

REVIEW

Coping with antibiotic resistance: contributions from genomics

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Abstract

Antibiotic resistance is a public health issue of global dimensions with a significant impact on morbidity, mortality and healthcare-associated costs. The problem has recently been worsened by the steady increase in multiresistant strains and by the restriction of antibiotic discovery and development programs. Recent advances in the field of bacterial genomics will further current knowledge on antibiotic resistance and help to tackle the problem. Bacterial genomics and transcriptomics can inform our understanding of resistance mechanisms, and comparative genomic analysis can provide relevant information on the evolution of resistant strains and on resistance genes and cognate genetic elements. Moreover, bacterial genomics, including functional and structural genomics, is also proving to be instrumental in the identification of new targets, which is a crucial step in new antibiotic discovery programs.

Antibiotic resistance as a global public health problem

The advent of antibiotics for treating bacterial infections is considered one of the major advances in modern medicine. However, compared with other drugs, the lifetime of antibiotics for clinical use has been substantially limited by the phenomenon of antibiotic resistance. Failures of antimicrobial chemotherapy due to resistance were observed soon after the introduction of penicillin in clinical practice and, thereafter, the emergence of resistance has always followed the release of new antibiotics, posing a major clinical challenge and at the same time acting as a major driver for drug discovery and development in the field of antimicrobial agents.

Antibiotic resistance is now recognized as a major public health problem because of its magnitude and global scale. In fact, resistance does not affect only industrialized countries (where this is expected given the unlimited access to the antibiotic market) but also low-income settings, where resistance rates have been found to be even higher because of less well regulated antimicrobial policies and worse hygiene conditions and infection-control practices [1]. It is also well documented that resistance has a significant impact in terms of morbidity, mortality and healthcare-associated costs [2]. For instance, infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA, one of the most widespread and challenging resistant superbugs) were shown to be associated with an increased risk of mortality (almost twofold for bacteremias; more than threefold for surgical-site infections) in comparison with infections of the same type caused by methicillin-susceptible *S. aureus* (MSSA). There is also a positive correlation with the length of stay in hospital and the healthcare-associated costs of handling MRSA infections compared with MSSA infections [2].

Although antibiotic resistance continues to evolve at a steady pace and the spreading of resistant strains has been facilitated by increasing international travel and migration, most pharmaceutical companies have also recently chosen to restrict programs aimed at the discovery and development of new antibiotics. This has plunged the phenomenon of antibiotic resistance into a major crisis, with the increasing emergence of strains that are resistant to most or all antibiotics currently available for clinical use [3], which threatens to turn the clock back to the pre-antibiotic era.

Here, we briefly review and discuss how the recent advancement of bacterial genomic sciences can contribute to further current knowledge on resistance and to find new solutions to address this important problem.

The genetics of antibiotic resistance

Acquired antibiotic resistance can be due to a plethora of mechanisms (Table 1). These include, first, drug inactivation, for example by β -lactamases and aminoglycoside-modifying enzymes, which confer resistance to β -lactams and aminoglycosides, respectively; second, drug target

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Table 1. Examples of clinically relevant resistance mechanisms in major bacterial pathogens

