discovery platform is essential for allowing us to compete with the evolution of microbial resistance [10]. In the post-genomic era, with the availability of various genomics-based platforms including whole-genome sequencing, genotyping, and gene expression profiling, a new horizon opens that could revolutionize our pursuit of novel antimicrobial agents. In this review we will briefly discuss how genomics has transformed our understanding of antibiotics and impacted our approach toward antibacterial drug discovery.

Identification and validation of novel targets

Most commonly used antibiotics only target a limited number of crucial biological processes, including DNA replication, protein translation, and cell wall biosynthesis [11]. Genomics provides the tools for rapid identification of new classes of biological processes, which will be alternative targets for novel antimicrobial agents.

Identifying essential proteins and pathways, not present in the host

Identification of essential genes has been one of the primary contributions of genomics to antibiotic target discovery [12]. Strategies like transposon mutagenesis or allelic replacement, all relying on the genome sequence of the desired microorganism, were widely used for constructing recombinant strains and mapping putative essential genes in sequenced pathogens [4••]. Those efforts were later replaced by comparative genomics approaches that searched for broadly conserved genes among the desired bacterial spectrum, which are either absent or evolutionary distant in eukaryotes [12,13]. As a case in point fatty acid biosynthetic genes, more specifically *fabI*, have been identified as putative drug targets based on their conservation among *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenza* species [14]. Purified FabI protein was then screened against a compound library by GlaxoSmithKline (GSK), which led to the identification of AFN-1252 as a specific inhibitor [15•]. Other examples of genomics-derived targets are polypeptide deformylasese [16,17], aminoacyl-tRNA synthetases [15•,16,18] and components of the NAD(P) biosynthetic pathway [19].

Identifying virulence genes

Virulence factors and other genes that are expressed *in vivo* during the course of an infection are potential therapeutic targets since they are directly associated with either the disease or adaptation to host. Traditionally, signature tagged mutagenesis (STM), which recognizes mutants that survive and proliferate better in the host, and *in vivo* expression technology (IVET), which identifies genes that are induced during an infection, were used for assessing genes associated with the infection phase [20]. Microarrays and more recently transcriptome

sequencing (mRNA-seq) have significantly increased the throughput and sensitivity of the IVET technology [21]. Similarly, next-generation sequencing has extensively enhanced the resolution and capacity of the STM workflow [22].

Optimizing antibiotic biosynthesis

Optimizing the efficiency of antibiotic biosynthetic processes that are fully or partially synthesized by a microorganism is crucial for cost-effective large-scale production of the drug. This can be facilitated in different ways via genomics-associated technologies.

Dissecting the metabolic network of the natural producer

Whole-genome sequencing followed by functional annotation of the putative ORFs of any antibiotic-producing strain can help construct the *in silico* metabolic network of that strain which allows drug production optimization. This can be achieved by enhancing the flux of the desired pathways or removing any competitive biochemical pathways in the host [23].

Finding hosts for heterologous expression of secondary metabolites

Efficiency of engineered or naturally occurring antibiotic biosynthetic pathways can be enhanced by choosing a host that tolerates higher concentrations of the secondary metabolite or has a metabolic network engineered to be compatible with the biosynthesis of the desired compound. Availability of the genome sequences of a diverse spectrum of microorganisms facilitates identification of the ideal host. A genomics-driven engineering of microorganisms toward making reduced genome hosts for heterologous gene cluster expression can also enhance the production yield [24,25,26].

Understanding antibiotics mode of action and emergence of antibiotic resistance

Many antimicrobial agents are discovered via phenotypic screening based on growth-inhibitory effect of a compound identified by screening large libraries [15•]. Many of these identified compounds have no known mechanism of action. There are also other antimicrobial agents that have a proposed mode of action, but may have other activities of equal or greater importance [27]. There are several genomics-based platforms that could elucidate antibiotics mechanism of action.

Transcriptome profiling of response to antibiotics

Introduction of any antibiotic to a sensitive microorganism perturbs a number of biological processes that is reflected to some extent at the gene expression level. Therefore, comparative transcriptome profiling in the presence and absence of any given antibiotic can identify genes that show differential expression upon exposure to the drug. For example, gene expression microarrays elucidated that different classes of bactericidal antibiotics kill cells by inducing a common oxidative damage pathway [28]. A more recent example is a microarray-based transcription profiling of *S. aureus* challenged with daptomycin, a drug recently approved for treatment of gram-positive bacterial infections, which suggested inhibition of peptidoglycan biosynthesis and membrane depolarization are two major modes of action for the drug [29].

