Accepted Manuscript

Title: Resistance to rifampicin: at the crossroads between ecological, genomic and medical concerns

Authors: Audrey Tupin, Maxime Gualtieri, Françoise Roquet-Banères, Zakia Morichaud, Konstantin Brodolin, Jean-Paul Leonetti

PII: S0924-8579(10)00017-8
DOI: doi:10.1016/j.ijantimicag.2009.12.017
Reference: ANTAGE 3220

To appear in: *International Journal of Antimicrobial Agents*

Received date: 10-12-2009
Accepted date: 22-12-2009

Please cite this article as: Tupin A, Gualtieri M, Roquet-Banères F, Morichaud Z, Brodolin K, Leonetti J-P, Resistance to rifampicin: at the crossroads between ecological, genomic and medical concerns, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.12.017

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Resistance to rifampicin: at the crossroads between ecological, genomic and medical concerns

Audrey Tupin ^{a,b,*}, Maxime Gualtieri ^{a,b}, Françoise Roquet-Banères ^{a,b}, Zakia Morichaud ^{a,b}, Konstantin Brodolin ^{a,b}, Jean-Paul Leonetti ^{a,b}

^a *Université Montpellier 1, Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé (CPBS), CNRS, UMR 5236, 4 Bd Henri IV, CS 69033, F-34965 Montpellier, Cedex 2, France*

^b *Université Montpellier 2, CPBS, F-34095 Montpellier, France*

ARTICLE INFO

Article history:

Received 10 December 2009

Accepted 22 December 2009

Keywords:

Rifampicin resistance

Antibiotic

Transcription

Bacterial ecology

* Corresponding author. Tel.: +33 4 67 54 86 07; fax: +33 4 67 54 86 04.

E-mail address: audrey.tupin@univ-montp1.fr (A. Tupin).

Accepted Manuscript

ABSTRACT

The first antibiotic of the ansamycin family, rifampicin (RIF), was isolated in 1959 and was introduced into therapy in 1962; it is still a first-line agent in the treatment of diseases such as tuberculosis, leprosy and various biofilm-related infections. The antimicrobial activity of RIF is due to its inhibition of bacterial RNA polymerase (RNAP). Most frequently, bacteria become resistant to RIF through mutation of the target; however, this mechanism is not unique. Other mechanisms of resistance have been reported, such as duplication of the target, action of RNAP-binding proteins, modification of RIF and modification of cell permeability. We suggest that several of these alternative resistance strategies could reflect the ecological function of RIF, such as autoregulation and/or signalling to surrounding microorganisms. Very often, resistance mechanisms found in the clinic have an environmental origin. One may ask whether the introduction of the RIF analogues rifaximin, rifalazil, rifapentine and rifabutin in the therapeutic arsenal, together with the diversification of the pathologies treated by these molecules, will diversify the resistance mechanisms of human pathogens against ansamycins.

1. Introduction

Ansamycin derivatives such as rifampicin (RIF) are first-line antibiotics in the treatment of tuberculosis (TB), leprosy and a growing number of Gram-positive bacteria such as multidrug-resistant *Staphylococcus aureus*. Ansamycin was isolated in 1959 by Sensi et al. [1,2] from a strain of *Amycolatopsis mediterranei* (previously known as *Streptomyces mediterranei* and *Nocardia mediterranei*) and was introduced into therapy in 1962.

Ansamycins are active against a large variety of organisms, including bacteria and eukaryotes. They have a broad activity against Gram-positive bacteria and, to a lesser extent, against Gram-negative bacteria. Interestingly, ansamycins are also active against the transcription machinery of the eukaryotic parasite *Plasmodium falciparum* [3,4].

Ansamycins are structurally characterised by a planar naphthoquinone ring in which positions 3 and 4 have been extensively modified by hemisynthesis to yield commercial antibiotics such as RIF. Interest in these molecules has recently increased with the US Food and Drug Administration (FDA) approval of several new ansamycin derivatives for treatment of a broader range of infectious diseases. For now, the ansamycins currently in therapeutic use are RIF, rifaximin, rifapentine, rifalazil and rifabutin.

