Antimicrobial resistance: a complex issue

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Summary

The discovery of antibiotics represented a turning point in human history. However, by the late 1950s infections that were difficult to treat, involving resistant bacteria, were being reported. Nowadays, multiresistant strains have become a major concern for public and animal health.

Antimicrobial resistance is a complex issue, linked to the ability of bacteria to adapt quickly to their environment. Antibiotics, and antimicrobial-resistant bacteria and determinants, existed before the discovery and use of antibiotics by humans. Resistance to antimicrobial agents is a tool that allows bacteria to survive in the environment, and to develop. Resistance genes can be transferred between bacteria by horizontal transfer involving three mechanisms: conjugation, transduction and transformation. Resistant bacteria can emerge in any location when the appropriate conditions develop.

Antibiotics represent a powerful selector for antimicrobial resistance in bacteria. Reducing the use of antimicrobial drugs is one way to control antimicrobial resistance; however, a full set of measures needs to be implemented to achieve this aim.

Keywords

Antimicrobial determinant – Antimicrobial resistance – Complexity – Dissemination – Resistance gene – Superbug.

Introduction

Once upon a time, the magic bullets made from arsenical derivatives were replaced with new compounds. Following the sulphonamides, penicillin came into use in human medicine in the 1940s and changed our lives. There is no need to provide details of the importance and the role of antibiotics in medicine. Selman Waksman discovered streptomycin in 1943 and this led to important findings related to the 'secondary metabolites' produced by actinomycetes. The golden era lasted 40 years; new classes of antibiotics were discovered, modifications of existing antibiotics were made, and synthetic components were constantly adjusted to combat emerging resistant bacteria and to improve the clinical qualities of existing antibiotics (6).

The discovery of antibiotics represented a turning point in human history. Besides being important for the treatment and control of infectious diseases in humans and domestic animals, antibiotics used for treatment and prophylaxis were critical to the success of complex surgery, intensive care, organ transplants, survival of the immunosuppressed and the elderly, and the production of food animals.

It is important to note that, since the discovery of antibiotics, the number of known pathogenic bacterial species has expanded continuously. In developed countries, people no longer die early in life because of meningitis, typhoid fever, or serious streptococcal, pneumococcal or anaerobic infections. However, large numbers of new pathogens have been discovered, *Staphylococcus aureus* infections have become more frequent, and the so-called opportunistic pathogens are commonly responsible for nosocomial and community-acquired infections. A number of opportunistic pathogens, such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*, are innately resistant to many antibiotics.

As mentioned by Davies and Davies, 'the word antibiotic describes a laboratory effect of a chemical compound, it does not define its natural function or a class of compound' (17). In a similar way, mechanisms of resistance are named with respect to the antibiotic targeted by the mechanism (e.g. penicillin, penicillinase). This name does not relate to

the natural function of that mechanism, its origin or its age with respect to the history of the bacterium. Antibiotics naturally have a variety of biological activities in addition to antibacterial activity.

Pre-existence of antimicrobial resistance determinants in microbiota and nature

Antimicrobial resistance determinants existed before the use of antibiotics in therapeutics. All bacterial species that are not included in the spectrum of activity (the list of susceptible bacterial species) of an antibiotic are by definition resistant to it. Consequently, these bacteria are able to proliferate in the presence of that antibiotic. The resistant species are referred to as 'intrinsically' or 'naturally' resistant, or preferably as 'resistant due to an innate mechanism'. Therefore the composition and the balance of any mixed bacterial population in an ecosystem will be changed by an antibiotic.

In addition to strains with innate resistance, resistance may pre-exist in susceptible species, linked to various mechanisms of cross-resistance. Broad-spectrum enzymes and efflux pumps with broad specificity are two mechanisms that govern resistance to multiple antibiotics and other chemicals. They may have been selected previously by antibiotics or chemicals other than the antibiotic under consideration (36).

Co-resistance refers to the linkage of resistance determinants on a common genetic element. In this case one antibiotic may select for the whole set of resistance genes. Furthermore, co-selection may involve resistance genes associated with non-antibiotic substances, which can be located on the same genetic element, e.g. heavy metals (mercury, zinc) or biocides (15).

