ACTA ABP BIOCHIMICA POLONICA

Vol. 61, No 4/2014
633–638
on-line at: www.actabp.pl

Review

Antimicrobial resistance in *Rhodococcus equi**

Agata A. Cisek[1]✉, Magdalena Rzewuska[1], Lucjan Witkowski[2] and Marian Binek[1]

[1]Departament of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland; [2]Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

***Rhodococcus equi* is an important etiologic agent of respiratory- and non-respiratory tract infections, diseases of animals and humans. Therapy includes the use of various group of chemotherapeutic agents, however resistance acquirement is quite common. To date there is no preferred treatment protocol for infections caused by isolates resistant to macrolides and rifampicin. The resistance acquirement is a result of many molecular mechanisms, some of which include alterations in the cell envelope composition and structure, activity of the efflux pumps, enzymatic destruction or inactivation of antibiotics, and changes in the target site. This paper contains an overview of antimicrobial susceptibility of *R. equi*, and explains the possible molecular mechanisms responsible for antimicrobial resistance in this particular microorganism.**

Key words: antimicrobial susceptibility, *Rhodococcus equi*

Received: 17 November, 2013; revised: 19 August, 2014; accepted: 17 October, 2014; available on-line: 04 November, 2014

INTRODUCTION

Rhodococcus equi is a Gram-positive, pleomorphic rod, commonly found in soil. It is one of the most important pathogens of foals, in which it causes rhodococcosis, a disease manifesting in pyogranulomatous bronchopneumonia, abscesses, lymphadenitis or ulcerative enterocolitis. *R. equi* can be pathogenic to other domestic and wild animals (especially pigs and cattle), and humans as well (Weinstock & Brown, 2002; Yamshchikov *et al*., 2010; Giguère *et al*., 2011; Witkowski *et al*., 2011; Muscatello, 2012).

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in development of the antibiotic resistance. Prolonged therapy of *R. equi* infections with lipophilic antibiotics leads to a progressive increase in resistance of currently isolated strains. Combination of erythromycin and rifampin has been the treatment of choice for *R. equi* pneumonia in foals for the past two decades. The existing data indicates that the low-level resistance towards erythromycin and rifampin may be clinically relevant, and these drugs should be carefully considered for the further treatment of diseased horses. In recent years, newer macrolides, such as azithromycin, clarithromycin and tulathromycin, have become available as promising alternatives for the veterinary use. So has the gallium maltolate, which was also proven to inhibit *R. equi* growth in both rifampicin and macrolide-susceptible and rifampicin and macrolide-resistant strains (Chaffin *et al*., 2009; Coleman *et al*., 2010). Other drugs, such as imipenem, gentamicin, amikacin or vancomycin, have no application in treatment of foals but along with erythromycin and rifampicin are proposed to be used in a human therapy of *R. equi* infections (Gilbert *et al*., 2010).

Our pervious study (Cisek *et al*., 2013) reported differences of susceptibility profiles of *R. equi* strains isolated from wild boars (*Sus scrofa*) in comparison to data obtained from the literature on prevalence over time (Woolcock & Mutimer, 1980; Nordmann & Ronco, 1992; Soriano *et al*., 1995; Hsueh *et al*., 1998; Bowersock *et al*., 2000; Tomlin *et al*., 2001; Jacks *et al*., 2003; Niwa *et al*., 2005). Differences regarded five antimicrobials including tetracycline, rifampicin, clindamycin, cephalothin and amoxicillin-clavulanate. For the latter two β-lactam antibiotics, *R. equi* isolated from wild boars was more resistant in comparison to the strains isolated from other sources (literature data). In contrast, tetracycline, rifampicin, and clindamycin were more active against wild-boar isolates. Differences between strains isolated from various sources were also the subject-matter of the study of Girardini *et al.* (2013), in which it was observed that human and environmental isolates possess different susceptibility profiles. This could be due to the antimicrobial pressure, which comes from the fact that humans and farm animals are simply more exposed on antibiotic administration, sometimes inappropriate or unnecessary. This paper contains an overview of *R. equi* susceptibility, and it clarifies the possible molecular mechanisms of resistance acquirement of *R. equi* strains.