The antimicrobial activity of ansamycins is due to their inhibition of bacterial RNA polymerase (RNAP). RIF binds to conserved amino acids in the active centre of the enzyme and blocks transcription initiation. A large proportion of the encountered resistance to RIF is due to mutations of these amino acids. These mutations often occur

with high frequency, which compels the use of RIF almost exclusively in drug combinations, most commonly with isoniazid, as well as the restriction of its use to the treatment of TB and to emergencies.

Resistance to RIF by mutation of the target is widespread but not unique. Other mechanisms of resistance have been reported, such as duplication of the target, action of RNAP-binding proteins, modification of RIF and modification of cell permeability (Table 1). Bacteria and fungi are not isolated in nature, but exist in communities and need to communicate to be competitive. A majority of the low molecular weight compounds synthesised by microbes at low concentrations apparently act as cell-signalling molecules and directly or indirectly modulate transcription to favour symbiosis between these microorganisms [5]. As expected from a key player in the bacterial signalling network, RNAP is targeted by a variety of antibiotics that contain similar binding sites to RIF [6], and bacteria have developed highly diverse strategies to grow in the presence of these antibiotics. Some of these mechanisms confer resistance to very high levels of RIF, whilst others only protect the bacteria against lower concentrations. These natural resistances cast light on the potential evolution of resistance to RIF and on other new transcription inhibitors in clinical development in the future.

2. Resistance by mutation of the target

Many RIF-resistant (RIF^R) strains of *Escherichia coli*, *S. aureus*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, *Mycobacterium avium*, *Mycobacterium leprae*, etc. have been characterised, showing that resistance is most frequently conferred by mutations in

the RNAP active centre. RIF interacts only with the β -subunit of RNAP encoded by *rpoB* [7] and its binding site is highly conserved among bacteria (Fig. 1). Binding of the molecule to RNAP involves 12 amino acid residues (Fig. 2). Mutagenesis of each of these residues, except one, generates a resistant phenotype [7]. Spontaneous resistance to RIF is mainly associated with single point mutations resulting in amino acid substitutions and is less frequently associated with a few insertions or deletions. Ninety-five percent of these mutations map to four regions in the *N*-terminal half of the β -subunit polypeptide [8] involved in the binding of RIF. In *E. coli*, RIF resistance mutations are usually located in the central region of the polypeptide, within cluster I (amino acids 507–533), cluster II (amino acids 563–572) and cluster III [9,10], and they can also occur near the *N*-terminus of the β -subunit [11]. In *M. tuberculosis*, resistance to RIF is mainly associated with mutations in an 81-bp hot-spot region (codons 432–458 in cluster I) of *rpoB* [12], with 41% of resistant clinical isolates carrying a mutation of S455, 36% with a mutation of H440 and 9% with a mutation of D430 [13]. A mutation at the beginning of the *rpoB* gene, the amino terminal of cluster I, has also been reported (V176F) [14,15]. This mutation confers high-level resistance to RIF and may account for more than 1% of all RIF^R strains [16]. RIF resistance mutations have also been found in the *N*-terminus (V135F) and in cluster I (Q469K, Q469L, Q469R, H482D, H482P, H482R, H482Y, S487F, S487L and S487Y) of *B. subtilis* *rpoB* [17] as well as in *Helicobacter pylori* (V149F) [16].

In most cases, a point mutation in *rpoB* only confers RIF resistance, but recently a point mutation was shown to confer resistance to and dependence on RIF [18]. The molecular

basis of this effect is not yet understood, but the finding itself casts doubt on the historical view that RIF acts by plugging the path of the elongating RNA as mentioned by Campbell et al. [7].