Interaction of bacteria with antibiotics

The ability of bacteria to develop antimicrobial resistance is the adaptive response to antibiotic selective pressure and is part of the evolutionary aspect of microbial life. It occurs immediately, with any antibiotic, when the appropriate conditions are met (mixed bacterial populations, suboptimal antibiotic concentration, etc.). Between the start of the use of an antibiotic in treatment and the identification of bacterial resistance to this antibiotic, there is an inapparent period whose duration is related to several factors, in particular the pattern of usage, the amount of drug, and the bacterial species involved. It should also be highlighted that the identification of a new form of resistance is linked to the capability of existing surveillance programmes and the presence of a competent clinical microbiology laboratory.

There are several biochemical mechanisms by which a bacterial population can develop resistance to an antibiotic. Different mechanisms of resistance in a bacterial cell may cooperate and determine the final level of resistance. The mechanisms are usually grouped under six categories:

– active efflux, which prevents the antibiotic from reaching its target; many varieties of pump have been described

– reduced permeability, which occurs when the composition of the bacterial envelope is modified

– inactivation of the antibiotic by enzymes; these may alter the antibiotic within the bacterial cell or outside it

– alteration of the target of the antibiotic, reducing its affinity for it

– duplication of the drug target with a resistant form; this mechanism allows bypass of the sensitive target by use of the resistant one

– target amplification, which results from regulatory mutations that greatly increase the production of the target (40).

Origin of antimicrobial resistance determinants

The origin of resistance determinant genes was first associated with the antibiotic-producing microorganism and its need to protect itself when producing an antibiotic (29). In addition to that mechanism, there is a growing body of evidence showing that bacterial species with innate resistance, particularly environmental species, have the potential to mobilise and transfer resistance determinants to other bacteria. Some clinically relevant resistance genes have originated in environmental microbes (e.g. *Kluyvera*, *Shewanella*) (2, 5, 17, 33, 39). Recent studies using modern technology have demonstrated the existence of a vast environmental reservoir of resistance genes. Various environmental samples of different geological age have proved that resistance genes in the environment are much more numerous than those found in pathogens, and that they have existed for many thousands of years. The word 'resistome' refers to the population of resistant genes in nature (18, 48).

The large-scale use of antibiotics and other chemicals is responsible for the huge disposal of various substances in the biosphere. It is likely that, for the last 70 years, the anthropogenic environmental changes in microbiology and bacterial ecosystems have been much more important than imagined. Bacteria have responded to the massive outflow of antibiotics into the environment, and they continue to respond by a level of adaptation and evolution that demonstrates amazing genetic flexibility.

Evolutionary biology has provided some understanding of the extraordinary diversification and the swiftness of the adaptive changes of resistance mechanisms: the evolution of the β-lactamases is an impressive example (12, 37).

Resistance to an antibiotic can be acquired

The most frequent mechanism governing the acquisition of antibiotic resistance by a bacterial cell is the occurrence of horizontal gene transfer (HGT) between a resistant bacterial cell and a susceptible one. The process happens in a mixed population where antibiotic-resistant bacterial cells are in contact with susceptible bacteria. Environmental ecosystems and the gut microbiome (human or animal) are privileged places for HGT (7).

Bacterial cells exchange genes by three mechanisms:

– transduction
– transformation
– conjugation.

There is a large range of mobile genetic elements involved in HGT that are key to the evolution of bacteria. They are classified as integrative and conjugative elements (ICE) (7, 47).

It is interesting to note that treatment of an infection with an antibiotic does not expose the pathogen to HGT because there is no donor in the infection site (unless the infection occurs in the digestive tract). In contrast, HGT between commensal microorganisms can occur as a collateral effect of antibiotic treatment.

In rare clinical cases a pathogen that was susceptible at the onset of treatment can be isolated again during or at the end of the treatment period, but as a resistant form. Such an occurrence can be explained by two mechanisms:

a) the pathogen had an inducible mechanism of resistance such as an inducible cephalosporinase or an inducible resistance to macrolides and lincosamides

b) the patient was treated with an antibiotic for which the rate of mutation to resistance is high, ~10^{-9} or more (e.g. fusidic acid, rifampin, fluoroquinolones); in these cases the mutation affects the main target of the antibiotic.

Acquisition of resistance by a susceptible strain can be the result of HGT, the presence of various mutations, or both mechanisms.

Selection of a resistant bacterial population

From the starting point of its use to its final destruction, an antibiotic encounters many different ecosystems, and occurs in different concentrations, for variable periods of time.