Mapping adaptive mutations

Upon exposure to antimicrobial agents, most bacteria efficiently accumulate adaptive mutations owing to their short generation time [30]. Identity of these mutations, if not associated with efflux pumps or other generic cellular stress responses, could help identify the antibiotic targets or its mechanism of action [27]. Whole-genome sequencing combined with methods that can discriminate adaptive from neutral mutations [31] can provide an accurate genetic map of the mutations responsible for the emerged resistance phenotype.

Functional genomics and high throughput profiling of complex bacterial populations

Microarray-based or sequencing-based high-throughput genetic footprinting of complex bacterial populations has turned into a powerful platform for dissecting the genetic basis of different bacterial behaviors [32–34]. A diverse transposon-mutagenized library of a microorganism can be challenged with an antibiotic and the abundance of all mutants in the enriched population can be quantified on microarrays or by sequencing, reflecting the contribution of each locus in the genome to the observed phenotype. This has been done for a wide range of drugs in *E. coli*, successfully identifying both known and novel loci contributing to antibiotic resistance [35].

Tracking population-wide resistance

Recently, population-level antibiotic resistance behaviors mediated by signaling molecules have been observed in bacteria. In a continuous culture of *E. coli* evolved in increasing levels of norfloxacin, a small number of highly resistant mutants produced indole at a fitness cost to them. The more sensitive members of the population then sensed the indole and increased production of efflux pumps and oxidative-stress protections, increasing their resistance beyond that exhibited in a homogeneous population [36••]. Deep sequencing of heterogeneous bacterial samples can identify even rare mutations in the highly resistant and more sensitive subpopulations that are driving these population-level behaviors.

Theragnostics and personalized treatment

Theragnostics combines therapeutics with diagnostics to come up with a treatment strategy for individual patients. A diagnostic test is conducted to identify whether any given patient is more likely to be helped or harmed by a medication and suggests targeted drug therapy based on

the results [37]. Genomics, in the theragnostics setting, can help rapidly genotype patients and pathogens isolated from them and use that information to pick the most effective therapy or potentially identify risks and side effects associated with a given therapy regime [37].

Looking for genetic determinants of antibiotic resistance at the individual level

The success of the widely used triple combination therapy based on a proton pump inhibitor (PPI), amoxicillin (AMPC), and clarithromycin (CAM) against *Helicobacter pylori* is known to be associated with cytochrome P450 2C19 (CYP2C19) enzyme activity among patients [38] and presence of CAM resistance mutation (usually in the 23S rRNA gene) in the pathogen [39]. By genotyping the patients and bacteria, the efficacy of the candidate antibiotic can be predicted [38] and when necessary, appropriate alternatives can be sought.

Profiling drug targets

Triclosan is a commonly used antimicrobial agent that acts by inhibiting FabI or enoyl-ACP reductase, an enzyme involved in fatty acid biosynthesis. However, many pathogens including *S. pneumoniae* do not possess FabI, but instead have an alternative isozyme, FabK, that is resistant to triclosan [40]. Other pathogens like *Pseudomonas aeruginosa* encode two isozymes, one of which (FabK) is non-responsive to triclosan [41]. Using genomics, the clinical samples isolated from a patient can be analyzed by a quick genotyping assay that screens for the presence of the drug target and absence of redundant isoforms that are drug-resistant.