Resistance to transcription inhibitors that arise through point mutations of the target is not neutral for bacteria. In the case of RIF, these alterations in the target were repeatedly involved in deregulation of gene expression [19–23], possibly due to altered *rho*-dependent transcription termination [23]. A striking example is the recent report that many alterations in substrate utilisation patterns were observed in *B. subtilis* RIF^R mutants [17]. The consequences of this deregulation are, in most cases, reduced virulence [24,25] of the RIF^R mutants and a reduced fitness [26,27]. More prolonged treatment with RIF can result in strains with no fitness defect owing to compensatory mutations, and these strains with low- or no-cost resistance mutations are also the most frequent among clinical isolates [26].

3. Resistance by duplication of the target

Several bacterial species are naturally resistant to RIF owing to mutations in *rpoB*. *Nocardia* sp. are Gram-positive saprophytes present in the environment, with several species causing severe infections. *Nocardia farcinica* is an opportunistic pathogen that is considered a major multidrug resistance problem that is particularly frequent in Japan [28] and Europe [29,30]. The genome of *N. farcinica* IFM 10152 was recently sequenced [31] and the presence of two different RNAP β -subunit genes, *rpoB* and *rpoB2*, was detected. These two genes share a high degree of homology (88.8%) and careful

comparison of their amino acid sequences revealed that the *Nocardia rpoB2* protein contains amino acid substitutions in RIF clusters I and II that are likely to confer RIF resistance [32]. The role of the altered gene in conferring resistance was confirmed by knockout of *rpoB2*, shedding light on a novel resistance mechanism. Such a mechanism may not be a rare event, as *rpoB* duplication is apparently widespread in *Nocardia* sp. and has recently been found in another closely related actinomycete, *Actinomadura* sp. strain ATCC 39727 [33]. It is not yet known whether the actinomycete *A. mediterranei*, which produces RIF, uses this strategy of duplication to resist this antibiotic. RIF mutations are known to affect gene expression and may result in an increase in antibiotic production in *Streptomyces* [21] and a change in carbon source assimilation in *B. subtilis* [34]. In light of these findings, the abovementioned duplications of mutated *rpoB* could either reflect signalling mechanisms between *N. farcinica* or *Actinomadura* sp. and other bacteria producing molecules sharing the same binding site as RIF, or the presence of dormant genes coding for molecules targeting transcription in their genomes.

4. Resistance induced by RNA polymerase-binding proteins

Although most cases of RIF resistance are due to mutations in the *rpoB* gene, some bacteria use other strategies. The actinomycete *Streptomyces coelicolor* is intrinsically resistant to low concentrations of RIF, and recently Newell et al. [35] showed that an RNAP-binding protein, RbpA, confers basal levels of RIF resistance to this microorganism. Experiments have demonstrated that *rbpA* expression is induced by low concentrations of RIF and that this molecule inhibited a promoter directing the

transcription of a ribosomal RNA operon. In vitro experiments confirmed that RIF induces σ^R -dependent transcription of the *rbpA* gene and that RbpA stimulates σ^{HrdB} -dependent transcription from *rrnDp3*. The data presented also suggest that RbpA confers basal levels of resistance to RIF and that it is a novel regulator of rRNA synthesis in *S. coelicolor*. The authors point out that genes homologous to *rbpA* are present in *M. tuberculosis*, *M. leprae* and *Corynebacterium diphtheriae*. These genes could account for the transcriptional activity detected in *M. tuberculosis* following treatment with RIF [36].

DnaA is another RNAP-binding protein that partially protects RNAP from the effect of RIF. Transcription and replication are intricate mechanisms [37], since RNA is used as a primer for replication. The RNAP located at a replication origin provides the transcription activity necessary for initiation of replication and is protected from the action of RIF by origin-bound DnaA. In vitro experiments showed that the DnaA protein interacts directly with RNAP, and both the ATP-form and the ADP-form of the DnaA protein were found to alleviate the inhibition of RNAP by RIF at the *dnaA* promoter, although a higher concentration was necessary to achieve the effect when the ADP-form of DnaA was used [38].