Resistance mechanism	Molecular basis	Antibiotics affected	Relevant clinical pathogens
Drug inactivation	β -Lactamases	β -Lactams (variable spectrum, depending on the enzyme type)	Many Gram-positive and Gram-negative pathogens
	Aminoglycoside-modifying enzymes	Aminoglycosides (variable spectrum, depending on the enzyme type)	Many Gram-positive and Gram-negative pathogens
Target modification	Mutated DNA topoisomerases	Quinolones and fluoroquinolones	Many Gram-positive and Gram-negative pathogens
	Mutated RNA polymerase	Rifampin	<i>Staphylococcus aureus</i> , <i>Mycobacterium tuberculosis</i>
	Altered peptidoglycan (presence of D-Ala-D-Lac depsipeptide)	Glycopeptides	Enterococci
Target protection	23S ribosomal RNA methylation by Erm methylases	Macrolides	Streptococci, Staphylococci, anaerobes
	16S ribosomal RNA methylation by Arm/Rmt-like methylases	Aminoglycosides	Enterobacteriaceae
	Ribosomal protection by Tet proteins	Tetracyclines	Many Gram-positive pathogens
	Topoisomerase protection by Qnr proteins	Quinolones and fluoroquinolones	Enterobacteriaceae
Bypass of target function	Production of PBP2a, which takes over the functions of other PBPs	Most β -lactams	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)
Impermeability	Loss of OprD porin	Carbapenems	<i>Pseudomonas aeruginosa</i>
Drug efflux	Tet MFS-type pumps	Tetracyclines	Enterobacteriaceae, Staphylococci
	Upregulation of MexAB RND-type pump	Fluoroquinolones and most anti-pseudomonal β -lactams	<i>Pseudomonas aeruginosa</i>
	Upregulation of MexXY RND type pump	Fluoroquinolones, aminoglycosides, cefepime and meropenem	<i>Pseudomonas aeruginosa</i>

PBP: penicillin-binding-protein.

modification by mutation, such as DNA topoisomerase or RNA polymerase modifications, conferring resistance to quinolones and rifampicin, respectively; third, drug target protection, for example ribosomal methylation conferring resistance to aminoglycosides or macrolides, or topoisomerase protection by Qnr proteins conferring resistance to quinolones; fourth, drug target bypass, for example peptidoglycan synthesis by a novel penicillin-binding-protein, PBP2a, conferring resistance to β -lactams; fifth, impermeability, for example by loss of outer membrane porin channels, conferring resistance to carbapenems; and finally drug efflux, for example by Tet major facilitator superfamily (MFS)-type pumps, conferring resistance to tetracyclines, or by tripartite resistance-nodulation-cell division superfamily (RND)-type pumps, conferring a multidrug resistance phenotype. This diversity of resistance mechanisms reflects the broad repertoire of available agents, the multiplicity of their mechanisms of action, the biological diversity of bacteria, and the plasticity of their genomes. From a genetic standpoint, resistance mechanisms can arise either by mutation or by the acquisition and expression of heterologous genes encoding the resistance determinant through

horizontal gene transfer mechanisms such as conjugation, transformation or phage transduction.

Once acquired, the resistance determinants confer a selective advantage to the host when it is exposed to antibiotics, which can result in an expansion of the resistant clone relative to susceptible ones; this expansion can assume epidemic or even pandemic dimensions. Typical examples of resistant clones of Gram-positive pathogens that underwent an international dissemination, with remarkable clinical and epidemiological impact, include the MRSA strains belonging to clonal complexes (CCs) 5 and 8 [4], the glycopeptide-resistant *Enterococcus faecium* strains of CC17 [5] and the several penicillin-nonsusceptible pneumococcal clones spreading worldwide [6]. Similar examples of resistant clones of Gram-negative pathogens with high spreading potential include the *Escherichia coli* clone O25:H4-ST-131, which produces the CTX-M-15 extended-spectrum β -lactamase (ESBL) [7], the *Klebsiella pneumoniae* clone (KPC) ST-258, which produces acquired KPC-type carbapenemases [8], and the *Acinetobacter baumannii* derivatives of major European clone II, which produces oxacillin (OXA)-type carbapenemases [9].

Mobile genetic units, such as conjugative plasmids or transposons and lysogenic bacteriophages, can also be very successful in transferring across different strains, leading to a multi-clonal emergence of resistant strains. A typical example is represented by several conjugative plasmids encoding CTX-M-type ESBLs that were responsible for rapid and efficient dissemination of these resistance determinants, conferring resistance to the expanded-spectrum cephalosporins, among *E. coli* [10]. Other examples of mobile genetic elements that contributed significantly to horizontal dissemination of resistance genes are the large conjugative transposons and phage-like elements carrying macrolide resistance determinants of the *erm* and *mef* type, responsible for the dissemination of these resistance determinants among different clones of pneumococci and group A streptococci [11].