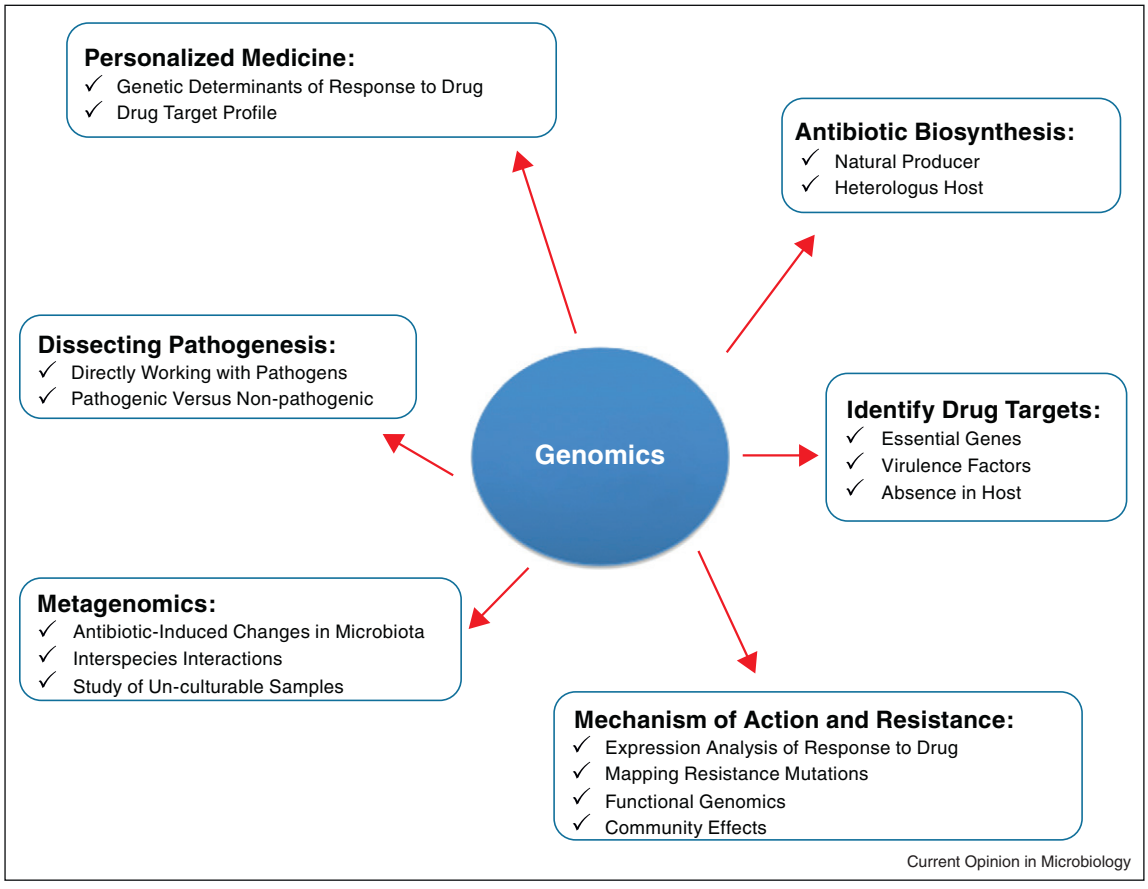
Better understanding of pathogenesis

Genomics can facilitate the study of the pathogenesis phenomenon, which would set the stage for development of new therapeutics and expand our knowledge of currently used antimicrobial agents.

Directly working with pathogens

There is considerable genetic diversity among different bacterial species and different strains of the same species. The affordable cost of DNA sequencing allows direct whole-genome sequencing of any culturable pathogen

Figure 1



Contribution of Genomics to Antibiotics: Genomics has transformed our overall knowledge of antibiotics and our approach toward antibacterial drug discovery. This includes but is not limited to new drug target identification, understanding the mechanism of antibiotic action and emergence of resistance, better understanding of pathogenesis, optimizing antibiotic biosynthetic processes, and personalized medicine.

isolated from a patient, providing a precise genetic profile of that pathogen in the context of its associated disease. For example, whole-genome sequencing has been applied to identify resistance mutations that emerge in the nosocomial pathogen, *Acinetobacter baumannii* upon antibiotic therapy *in vivo* [42].

Pathogenic versus non-pathogenic strains

Many bacterial genera including *Listeria* contain both pathogenic and non-pathogenic species. Comparative genomics allows identification of homologous virulence gene clusters such as *prfA* [43], which could be potential drug targets. It would also provide some insight into the evolution of the pathogen and its pathogenic traits [43] with potential therapeutic applications [19].

Metagenomics and antibiotics

With the rapid evolution of high-throughput sequencing platforms, highly complex environmental (i.e. metagenomics) samples can be directly analyzed, circumventing the need for culturing the samples [44]. Metagenomic studies of bacterial communities have also the potential of providing a wealth of insights into antibiotic utilization in the native ecological context.