Antibiotics excreted from humans or animals follow the path of waste water to rivers or treatment plant. They remain in soil, manure and/or water for a duration that depends on their stability in the local environmental conditions; they are removed by adsorption, complex formation and degradation (chemical and biological). Biological degradation is frequent because resistant bacteria are present all along the antibiotic journey (1, 10, 22, 23). Some of the resistant bacteria may even grow efficiently on antibiotic residues; moreover some of them can be antibiotic dependent. 'Subsistomes' refer to soil bacteria that inactivate and use antibiotics as nutrients (16, 17).

It is obvious that antibiotics are powerful selectors for resistant bacteria. The interaction between the bacterial population and an antibiotic varies according to the concentration of the antibiotic and the composition of the bacterial community. The pre-existing resistant bacterial populations are selected first. Subsequently, it is very likely that the surviving susceptible bacterial populations may acquire one or more determinants of resistance by HGT and/or by various mutations.

At a low level, antibiotics are stress agents; they trigger adaptive responses and the SOS system (an inducible DNA repair system). They favour HGT, activate prophages, modulate transcription in a dose-dependent manner and have a role as signalling molecules. They also activate efflux pumps (3, 5, 17, 20, 21, 27, 38). In a large range of concentrations, and especially at low concentrations, antibiotics are important for the enrichment and maintenance of resistance (25).

Bacteria may acquire resistance mechanisms that confer different levels of resistance. A bacterial population with a low level of resistance may have a survival advantage that confers on the population the ability to acquire a higher level of resistance, in a system that involves multiple steps. Such acquisition of resistance has been studied in

detail for the fluoroquinolones. The plasmid-mediated fluoroquinolone resistance confers a low level of resistance. Subsequent mutations may occur to give a higher level of resistance (42). Low-level resistance in bacteria can be difficult to detect using common testing methods.

The development of 'superbugs'

As early as the late 1950s, 'difficult to treat infections' were reported that involved bacteria resistant to the antibiotics available at the time. The recognition of multiple resistance in *Shigella* spp. isolated in Japan initiated studies of plasmid-mediated multiple resistance. The 1960s saw the expansion of the study of plasmids; *Salmonella* isolates from humans and other animals were found to harbour multiple resistance plasmids (14, 45). In 1987, integrons were identified. They are the critical intermediates in the acquisition and expression of resistance genes and play important roles in genome evolution (26, 35, 40).

Multidrug-resistant strains (which show resistance to three or more classes of antibiotics) have become a major concern, and they have more frequently involved nosocomial infections (in hospitals and veterinary hospitals) since the flow of antibiotic discovery has ceased. *Salmonella*, *Klebsiella*, *Escherichia coli* and meticillin-resistant staphylococci are the most feared pathogens, along with *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

It is possible that the number of resistance determinants that can occur in a bacterial cell is practically unlimited. Moreover, the presence of multiple resistance determinants addressing the same antibiotic is common (e.g. most Enterobacteria harbour two or more β-lactamases). Virulence function may be encoded on the same genomic island as resistance determinants.

Contrary to earlier belief, antibiotic resistance determinants can persist and become stable in resistant bacteria. It was assumed in the past that, because the acquisition of resistance was associated with a fitness cost to the microorganism, multidrug-resistant bacteria would be unstable. Recent studies suggest that resistant bacteria develop compensatory mutations, survive successfully and moreover may be able to acquire more resistance genes and mutations (43, 44). Systems that confer plasmid stability have been also described (e.g. the loss of suicide plasmids that destroy the bacterial cell). Bacteria with resistant determinants have been shown to persist in the absence of antibiotics (8, 19, 31). Reversion to susceptibility is extremely rare. In the absence of selection pressure, the prevalence of resistant strains tends to decrease slowly, but this does not indicate reversion.

For this reason, control of pathogen transmission is essential. Cessation or a decrease in the use of a specific antibiotic will modify the balance between resistant and susceptible strains, slowly reducing the number of resistant bacteria (as the selection pressure decreases). However, resistant bacteria will still be present and can increase again.

'Superbugs' are the product of an astounding bacterial evolutionary process that started with the use of antibiotics 70 years ago. Those natural secondary metabolites that bacteria had produced and handled for four billion years were used at a colossal level to improve the health of humans and domestic animals, and they have changed our microbiosphere. The evolution and adaptation of bacteria to the use of antibiotics as therapeutic agents are unfolding in front of us, and are challenging our understanding.