The role of genomics in surveillance and control of resistance

Tracing the epidemiology of resistant strains and resistance genes is of paramount importance for surveillance and control of antibiotic resistance, which can no longer rely on the simple phenotypic characterization of bacterial isolates. Molecular epidemiology is the discipline that studies the epidemiology of resistant strains and resistance genes by characterizing them at the molecular level, and it has provided major breakthroughs in the understanding of this phenomenon, with practical implications for resistance control strategies. The information provided by molecular analysis has a variable degree of resolution depending on the analytical tools, with genetic ones being the most versatile and of highest resolution. From this perspective, full genomic analysis of major resistant clones can be very informative for understanding their lifestyle and evolution and would be the golden standard for their comparison, as it was clearly shown with MRSA [12].

Genomic knowledge can also be instrumental to the development of sets of molecular probes for easy and specific identification of resistant clones of high spreading propensity and clinical impact, such as those described above, which is crucial in infection control practices. These probes can be used by reference laboratories or even by the largest diagnostic laboratories, in multiplexed amplification or DNA microarray technologies, for identification of such 'high risk' resistant clones involved in hospital or community outbreaks.

The importance of identifying high risk resistant clones and understanding their evolution explains the considerable effort that has recently been undertaken to sequence the genomes of multiresistant strains of bacterial pathogens [13], such as MRSA (eight projects), *Acinetobacter baumannii* (five projects), *Mycobacterium tuberculosis* (three projects) and *Pseudomonas aeruginosa*

(two projects), and also to sequence plasmids involved in the dissemination of important resistance determinants, such as ESBLs, AmpC-type β -lactamases or (quinolone resistance) Qnr proteins [14].

Genomics can inform our understanding of resistance mechanisms

Understanding resistance mechanisms to novel drugs is crucial in the process of discovery and development of antimicrobial drugs. Knowledge on newly emerging resistance mechanisms to antibiotics already available for clinical use is also of paramount importance for the prediction of resistance evolution, antibiotic policies and resistance surveillance and control strategies. Genome-scale investigations may provide relevant insights into unknown mechanisms of antibiotic resistance.

For instance, a comparative genomic analysis between *S. aureus* strains showing heterogeneous or homogeneous intermediate resistance to vancomycin (hVISA or VISA strains) and the susceptible parent strain has recently revealed the role of mutations of the genes encoding the *vraSR* and *graSR* two-component regulatory system in conferring this resistance phenotype, which is associated with clinical failures of glycopeptides [15]. A similar comparative genomic approach, carried out using array-based methodology, provided insights into mutational mechanisms that, in *S. aureus*, could be responsible for the emergence of reduced susceptibility to daptomycin, a new anti-Gram-positive antibiotic recently released for the treatment of staphylococcal infections [16]. Another genome-scale investigation that advanced our knowledge of resistance mechanisms was that by Breidenstein *et al.* [17], who, using a comprehensive *P. aeruginosa* mutant library, identified a number of new genes that, when inactivated, are associated with decreased susceptibility to fluoroquinolones in this species. These genes include *PA2047* and *PA3574*, which encode transcriptional regulators, the *nuo* genes, which encode NADH dehydrogenase I subunits, and several other genes encoding hypothetical proteins.

Transcriptomics at a genome-wide scale has also been used successfully to investigate resistance mechanisms. For example, transcription profiling following ciprofloxacin exposure has revealed an important role of the *mfd* gene, which encodes a transcription-repair coupling factor involved in strand-specific DNA repair, in the development of fluoroquinolone resistance in *Campylobacter jejuni* [18]. Transcriptome analysis of *S. aureus* treated with sublethal concentrations of cationic antimicrobial peptides has revealed a role for several genes, including the *vraSR* cell wall regulon, in the bacterial response to these agents, as well as a role of the *VraDE* putative ATP-binding cassette (ABC) transporter in conferring resistance to bacitracin [19].