ANTIMICROBIAL SUSCEPTIBILITY AND TREATMENT

Standard therapy of rhodococcosis consist of a few antibiotics (table 1). Still, the treatment of rhodococcal infection may be difficult, because strains seem to become more resistant, and there is no clear and straightforward protocol that would indicate the preferred antimicrobial combination for the therapy of animals infected by the antimicrobial resistant strains. Another problem comes due to the fact that *R. equi* is an intracellular pathogen, which only shortens the list of active antimicrobials to just a few groups of drugs that are suitable for use.

Among active antimicrobials, macrolides (erythromycin, azithromycin and clarithromycin) demonstrate good inhibitory activity against *R. equi* in comparison to other classes of drugs (Muscatello, 2012). However, in case of

✉e-mail: gutkac@op.pl
*Presented at the 3rd Workshop on Microbiology in Health and Environmental Protection MIKROBIOT 2013, Łódź, Poland
Abbreviations: aa, amino acid; MIC, minimum inhibitory concentration

Table 1. Antimicrobials used for treatment of *Rhodococcus equi* infections in horses and humans (Wilson, 2001; Gilbert *et al.*, 2010; Yamshchikov *et al.*, 2010; Giguère *et al.*, 2011; de Bruijn *et al.*, 2013; Venner *et al.*, 2013)

Horses	Humans
First choice drugs: erythromycin with rifampicin clarithromycin with rifampicin azithromycin with rifampicin	First choice drugs*: erythromycin rifampicin imipenem vancomycin levofloxacin aminoglycosides: tobramycin gentamycin amikacin
Alternative choices: gamithromycin with or without rifampicin tulathromycin erythromycin azithromycin gentamicin with rifampicin doxycycline with rifampicin trimethoprim-sulfamethoxazole with or without rifampicin	Alternative choices*: clindamycin ciprofloxacin trimethoprim-sulfamethoxazole tetracyclines linezolid cephalosporins
	Also effective** azithromycin clarithromycin teicoplanin meropenem amoxicillin-clavulanate

*consider 2 agents, **based on clinical studies that ended in patient's recovery

erythromycin increasing MIC values are well documented in the 10-year period of time starting from the late 1990s (Buckley *et al.*, 2007). Moreover, usage of erythromycin may contribute to serious side effects. For these two reasons newer and safer macrolides, such as azithromycin and clarithromycin, are proposed as an alternative in the treatment of foals and humans. In fact, study of Villarino & Martín-Jiménez (2013) reports that newer macrolides demonstrate accumulation inside the bronchoalveolar lavage cells at higher extent than erythromycin, which explains why the former are more effective than erythromycin in pneumonia caused by *R. equi*. Moreover, the use of azithromycin monotherapy provided nearly as high survival rate values in foals as a combination of azithromycin with rifampicin (Venner *et al.*, 2013). Other two macrolides, tulathromycin and tilmicosin, demonstrate low *in vitro* activity against *R. equi*, which should be taken under consideration by veterinarians (Giguère *et al.*, 2011). Finally, it should be noted that some study highlights the occurrence of cross-resistance to macrolides, especially among rifampicin-resistant strains (Giguère *et al.*, 2010; Muscatello, 2012).

Erythromycin and other macrolides are preferably coupled with rifampicin that was found very useful in therapy against rhodococcal infections. It has been used so often and eagerly that rifampicin resistance of *R. equi* became a therapeutic problem. Occurrence of this phenomenon was well documented by Buckley *et al.* (2007), who demonstrated that increase in prevalence of rifampin resistance is even faster than that of erythromycin. Because of this ready development of rifampicin resistance, this drug should be combined with other antimicrobials.

Lincosamides (clindamycin and lincomycin), fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin), tetracyclines (tetracycline, doxycycline and minocycline) and chloramphenicol are another antibiotics able to penetrate macrophages and kill intracellular pathogens such as *R. equi*. It is important to know that not all these drugs are entirely effective. Some strains of *R. equi* demonstrate variable *in vitro* susceptibility, and the number of multidrug-resistant strains is increasing. For instance, in two studies from the year 1992 and 2010 percentage of drug resistant human strains were ranging between 50% and 90.2% for clindamycin, 9.8% and 16% for norfloxacin, 7.84% and 18% for ciprofloxacin, and 4% and 21.57% for tetracycline (McNeil & Brown, 1992; Silva *et al.*, 2010).