Dissemination of bacteria and their mobile gene elements

Resistant bacterial populations are a component of the large number of bacterial communities (the microbiota) that are distributed in various ecosystems. Bacteria are the oldest form of life, the most numerous and the most diverse. They are everywhere, all around, over and inside every living being, in soil, air and water. With no borders between ecosystems, continuous bacterial exchange is the normal situation (4, 32).

The topography of bacterial dissemination is linked to human life, activity and travel; animals and the food trade; wild animals; migration; transportation; the flow of water and the wind. The magnitude of dissemination and transmission of resistant bacteria is variable; it increases in large and concentrated communities and where antibiotics offer an advantage for their proliferation. It is not surprising that resistant bacteria are everywhere, even in remote places (46). As mentioned previously, resistant bacteria can emerge in any location in which appropriate conditions are met. It is likely that two concomitant mechanisms interact: local emergence and dissemination from distant locations (41).

Recent studies have employed epidemiological analyses, combining molecular markers and resistance profiles with the identification of communities and clusters. Study of the temporal and geographical distributions of resistant bacteria gives the basis for interventions to control them (24, 34).

Some resistant bacteria and resistance determinants are more successful than others. Clonal epidemics of highly transmissible strains are well known. Meticillin-resistant

Staphylococcus aureus and penicillin-resistant pneumococci are examples of the global dissemination of specific clonal complexes (28, 30). More mysterious is the dissemination of certain resistance determinants. Among the many β-lactamases, the CTX-M family has become the most successful worldwide (13). In contrast, penicillinase-producing *Streptococcus faecalis* has not been able to expand. Epidemiological analysis of antibacterial resistance requires cooperation among countries and an approach that integrates research on humans and on other animals.

The genetic structure of a mobile element accounts for its expression and the success of the determinant, as well as for the frequency of HGT. Horizontal gene transfer is a universal property of genes; it occurs naturally with a higher frequency than observed under laboratory conditions. Antibiotics may increase the rate of HGT. More studies are needed on the factors that facilitate and increase HGT in soil, water and in the gastrointestinal tracts of animals and humans. Such studies will contribute to the developement of better strategies to deal with the expansion of antimicrobial resistance.

The complexity of the resistance problem

The control of antimicrobial resistance has recently become a health priority in many countries. There is overwhelming evidence that antibiotic use has been a powerful selector of resistance. Antibiotics are also a powerful evolutionary force in the microbiosphere. Bacterial populations with multiple forms of resistance, and cross-resistance between antibiotics and many other chemicals, are extremely diverse and their resistance is driven by several selectors.

It is unknown whether control of, and reduction in, antibiotic use will improve the situation. Although the evolution of bacteria is not likely to be reversible, it is thought that improvement is possible. It may be possible at least to minimise the incidence and the transmission of resistant bacteria, provided that the other interventions listed below are also achieved and implemented.

Restrictions, and rules for the application of antibiotics in humans and in domestic animals, must be supported concomitantly by:

– discovery and development of new antibiotics and, as discussed recently, other compounds able to affect the ecology and evolution of bacterial populations (9)

– improvement of rapid methods for diagnosis of infections

– control of bacterial transmission within defined communities (day care centres, nursing homes, hospitals)

– control of the release of antibiotics and other chemicals from cities, hospitals, factories, farms, etc., to nature

– control of the dissemination of resistant bacteria, not only in hospitals but also along privileged pathways, e.g. from waste water to drinking water, and from food-processing plants to the table

– identification of the reservoir of resistant clones in and on living beings, as well as those found in the environment; information about their communities, diversity, and geographical distribution should be part of the surveillance programme.

The task is immense, and collaboration should be encouraged among all disciplines concerned by the problem in order to empower decisions with sound science (4, 11, 17, 32). The danger is to human health. The problem is no longer limited to medical science; it has come to involve the whole of society: industry, urban architects, a variety of engineers, economists, regulatory agencies, politicians, the general public, consumers, etc. Research is essential and should bridge microbiology, evolutionary biology, epidemiology, ecology and sociology.

Recently, at the conclusion of a lecture, Stuart Levy proposed that we should 'make peace with bacteria' (Infectious Disease Society of Massachusetts, Boston, 2012). We have to coexist with bacteria (even resistant forms), there is no other choice. Living in harmony with bacteria requires continual study and an increase in knowledge, in order to be able to control their dissemination when and where necessary, and to continue to use antibiotics and vaccines for the sake of human and animal health.