σ factors are accessory proteins that bind to the core of RNAP to form the RNAP holoenzyme. Binding of the σ factor allows transcription initiation by directing specific recognition of the promoter and isomerisation of the RNAP–DNA complex. Wegrzyn et al. [39] have reported a differential effect on the inhibition of transcription by RIF

depending on the σ factor. RIF was shown to inhibit transcription from the σ^{70} -dependent P_L promoter at a significantly lower concentration than transcription from the σ^{32} -dependent P_{groE} promoter. These results may only reflect a selectivity of RIF according to the nature of the σ factor bound to the core of RNAP and are probably more related to a response to stress rather than a true resistance.

5. Resistance by modification of rifampicin

Another strategy of resistance is to neutralise the antibiotic by covalent modification. Bacteria can inactivate RIF in a variety of ways, such as glucosylation, ribosylation, phosphorylation and decolorisation (Fig. 3). *Mycobacterium smegmatis* DSM 43756, a pathogen intrinsically resistant to this drug, inactivates RIF by ribosylation [41]. Structural studies have shown that the final product is obtained by formation of 23-(O-ADP-ribosyl)-RIF followed by removal of AMP and dephosphorylation, leading to 23-ribosyl-RIF. The strain expresses Arr enzymes that act as mono (ADP-ribosyl) transferases and catalyse the reaction of ADP-ribosylation of rifamycins. Arr homologues are widely distributed in environmental bacteria and in *Stenotrophomonas maltophilia*, *Burkholderia cenocepacia*, *S. coelicolor*, *Corynebacterium glutamicum*, *Desulfitobacterium hafniense* and *Rhodopseudomonas palustris*. It was shown that RIF analogues modified at position C25 generate derivatives that are resistant to inactivation by ADP-ribosyl transferases from *M. smegmatis* [42]. Other species use a different mechanism of modification. For example, *Nocardia* sp. modify the antibiotic by glucosylation (23-OH group) and phosphorylation, but ribosylation is not observed. *Bacillus* strains and actinomycete-related species were also found to inactivate RIF

through phosphorylation of the 21-OH group, RIF glucosylation (23-OH group) or decolorisation [43,44].

6. Resistance by modification of permeability

To be effective, an antibiotic has to reach its specific target in an efficient time frame and at sufficient concentration. One strategy to impair the action of an antibiotic is to prevent its entry to the cell. Some bacterial species that do not allow multiple antibiotics to accumulate in the cytoplasm are called 'intrinsically' resistant, whilst other antibiotic-susceptible bacteria can become resistant to multiple antibiotics. This occurs through two mechanisms: (i) the bacteria can be resistant or acquire resistance by decreasing the permeability of the membrane barriers; and (ii) they can also overexpress membrane-associated energy-driven efflux pumps reminiscent of the eukaryotic multidrug resistance pumps.

Mycobacteria are intrinsically resistant to many antibiotics and this property is often attributed to a low permeability of the mycobacterial cell wall and its specific lipid-rich character. Decades ago, a strain of *Mycobacterium intracellulare* exhibiting natural resistance to RIF was isolated. Its RNAP was found to be susceptible to RIF and experiments suggested that a permeability barrier against RIF was responsible for this resistance [45]. Several efflux pumps are also suspected to contribute to mycobacteria multidrug resistance. Siddiqi et al. [46] have pointed out a correlation between drug resistance and *Rv1258c* gene transcription levels of a clinical isolate of *M. tuberculosis* resistant to multiple antibiotics (RIF, ofloxacin, isoniazid and minomycin). Reverse

transcriptase polymerase chain reaction (RT-PCR) showed that the level of transcription of *Rv1258c* increased by a factor of 10 when the bacteria are grown in the presence of RIF or ofloxacin. These results suggest that efflux pumps can be induced in response to antibiotic treatment in *Mycobacteria*, consistent with the hypothesis of the involvement of efflux pumps in detoxification [47]. The *mtr* operon of *Neisseria gonorrhoeae* encodes an efflux pump system composed of the proteins MtrCDE [48,49]. This pump transports several antibiotics, and mutations in the *mtrR* gene, a transcriptional repressor of *mtrCDE*, induce overexpression of this efflux pump. This confers resistance to several antimicrobials, including rifamycins.