Genomic knowledge and antibacterial drug discovery

Knowledge on bacterial genomes has progressed at a fast pace during recent years, with almost 1,500 completed bacterial genomes and more than 600 additional genome projects in progress at the beginning of 2010 [13]. Indeed, since its very beginning, the advent of bacterial genomics was not only regarded as a fascinating scientific tool, but also raised great hopes for renewing the golden era of antimicrobial discovery at a time when this is sorely needed because of the growing impact of bacterial resistance. The rationale behind this expectation was that comparative genomic analysis could reveal valuable information on bacterial genes that presumably encode proteins that are essential to survival or fitness of bacterial pathogens and do not have close eukaryotic counterparts; these proteins could then be potential targets for new antimicrobial agents.

This approach has been extensively pursued and has returned some potential targets for new antimicrobial agents that fulfilled the above criteria, including enzymes involved in biosynthetic pathways (for example coenzyme A, chorismate, lipid A and fatty acids), protein synthesis (such as aminoacyl tRNA synthetases and peptide deformylase), protein secretion (such as signal peptidase 1) and DNA replication (such as FtsZ/FtsA) [20,21]. Some two-component signal transduction systems have also been considered [20].

However, it was soon evident that, although identification of potential targets is important, a comprehensive understanding of bacterial biochemistry, physiology and pathogenicity are essential for exploiting this information for antimicrobial drug discovery. After the identification of the potential targets, there are several bottlenecks to be overcome in the process of developing new drugs; in particular, the need to set up high-throughput screening of banks of small molecules to obtain potential hits, which has prompted great efforts in the fields of functional and structural genomics [22]. Moreover, many screenings have turned out to be unproductive, or it was discovered that effective drugs could not be developed from the potential inhibitors [20].

This accounts for the overall dearth of new drugs discovered using genomic approaches that have made it into advanced clinical phases of the antibiotic pipeline after almost 15 years of efforts in this area. However, the natural products platensimycin and platenmycin, produced by *Streptomyces platensis*, have been discovered to be inhibitors of FabF and/or FabH (enzymes involved in bacterial type II fatty-acid biosynthesis) [23] and are now awaiting further preclinical studies, and some peptide deformylase inhibitors have already entered clinical trials [20,21]. Therefore, the potential of microbial genomics for discovery of new drugs should not be

underestimated. Indeed, a novel screening technique, combining chemical genomics with a yeast-based assay, has enabled the discovery of both a *P. aeruginosa* virulence-associated target (ExoS) and its inhibitor [24].

Finally, cloning and sequencing metagenomes from various ecological niches in which antibiotic-producing bacteria are expected to be present could reveal new antimicrobial synthetic gene clusters from unknown or uncultured bacteria. This strategy has already yielded some results such as the gene clusters for synthesis of turbomycins A and B, new glycopeptides and other compounds with antimicrobial activity cloned from environmental DNA (eDNA) libraries [25].

Concluding remarks

Antibiotic resistance is one of the greatest clinical challenges in the treatment of infectious diseases, given that it is a complex phenomenon whose emergence and evolution is still only partially understood. Bacterial genomics provides an invaluable contribution to furthering current knowledge on antibiotic resistance mechanisms and evolution, and on molecular epidemiology of resistance, which is instrumental to infection control practices. Moreover, information from functional and structural genomics has a key role in the identification of novel antimicrobial targets. The number of new agents discovered following this approach that are found in the advanced stages of the antibiotic pipeline is still very limited; however, the exploitation of bacterial genomics has been a major breakthrough for discovery and development of new antibiotics active against multiresistant pathogens.

Abbreviations

CC, clonal complex; ESBL, extended-spectrum β -lactamase; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors contributed to reviewing the relevant literature. MCT analyzed microbial genome databases. GMR wrote the article.

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