■

L'antibiorésistance : une question complexe

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Résumé
La découverte des antibiotiques a représenté un véritable tournant dans l'histoire de l'humanité. Toutefois, dès la fin des années 50, des rapports ont fait état d'infections difficiles à traiter, impliquant des bactéries résistantes. À l'heure actuelle, les souches multirésistantes sont un sujet majeur de préoccupation en santé animale et en santé publique.
L'antibiorésistance est une question complexe, liée à la capacité des bactéries de s'adapter rapidement à leur environnement. Les antibiotiques, tout comme les bactéries résistantes aux antimicrobiens et les déterminants de cette résistance existaient bien avant la découverte des antibiotiques et leur utilisation par l'homme. La résistance aux agents antimicrobiens est un mécanisme grâce auquel les bactéries peuvent survivre dans leur environnement et s'y développer. Les gènes codant pour la résistance peuvent être transférés d'une bactérie à l'autre de manière horizontale, au moyen de trois mécanismes : la conjugaison, la transduction et la transformation. Des bactéries résistantes peuvent émerger en tout lieu, dès lors que les conditions appropriées sont réunies.
Les antibiotiques constituent un facteur puissant de sélection de la résistance aux antimicrobiens chez les bactéries. La réduction de l'utilisation des antibiotiques est l'un des moyens permettant de maîtriser la résistance à ces agents ; néanmoins, cet objectif requiert la mise en œuvre d'un large éventail de mesures appropriées.

Mots-clés
Antibiorésistance – Bactérie multirésistante aux antibiotiques – Complexité – Déterminant de l'antibiorésistance – Dissémination – Gène codant pour la résistance.

La resistencia a los agentes antimicrobianos, un tema complejo

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Resumen
El descubrimiento de los antibióticos supuso un punto de inflexión en la historia del hombre. Sin embargo, a finales de los años cincuenta ya se empezaron a describir infecciones difíciles de tratar, causadas por bacterias resistentes. Hoy en día, las cepas multirresistentes constituyen un grave problema de salud pública y sanidad animal.
La resistencia a los agentes antimicrobianos es un tema complejo, ligado a la capacidad de las bacterias de adaptarse rápidamente a las condiciones ambientales. Los antibióticos, y por ende las bacterias resistentes y los factores determinantes de la resistencia, ya existían antes de que el ser humano los descubriera y utilizara. La resistencia es una herramienta de la que se sirven las bacterias para sobrevivir y reproducirse en el medio. Los genes que la confieren pueden transmitirse de un organismo a otro por un mecanismo de transferencia horizontal en el que intervienen tres procesos: conjugación, transducción y transformación. En cualquier lugar, cuando las condiciones son propicias, pueden surgir bacterias resistentes.

Los antibióticos representan un poderoso factor de selección de bacterias resistentes a los antimicrobianos. Una forma de contener las resistencias es reducir el uso de esos fármacos. Tal objetivo, sin embargo, pasa por la aplicación de un arsenal completo de medidas.

Palabras clave
Complejidad – Diseminación – Factor determinante de resistencia a los agentes antimicrobianos – Gen de resistencia – Resistencia a los agentes antimicrobianos – Superbacteria.

References

1. Akiyama T. & Savin M.C. (2010). – Populations of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream. *Sci. Total Environ.*, **408** (24), 6192–6201.

2. Allen H.K., Donato J., Huimi Wang H., Cloud-Hansen K.A., Davies J. & Handelsman J. (2010). – Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.*, **8**, 251–259.

3. Allen H.K., Looft T., Bayles D.O., Humphrey S., Levine U.Y., Alt D. & Stanton T.B. (2011). – Antibiotics in feed induce prophages in swine fecal microbiomes. *Am. Soc. Microbiol.*, **2** (6). doi: 10.1128/mBio.00260-11.

4. American Academy of Microbiology (2009). – Antibiotic resistance: an ecological perspective on an old problem (Chairs: J. Acar & J. Davies). *In* Colloquium of the AAM. American Society for Microbiology (ASM) Press, Washington DC, United States of America.

5. Aminov R.I. (2009). – The role of antibiotics and antibiotic resistance in nature. *Environ. Microbiol.*, **11** (12), 2970–2988.