7. Conclusions

Bacteria use a variety of mechanisms to counter the actions of antibiotics, and RIF resistance or tolerance is a perfect example of this diversity. One may wonder what these genes are doing in these bacteria and whether they have additional functions. The RIF-binding domain is located in the catalytic site of the bacterial RNAP and is highly conserved among bacteria, but it has a high propensity to mutate, as observed, when RIF selection pressure is applied. These discrepancies remain to be understood.

The existence of multiple antibiotics sharing, at least partially, the same binding site as RIF, the presence of duplicated genes resistant and sensitive to RIF in the same bacteria, and the involvement of several RNAP-binding proteins in adaptation to low RIF concentrations are probably more revealing of the true function of this antibiotic.

Bacteria live in a community and they must be competitive to survive in their

environment. Apart from their killer function, RIF and these various other molecules at low concentrations could also find their usefulness in regulating the genes of surrounding bacteria to the benefit of the producer or the genes of the producer itself. We now have strong genomic evidence that these resistance mechanisms found in the clinic have an environmental origin. The most common soil bacteria are a reservoir of a previously underappreciated diversity of resistance genes that can be mobilised under selection pressure such as antibiotic therapy. This raises concerns about the future of our antibacterial arsenal. One may ask whether the introduction of the RIF analogues rifaximin, rifapentine, rifabutin and rifalazil to the therapeutic arsenal together with the diversification of the pathologies treated by these molecules will diversify the resistance mechanisms of human pathogens to ansamycins.

Acknowledgments

KB is supported by a fellowship from the RTRS Infectiopole Sud (CPBS, Montpellier, France). AT is supported by the CNRS (Montpellier, France) and the Region Languedoc-Roussillon.

Funding

This work was funded by the CNRS (Montpellier, France).

Competing interests

None declared.

Ethical approval

Not required.

Accepted Manuscript

References

- [1] Sensi P, Greco AM, Ballotta R. Rifomycin. I. Isolation and properties of rifomycin B and rifomycin complex. *Antibiot Annu* 1959;7:262–70.
- [2] Sensi P, Ballotta R, Greco M. Rifomycin. V. Rifomycin O, a new antibiotic of the rifomycin family. *Farmaco Sci* 1960;15:228–34.
- [3] Gardner MJ, Williamson DH, Wilson RJM. A circular DNA in malaria parasites encodes an RNA polymerase like that of prokaryotes and chloroplasts. *Mol Biochem Parasitol* 1991;44:115–23.
- [4] Pukrittayakamee S, Viravan C, Charoenlarp P, Yeamput C, Wilson RJ, White NJ. Antimalarial effects of rifampin in *Plasmodium vivax* malaria. *Antimicrob Agents Chemother* 1994;38:511–4.
- [5] Yim G, Huimi HH, Davies J. Antibiotics as signalling molecules. *Philos Trans R Soc Lond B Biol Sci* 2007;362:1195–200.
- [6] Villain-Guillot P, Bastide L, Gualtieri M, Leonetti J-P. Progress in targeting bacterial transcription. *Drug Discov Today* 2007;12:200–8.
- [7] Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell* 2001;104:901–12.
- [8] Floss HG, Yu TW. Rifamycin—mode of action, resistance, and biosynthesis. *Chem Rev* 2005;105:621–32.
- [9] Jin DJ, Gross CA. RpoB8, a rifampicin-resistant termination-proficient RNA polymerase, has an increased K_m for purine nucleotides during transcription elongation. *J Biol Chem* 1991;266:14478–85.