Studying the effect of antibiotics on microbiota

Antibiotic usage can disrupt the body's natural microbiota, which could have adverse health consequences, a phenomenon that in some cases can be addressed directly using the genomics platforms. An illustrative example arises from deep sequencing of the metagenomic samples isolated from the distal gut of three healthy individuals before and after ciprofloxacin treatment, which showed dramatic changes in the diversity and distribution of the microbial community upon treatment [45•]. The same platform can be used to compare the diversity of bacterial communities pre-antibiotic and post-antibiotic exposure during infections. A metagenomics study of a mouse model of *Enterococcus* infection surprisingly suggested that antibiotic treatment set the stage for the pathogen to dominate the intestinal microbiota [46].

Dissecting interspecies interactions

Interspecies interactions can influence many physiological aspects of microbial communities including their response to antibiotics. Whole genome sequencing provided a good example for horizontal transfer of a methicillin-resistant gene cassette from *Staphylococcus epidermidis* to *S. aureus* in a patient [47].

Identifying novel antibiotic biosynthetic gene clusters from un-culturable samples

Using metagenomics, secondary metabolites produced by un-culturable members of natural bacterial populations can be explored and cloned in heterologous hosts and assayed for antimicrobial activity. When this approach was applied to screen soil DNA libraries for variant glycopeptide antibiotics, two novel biosynthetic gene clusters were found [48].

Conclusions

The contribution of genomics to our knowledge of antibiotics will expand owing to the dropping cost of next-generation sequencing technologies. This will have a multi-dimensional impact on various aspects of the antibiotic development field, including but not limited to new drug target identification, understanding the mechanism of antibiotic action, drug safety and efficacy assessment, bacterial resistance development, understanding the pathogenesis process, optimizing antibiotic biosynthetic process, and devising personalized treatments for specific instances of infectious disease (Figure 1). This combination will be a valuable asset in our endless battle against drug resistant bacterial infections.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Scholar EM, Pratt WB: *The Antimicrobial Drugs*. New York: Oxford University Press; 2000.

2. •• Fischbach MA, Walsh CT: **Antibiotics for emerging pathogens**. *Science* 2009, **325**:1089-1093.
This article provides a concise history of antibiotic development and introduces new strategies for novel antibiotic discovery, including exploring new microbial niches for natural products, using synthetic molecule libraries as antibiotics, and redesigning drug discovery screens to avoid rediscovering previously characterized antibiotics.

3. Mather R, Karenchak LM, Romanowski EG, Kowalski RP: **Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics**. *Am J Ophthalmol* 2002, **133**:463-466.

4. •• Mills SD: **When will the genomics investment pay off for antibacterial discovery?** *Biochem Pharmacol* 2006, **71**:1096-1102.
A comprehensive review on the promise of genomics for antibacterial discovery.

5. Herrmann DJ, Peppard WJ, Ledeboer NA, Theesfeld ML, Weigelt JA, Buechel BJ: **Linezolid for the treatment of drug-resistant infections**. *Expert Rev Anti Infect Ther* 2008, **6**:825-848.

6. Endimiani A, Blackford M, Dasenbrook EC, Reed MD, Bajaksouszian S, Hujer AM, Rudin SD, Hujer KM, Perreten V, Rice LB *et al.*: **Emergence of linezolid-resistant *staphylococcus aureus* after prolonged treatment of cystic fibrosis patients in cleveland, Ohio**. *Antimicrob Agents Chemother* 2011, **55**:1684-1692.

7. Sanchez Garcia M, De la Torre MA, Morales G, Pelaez B, Tolon MJ, Domingo S, Candel FJ, Andrade R, Arribi A, Garcia N *et al.*: **Clinical outbreak of linezolid-resistant *staphylococcus aureus* in an intensive care unit**. *JAMA* 2010, **303**:2260-2264.

8. Hayden MK, Rezai K, Hayes RA, Lolans K, Quinn JP, Weinstein RA: **Development of daptomycin resistance in vivo in methicillin-resistant *staphylococcus aureus***. *J Clin Microbiol* 2005, **43**:5285-5287.

9. Breithaupt H: **The new antibiotics**. *Nat Biotechnol* 1999, **17**:1165-1169.

10. Davies J: **Inactivation of antibiotics and the dissemination of resistance genes**. *Science* 1994, **264**:375-382.

11. Walsh C: **Where will new antibiotics come from?** *Nat Rev Microbiol* 2003, **1**:65-70.

12. Chalker AF, Lunsford RD: **Rational identification of new antibacterial drug targets that are essential for viability using a genomics-based approach**. *Pharmacol Ther* 2002, **95**:1-20.