6. Aminov R.I. (2010). – A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.*, **1**, 134.

7. Aminov R.I. (2011). – Horizontal gene exchange in environmental microbiota. *Front. Microbiol.*, **2**, 158.

8. Andersson D.I. & Hughes D. (2011). – Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol. Rev.*, **35** (5), 901–911.

9. Baquero F., Coque T.M. & de la Cruz F. (2011). – Ecology and evolution as targets: the need for novel eco-evo drugs and strategies to fight antibiotic resistance. *Antimicrob. Agents Chemother.*, **55** (8), 3649–3660.

10. Baquero F., Martinez J.L. & Canton R. (2008). – Antibiotics and resistance in water environments. *Curr. Opin. Biotechnol.*, **19** (3), 260–265.

11. Bush K., Courvalin P., Dantas G., Davies J., Eisenstein B., Huovinen P., Jacoby G.A., Kishony R., Kreiswirth B.N., Kutter E., Lerner S.A., Levy S., Lewis K., Lomovskaya O., Miller J.H., Mobashery S., Piddock L.J., Projan S., Thomas C.M., Tomasz A., Tulkens P.M., Walsh T.R., Watson J.D., Witkowski J., Witte W., Wright G., Yeh P. & Zgurskaya H.I. (2011). – Tackling antibiotic resistance. *Nat. Rev. Microbiol.*, **9** (12), 894–896.

12. Bush K. & Fisher J.F. (2011). – Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. *Annu. Rev. Microbiol.*, **65**, 455–478.

13. Cantón R. & Coque T.M. (2006). – The CTX-M beta-lactamase pandemic. *Curr. Op. Microbiol.*, **9** (5), 466–475.

14. Chabbert Y.A. & Baudens J.G. (1965). – Transmissible resistance to six groups of antibiotics in *Salmonella* infections. *Antimicrob. Agents Chemother.*, **5**, 380–383.

15. Ciric L., Mullany P. & Roberts A.P. (2011). – Antibiotic and antiseptic resistance genes are linked on a novel mobile genetic element: Tn6087. *J. antimicrob. Chemother.*, **66** (10), 2235–2239.

16. Dantas G., Sommer M.O., Oluwasegun R.D. & Church G.M. (2008). – Bacteria subsisting on antibiotics. *Science*, **320** (5872), 100–103.

17. Davies J. & Davies D. (2010). – Origins and evolution of antibiotic resistance. *Microbiol. molec. Biol. Rev.*, **74** (3), 417–433.

18. D'Costa V.M., King C.E., Kalan L., Morar M., Sung W.W., Schwarz C., Froese D., Zazula G., Calmels F., Debruyne R., Golding G.B., Poinar H.N. & Wright G.D. (2011). – Antibiotic resistance is ancient. *Nature*, **477** (7365), 457–461.

19. Depardieu F., Podglajen I., Leclercq R., Collatz E. & Courvalin P. (2007). – Modes and modulations of antibiotic resistance gene expression. *Clin. Microbiol. Rev.*, **20** (1), 79–114.

20. El Garch F., Lismond A., Piddock L.J., Courvalin P., Tulkens P.M. & Van Bambeke F. (2010). – Fluoroquinolones induce the expression of patA and patB, which encode ABC efflux pumps in *Streptococcus pneumoniae*. *J. antimicrob. Chemother.*, **65** (10), 2076–2082.

21. Fajardo A. & Martinez J.L. (2008). – Antibiotics as signals that trigger specific bacterial responses. *Curr. Op. Microbiol.*, **11** (2), 161–167.

22. Fick J., Söderström H., Lindberg R.H., Phan C., Tysklind M. & Larsson D.G. (2009). – Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ. Toxicol. Chem.*, **28** (12), 2522–2527.

23. Girlich D., Poirel L., Szczepanowski R., Schlüter A. & Nordmann P. (2012). – Carbapenem-hydrolyzing GES-5-encoding gene on different plasmid types from a sewage treatment plant. *Appl. environ. Microbiol.*, **78** (4), 1292–1295.

24. Grundmann H., Aanensen D.M., van den Wijngaard C.C., Spratt B.G., Harmsen D., Friedrich A.W. *et al*. (2010). – Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med.*, **7** (1), e1000215.

25. Gulberg E., Cao S., Berg O.G., Ilbäck C., Sandegren L., Hughes D. & Andersson D.I. (2011). – Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.*, **7** (7), e1002158.