- [10] Severinov K, Soushko M, Goldfarb A, Nikiforov V. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the β subunit of *Escherichia coli* RNA polymerase. J Biol Chem 1993;268:14820–5.
- [11] Severinov K, Soushko M, Goldfarb A, Nikiforov V. Rif^R mutations in the beginning of the *Escherichia coli rpoB* gene. Mol Gen Genet 1994;244:120–6.
- [12] Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993;341:647–50.
- [13] Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis 1998;79:3–29.
- [14] Heep M, Odenbreit S, Beck D, Decker J, Prohaska E, Rieger U, et al. Mutations at four distinct regions of the *rpoB* gene can reduce the susceptibility of *Helicobacter pylori* to rifamycins. Antimicrob Agents Chemother 2000;44:1713–5.
- [15] Heep M, Rieger U, Beck D, Lehn N. Mutations in the beginning of the *rpoB* gene can induce resistance to rifamycins in both *Helicobacter pylori* and *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2000;44:1075–7.
- [16] Heep M, Brandstatter B, Rieger U, Lehn N, Richter E, Rusch-Gerdes S, et al. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. J Clin Microbiol 2001;39:107–10.

- [17] Perkins AE, Nicholson WL. Uncovering new metabolic capabilities of *Bacillus subtilis* using phenotype profiling of rifampin-resistant *rpoB* mutants. J Bacteriol 2008;190:807–14.
- [18] Maguire BA, Wild DG. Identification of a mutation conferring antibiotic dependence in *Escherichia coli*. In: 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC); 25–28 October 2008; Washington, DC. Washington, DC: ASM Press; 2008. p. 124.
- [19] Sonenshein AL, Cami B, Brevet J, Cote R. Isolation and characterization of rifampin-resistant and streptolydigin-resistant mutants of *Bacillus subtilis* with altered sporulation properties. J Bacteriol 1974;120:253–65.
- [20] Kawamura N, Kurokawa K, Ito T, Hamamoto H, Koyama H, Kaito C, et al. Participation of Rho-dependent transcription termination in oxidative stress sensitivity caused by an *rpoB* mutation. Genes Cells 2005;10:477–87.
- [21] Hu H, Zhang Q, Ochi K. Activation of antibiotic biosynthesis by specified mutations in the *rpoB* gene (encoding the RNA polymerase β subunit) of *Streptomyces lividans*. J Bacteriol 2002;184:3984–91.
- [22] Bergval IL, Klatser PR, Schuitema ARJ, Oskam L, Anthony RM. Specific mutations in the *Mycobacterium tuberculosis rpoB* gene are associated with increased *dnaE2* expression. FEMS Microbiol Lett 2007;275:338–43.
- [23] Jin DJ, Walter WA, Gross CA. Characterization of the termination phenotypes of rifampicin-resistant mutants. J Mol Biol 1988;202:245–53.