13. Thanassi JA, Hartman-Neumann SL, Dougherty TJ, Dougherty BA, Pucci MJ: **Identification of 113 conserved essential genes using a high-throughput gene disruption system in *Streptococcus pneumoniae***. *Nucleic Acids Res* 2002, **30**:3152-3162.

14. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL: **Drugs for bad bugs: confronting the challenges of antibacterial discovery**. *Nat Rev Drug Discov* 2007, **6**:29-40.

15. • Brotz-Oesterhelt H, Sass P: **Postgenomic strategies in antibacterial drug discovery**. *Future Microbiol* 2010, **5**:1553-1579.

This is an excellent review that discusses how genomics was introduced and applied to antibacterial drug discovery, and to what extent it was successful. It also describes how drug discovery strategies have changed over time in the postgenomic era.

16. McDevitt D, Rosenberg M: **Exploiting genomics to discover new antibiotics**. *Trends Microbiol* 2001, **9**:611-617.

17. Zhang D, Jia J, Meng L, Xu W, Tang L, Wang J: **Synthesis and preliminary antibacterial evaluation of 2-butyl succinate-based hydroxamate derivatives containing isoxazole rings**. *Arch Pharm Res* 2010, **33**:831-842.

18. Gutierrez-Lugo MT, Bewley CA: **Susceptibility and mode of binding of the mycobacterium tuberculosis cysteinyl transferase mycothiol ligase to trna synthetase inhibitors**. *Bioorg Med Chem Lett* 2011, **21**:2480-2483.

19. Bi J, Wang H, Xie J: **Comparative genomics of nad(p) biosynthesis and novel antibiotic drug targets**. *J Cell Physiol* 2011, **226**:331-340.

20. Andrews-Polymenis HL, Santiviago CA, McClelland M: **Novel genetic tools for studying food-borne salmonella**. *Curr Opin Biotechnol* 2009, **20**:149-157.

21. Woodhouse SD, Narayan R, Latham S, Lee S, Antrobus R, Gangadharan B, Luo S, Schroth GP, Klenerman P, Zitzmann N: **Transcriptome sequencing, microarray, and proteomic analyses reveal cellular and metabolic impact of hepatitis c virus infection in vitro**. *Hepatology* 2010, **52**:443-453.

22. Langridge GC, Phan MD, Turner DJ, Perkins TT, Parts L, Haase J, Charles I, Maskell DJ, Peters SE, Dougan G *et al.*: **Simultaneous assay of every salmonella typhi gene using one million transposon mutants**. *Genome Res* 2009, **19**:2308-2316.

23. Rokem JS, Lantz AE, Nielsen J: **Systems biology of antibiotic production by microorganisms**. *Nat Prod Rep* 2007, **24**:1262-1287.

24. Komatsu M, Uchiyama T, Omura S, Cane DE, Ikeda H: **Genome-minimized streptomyces host for the heterologous expression of secondary metabolism**. *Proc Natl Acad Sci USA* 2010, **107**:2646-2651.

25. Challis GL: **Engineering *Escherichia coli* to produce nonribosomal peptide antibiotics**. *Nat Chem Biol* 2006, **2**:398-400.

26. Posfai G, Plunkett G 3rd, Feher T, Frisch D, Keil GM, Umenhoffer K, Kolisnychenko V, Stahl B, Sharma SS, de Arruda M *et al.*: **Emergent properties of reduced-genome *Escherichia coli***. *Science* 2006, **312**:1044-1046.

27. Sandegren L, Lindqvist A, Kahlmeter G, Andersson DI: **Nitrofurantoin resistance mechanism and fitness cost in *Escherichia coli***. *J Antimicrob Chemother* 2008, **62**:495-503.

28. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ: **A common mechanism of cellular death induced by bactericidal antibiotics**. *Cell* 2007, **130**:797-810.

29. Muthaiyan A, Silverman JA, Jayaswal RK, Wilkinson BJ: **Transcriptional profiling reveals that daptomycin induces the *Staphylococcus aureus* cell wall stress stimulon and genes responsive to membrane depolarization**. *Antimicrob Agents Chemother* 2008, **52**:980-990.

30. Almahmoud I, Kay E, Schneider D, Maurin M: **Mutational paths towards increased fluoroquinolone resistance in legionella pneumophila**. *J Antimicrob Chemother* 2009, **64**:284-293.