26. Hall R.M. & Collis C.M. (1995). – Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Molec. Microbiol.*, **15** (4), 593–600.

27. Hastings P.J., Rosenberg S.M. & Slack A. (2004). – Antibiotic-induced lateral transfer of antibiotic resistance. *Trends Microbiol.*, **12** (9), 401–404.

28. Hermans P.W., Sluijter M., Dejsirilert S., Lemmens N., Elzenaar K., van Veen A., Goessens W.H. & de Groot R. (1997). – Molecular epidemiology of drug-resistant pneumococci: toward an international approach. *Microb. Drug. Resist.*, **3** (3), 243–251.

29. Hopwood D.A. (2007). – How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? *Molec. Microbiol.*, **63** (4), 937–940.

30. Lencastre H. de, Oliveira D. & Tomasz A. (2007). – Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. *Curr. Op. Microbiol.*, **10** (5), 428–435.

31. Levin B.R., Perrot V. & Walker N. (2000). – Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics*, **154** (3), 985–997.

32. Marshal B.M. & Levy S.B. (2011). – Food animals and antimicrobials: impacts on human health. *Clin. Microbiol. Rev.*, **24** (4), 718–733.

33. Martinez J.L. (2008). – Antibiotics and antibiotic resistance genes in natural environments. *Science*, **321** (5887), 365–367.

34. Mather A.E., Matthews L., Mellor D.J., Reeve R., Denwood M.J., Boerlin P., Reid-Smith R.J., Brown D.J., Coia J.E., Browning L.M., Haydon D.T. & Reid S.W. (2011). – An ecological approach to assessing the epidemiology of antimicrobial resistance in animal and human populations. *Proc. biol. Sci.*, **16**, doi: 10.1098/rspb.2011.1975.

35. Mazel D. (2006). – Integrons: agents of bacterial evolution. *Nat. Rev. Microbiol.*, **4** (8), 608–620.

36. Nikaido H. & Pagès J.M. (2011). – Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiol. Rev.*, doi: 10.1111/j.1574-6976.2011.00290.x.

37. Nordmann P., Naas T. & Poirel L. (2011). – Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg. infect. Dis.*, **17** (10), 1791–1798.

38. Piddock L.J. (2006). – Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin. Microbiol. Rev.*, **19** (2), 382–402.

39. Potron A., Poirel L. & Nordmann P. (2011). – Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in *Shewanella xiamenensis*. *Antimicrob. Agents Chemother.*, **55** (9), 4405–4407.

40. Rowe-Magnus D. & Mazel D. (2006). – The evolution of antibiotic resistance. *In* Evolution of microbial pathogens (H.S. Seifert & V.J. Dirita, eds), ASM Press, Washington, DC, 221–241.

41. Stokes H.W. & Gillings M.R. (2011). – Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. *FEMS Microbiol. Rev.*, **35** (5), 790–819.

42. Strahilevitz J., Jacoby G.A., Hooper D.C. & Robicsek A. (2009). – Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin. Microbiol. Rev.*, **22** (4), 664–689.

43. Trindade S., Sousa A., Xavier K.B., Dionisio F., Ferreira M.G. & Gordo I. (2009). – Positive epistasis drives the acquisition of multidrug resistance. *PLoS Genet.*, **5** (7), e1000578.

44. Walsh T.R. (2006). – Combinatorial genetic evolution of multiresistance. *Curr. Opin. Microbiol.*, **9** (5), 476–482.

45. Watanabe T. (1961). – Episome-mediated transfer of drug resistance in Enterobacteriaceae. *J. Bacteriol.*, **81**, 668–678.

46. Woerther P.L., Angebault C., Lescat M., Ruppé E., Skurnik D., Mniai A.E., Clermont O., Jacquier H., Costa A.D., Renard M., Bettinger R.M., Epelboin L., Dupont C., Guillemot D., Rousset F., Arlet G., Denamur E., Djossou F. & Andremont A. (2010). – Emergence and dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* in the community: lessons from the study of a remote and controlled population. *J. infect. Dis.*, **202** (4), 515–523.

47. Wozniak R.A. & Waldor M.K. (2010). – Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat. Rev. Microbiol.*, **8** (8), 552–563.

48. Wright G.D. (2010). – Antibiotic resistance in the environment: a link to the clinic. *Curr. Opin. Microbiol.*, **13** (5), 589–594.