- [24] Adone R, Ciuchini F, Marianelli C, Tarantino M, Pistoia C, Marcon G, et al. Protective properties of rifampin-resistant rough mutants of *Brucella melitensis*. *Infect Immun* 2005;73:4198–204.
- [25] Björkman J, Hughes D, Andersson DI. Virulence of antibiotic-resistant *Salmonella typhimurium*. *Proc Natl Acad Sci USA* 1998;95:3949–53.
- [26] Gagneux S, Long CD, Small PM, Van T, Schoolnik GK, Bohannan BJM. The competitive cost of antibiotic resistance in *Mycobacterium tuberculosis*. *Science* 2006;312:1944–6.
- [27] Enne VI, Delsol AA, Roe JM, Bennett PM. Rifampicin resistance and its fitness cost in *Enterococcus faecium*. *J Antimicrob Chemother* 2004;53:203–7.
- [28] Kageyama A, Yazawa K, Ishikawa J, Hotta K, Nishimura K, Mikami Y. Nocardial infections in Japan from 1992 to 2001, including the first report of infection by *Nocardia transvalensis*. *Eur J Epidemiol* 2004;19:383–9.
- [29] Schaal KP, Lee H-J. Actinomycete infections in humans—a review. *Gene* 1992;115:201–11.
- [30] Wauters G, Avesani V, Charlier J, Janssens M, Vaneechoutte M, Delmee M. Distribution of *Nocardia* species in clinical samples and their routine rapid identification in the laboratory. *J Clin Microbiol* 2005;43:2624–8.
- [31] Ishikawa J, Yamashita A, Mikami Y, Hoshino Y, Kurita H, Hotta K, et al. The complete genomic sequence of *Nocardia farcinica* IFM 10152. *Proc Natl Acad Sci USA* 2004;101:14925–30.

- [32] Ishikawa J, Chiba K, Kurita H, Satoh H. Contribution of *rpoB2* RNA polymerase β subunit gene to rifampin resistance in *Nocardia* species. *Antimicrob Agents Chemother* 2006;50:1342–6.
- [33] Vigliotta G, Tredici SM, Damiano F, Montinaro MR, Pulimeno R, di Summa R, et al. Natural merodiploidy involving duplicated *rpoB* alleles affects secondary metabolism in a producer actinomycete. *Mol Microbiol* 2005;55:396–412.
- [34] Inaoka T, Takahashi K, Yada H, Yoshida M, Ochi K. RNA polymerase mutation activates the production of a dormant antibiotic 3,3'-neotrehalosdiamine via an autoinduction mechanism in *Bacillus subtilis*. *J Biol Chem* 2004;279:3885–92.
- [35] Newell KV, Thomas DP, Brrekasis D, Paget MS. The RNA polymerase-binding protein RbpA confers basal levels of rifampicin resistance on *Streptomyces coelicolor*. *Mol Microbiol* 2006;60:687–96.
- [36] Hu Y, Mangan JA, Dhillon J, Sole KM, Mitchison DA, Butcher PD, et al. Detection of mRNA transcripts and active transcription in persistent *Mycobacterium tuberculosis* induced by exposure to rifampin or pyrazinamide. *J Bacteriol* 2000;182:6358–65.
- [37] Pomerantz RT, O'Donnell M. The replisome uses mRNA as a primer after colliding with RNA polymerase. *Nature* 2008;456:762–6.
- [38] Flåtten I, Morigen K, Morigen S. DnaA protein interacts with RNA polymerase and partially protects it from the effect of rifampicin. *Mol Microbiol* 2009;71:1018–30.
- [39] Wegrzyn A, Szalewska-Palasz A, Blaszcak A, Liberek K, Wegrzyn G. Differential inhibition of transcription from sigma70- and sigma32-dependent promoters by rifampicin. *FEBS Lett* 1998;440:172–4.