31. Goodarzi H, Hottes AK, Tavazoie S: **Global discovery of adaptive mutations**. *Nat Methods* 2009, **6**:581-583.

32. van Opijnen T, Bodi KL, Camilli A: **Tn-seq: high-throughput parallel sequencing for fitness and genetic interaction studies in microorganisms**. *Nat Methods* 2009, **6**:767-772.

33. Amini S, Goodarzi H, Tavazoie S: **Genetic dissection of an exogenously induced biofilm in laboratory and clinical isolates of *E. coli***. *PLoS Pathog* 2009, **5**:e1000432.

34. Girgis HS, Liu Y, Ryu WS, Tavazoie S: **A comprehensive genetic characterization of bacterial motility**. *PLoS Genet* 2007, **3**:1644-1660.

35. Girgis HS, Hottes AK, Tavazoie S: **Genetic architecture of intrinsic antibiotic susceptibility**. *PLoS ONE* 2009, **4**:e5629.

36. •• Lee HH, Molla MN, Cantor CR, Collins JJ: **Bacterial charity work leads to population-wide resistance**. *Nature* 2010, **467**:82-85.

This is a fascinating article that studies antibiotic resistance as a population-wide phenomenon. The authors identify a small number of highly resistant mutants in a bacterial population that induce antibiotic resistance at the population level at a fitness cost to themselves. Through transcriptional profiling, this behavior was linked to a signaling molecule.

37. Pene F, Courtine E, Cariou A, Mira JP: **Toward theragnostics**. *Crit Care Med* 2009, **37(1 Suppl)**:S50-S58.

38. Jinda S, Nakatani K, Nishioka J, Yasuda K, Soya Y, Hayashi A, Wada H, Nobori T: **Personalized treatment in the eradication therapy for *Helicobacter pylori***. *Int J Mol Med* 2011, **27**:255-261.

39. Xuan SH, Zhou YG, Shao B, Cui YL, Li J, Yin HB, Song XP, Cong H, Jing FX, Jin QH *et al.*: **Enzymic colorimetry-based DNA chip: a rapid and accurate assay for detecting mutations for clarithromycin resistance in the 23s rrna gene of *Helicobacter pylori***. *J Med Microbiol* 2009, **58(Pt 11)**:1443-1448.

40. Heath RJ, Rock CO: **A triclosan-resistant bacterial enzyme**. *Nature* 2000, **406**:145-146.

41. Zhu L, Lin J, Ma J, Cronan JE, Wang H: **Triclosan resistance of *Pseudomonas aeruginosa* pao1 is due to fabv, a triclosan-resistant enoyl-acyl carrier protein reductase**. *Antimicrob Agents Chemother* 2010, **54**:689-698.

42. Hornsey M, Loman N, Wareham DW, Ellington MJ, Pallen MJ, Turton JF, Underwood A, Gaulton T, Thomas CP, Doumith M *et al.*: **Whole-genome comparison of two *Acinetobacter baumannii* isolates from a single patient, where resistance developed during tigecycline therapy**. *J Antimicrob Chemother* 2011, **66**:1499-1503.

43. den Bakker HC, Cummings CA, Ferreira V, Vatta P, Orsi RH, Degoricija L, Barker M, Petrauskene O, Furtado MR, Wiedmann M: **Comparative genomics of the bacterial genus listeria: genome evolution is characterized by limited gene acquisition and limited gene loss**. *BMC Genomics* 2010, **11**:688.

44. Gilbert JA, Laverock B, Temperton B, Thomas S, Muhling M, Hughes M: **Metagenomics**. *Methods Mol Biol* 2011, **733**:173-183.

45. • Dethlefsen L, Huse S, Sogin ML, Relman DA: **The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16s rrna sequencing**. *PLoS Biol* 2008, **6**:e280.

This is one of the first papers that uses a genomics platform to look at the disruptive effect of antibiotics on the composition of bacterial communities in the human body.

46. Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, Viale A, Socci ND, van den Brink MR, Kamboj M, Pamer EG:

Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010, **120**:4332-4341.

47. Bloemendaal AL, Brouwer EC, Fluit AC: **Methicillin resistance transfer from staphylocccus epidermidis to methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy**. *PLoS ONE* 2010, **5**:e11841.

48. Banik JJ, Brady SF: **Cloning and characterization of new glycopeptide gene clusters found in an environmental DNA megalibrary**. *Proc Natl Acad Sci USA* 2008, **105**:17273-17277.