- [40] Vassilyev DG, Vassilyeva MN, Perederina A, Tahirov TH, Artsimovitch I. Structural basis for transcription elongation by bacterial RNA polymerase. *Nature* 2007;448:157–62.
- [41] Imai T, Watanabe K, Mikami Y, Yazawa K, Ando A, Nagata Y, et al. Identification and characterization of a new intermediate in the ribosylative inactivation pathway of rifampin by *Mycobacterium smegmatis*. *Microb Drug Resist* 1999;5:259–64.
- [42] Combrink KD, Denton DA, Harran S, Ma Z, Chapo K, Yan D, et al. New C25 carbamate rifamycin derivatives are resistant to inactivation by ADP-ribosyl transferases. *Bioorg Med Chem Lett* 2007;17:522–6.
- [43] Dabbs E, Yazawa K, Tanaka Y, Mikami Y, Miyaji M, Andersen S, et al. Rifampicin inactivation by *Bacillus* species. *J Antibiot (Tokyo)* 1995;48:815–9.
- [44] Tanaka Y, Yazawa K, Dabbs E, Nishikawa K, Komaki H, Mikami Y, et al. Different rifampicin inactivation mechanisms in *Nocardia* and related taxa. *Microbiol Immunol* 1996;40:1–4.
- [45] Hui J, Gordon N, Kajioka R. Permeability barrier to rifampin in mycobacteria. *Antimicrob Agents Chemother* 1977;11:773–9.
- [46] Siddiqi N, Das R, Pathak N, Banerjee S, Ahmed N, Katoch VM, et al. *Mycobacterium tuberculosis* isolate with a distinct genomic identity overexpresses a tap-like efflux pump. *Infection* 2004;32:109–11.
- [47] Fernandes P, Ferreira BS, Cabral JMS. Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics. *Int J Antimicrob Agents* 2003;22:211–6.
- [48] Maness M, Sparling P. Multiple antibiotic resistance due to a single mutation in *Neisseria gonorrhoeae*. *J Infect Dis* 1973;128:321–30.

- [49] Hagman K, Pan W, Spratt B, Balthazar J, Judd R, Shafer W. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the *mtrRCDE* efflux system. Microbiology 1995;141:611–22.

Fig. 1. Location of rifampicin (RIF) resistance mutations in the RNA polymerase (RNAP) β -subunit. The 1342-amino acid-long β -subunit is represented by the light bar, with grey shaded areas corresponding to the highly conserved sequence segments, designated A–I, and with red shaded areas corresponding to RIF resistance clusters: mutations in *Mycobacterium tuberculosis* (green triangle), *Escherichia coli* (yellow triangle) and *Bacillus subtilis* (purple triangle). Important contacts between RIF and the β -subunit (black sphere).

Fig. 2. Rifampicin (RIF)-binding site in the active site of RNA polymerase. The model was built from the Protein Data Bank co-ordinates of the *Thermus aquaticus* core complex (1I6V [7]) and from a *Thermus thermophilus* core scaffold complex (2O5I [40]). The α -subunit is represented in yellow, the β -subunit in blue, the β' -subunit in magenta, the DNA double helix in red and green, RNA in orange and RIF in bright yellow.

Fig. 3. ADP-ribosylation of rifampicin (RIF) by *Mycobacterium smegmatis*. RIF is inactivated by ADP-ribosylation in several bacteria, such as *M. smegmatis* DSM 43756. The different metabolites identified are presented.

Table 1

Summary of the different resistance mechanisms, listed together with the associated microorganisms and the resistance level

Resistance mechanism	Species	Level of resistance	References
Mutation	<i>Escherichia coli</i> , <i>Mycobacterium tuberculosis</i> , <i>Bacillus subtilis</i> , <i>Helicobacter pylori</i>	High to very high	[9,10,12,13,16,17]
Duplication of <i>rpoB</i>	<i>Nocardia farcinica</i> , <i>Amycolatopsis mediterranei</i> , <i>Actinomadura</i> sp.	High	[32]
RNAP-binding proteins	<i>Streptomyces coelicolor</i> , <i>M. tuberculosis</i> , <i>Mycobacterium leprae</i> , <i>Corynebacterium diphtheriae</i>	Intermediate to low	[35,38]
Modification of rifampicin	<i>Mycobacterium smegmatis</i> DSM 43756, <i>Bacillus</i> sp.	Intermediate to low	[41,43,44]
Modification of permeability	<i>Mycobacterium</i> sp., <i>Neisseria gonorrhoeae</i>	Low	[45,46]
RNAP, RNA polymerase.

Fig 2 : Rifampicin-binding site in the active site of RNA polymerase

Fig 3 :ADP-ribosylation of rifampicin
by *M.smegmatis* DSM 43756