The main advantage of macrolides, rifampicin and other cell-penetrating drugs is the fact that they reach high intracellular concentration in alveolar macrophages and neutrophils (Ribeiro *et al.*, 2006), which is essential for anti-rhodococcal activity. Drugs that inhibited *R. equi* growth *in vitro*, (such as combination of penicillin with gentamicin), but were not able to penetrate macrophages, were not effective in the treatment of foals (Giguère *et al.*, 2011). Therefore, antimicrobials that exhibit a poor intracellular activity, such as aminoglycosides, glycopeptides, and β-lactams, should be administered only as a supporting drug in a combination of two or more. In fact, the use of β-lactams should be limited to imipenem, for which the number of susceptible strains accounted for 98.04% (Silva *et al.*, 2010). Therapy based on combination of imipenem with teicoplanin, gentamicin, vancomycin or amikacin, as well as combinations of β-lactam antibiotics with β-lactamase inhibitors (i.e. amoxicillin with clavulanate, or ampicillin with sulbactam) may represent an alternative. For amoxicillin-clavulanate the *in vitro* susceptibility of *R. equi* was also 98.04% (Silva *et al.*, 2010). Of course, combination of two drugs that penetrate cells (e.g. rifampicin with a macrolide, rifampicin with a tetracycline, or a macrolide with a tetracycline) is recommended. In such case the use of two antibiotics from different classes decreases the risk of resistance development for either antimicrobial (Venner *et al.*, 2013).

Some antimicrobials demonstrate an antagonistic activity. For instance, use of amikacin alongside with a macrolide/rifampicin, or gentamicin with rifampicin is not recommended (Giguère *et al.*, 2012). Use of β-lactam antibiotics may be problematic since, according to Nor-

dmann *et al.* (1993), there are two groups of them. Antibiotics of the first group (imipenem, meropenem, moxalactam, cefoxitin, oxacillin and ceftriaxone) reduce *in vitro* activity of drugs from the other group (amoxicillin, penicillin, cephalothin and ticarcillin). Interestingly, antibiotics from the same group do not display any sign of antagonism (Nordmann *et al.*, 1993).

MOLECULAR MECHANISMS OF ANTIMICROBIAL RESISTANCE

The success of a therapy against *R. equi* relies on two major factors: the *in vitro* susceptibility of the strains to antibiotics, and the effectiveness of the penetration to the body cells where the drug plays its role.

In vitro susceptibility of *R. equi* varies among the strains, and this heterogeneity underlies in resistance of the entire population. In a study by McNeil & Brown (1992) less than 5% of human strains isolated from the HIV-infected patients were resistant to erythromycin, rifampicin, tetracycline, and trimethoprim-sulfamethoxazole (McNeil & Brown, 1992). The same study shows that resistance of human isolates were nearly 20% for fluoroquinolones (ciprofloxacin and norfloxacin), and 50% or more for clindamycin and some β-lactam antibiotics, such as ampicillin, cephalothin, oxacillin, and penicillin. Interestingly, human isolates demonstrate higher multidrug resistance than *R. equi* isolated from other sources (McNeil & Brown, 1992). It was also concluded that two out of eight strains of *R. equi* isolated from the HIV-infected patients showed evidence of acquired resistance to β-lactam antibiotics.

Other research made on human isolates presented similar results for rifampicin and erythromycin, which inhibited the *in vitro* growth of 98.04% of *R. equi* strains. Fluoroquinolones, i.e. levofloxacin, ciprofloxacin and norfloxacin, were very effective, inhibiting the growth of approx. 92–96% of strains. About 96% of strains was inhibited by doxycycline, whereas only 78.43% by tetracycline, and 84.31% by chloramphenicol. Much lower antimicrobial activity was observed in case of clindamycin and sulfamethoxazole with trimethoprim, to which only 9.8 and 41.16% of strains were susceptible, respectively. Susceptibility of strains to β-lactams varied, reaching from 1.96% for oxacillin, 5.88% for penicillin, 7.84% for ampicillin, 13.73% for cefazolin, 19.60% for cephalothin, 23.53% for cefotaxime, 45.10% for cefepime, 49.02% for cefoxitin, and 80.39% for ceftriaxone (Silva *et al.*, 2010). (Silva *et al.*, 2010).

The main mechanisms responsible for acquirement of resistance regard cell wall permeability, efflux pumps, metabolic pathways, and an acquisition of new genes that would result in the increase of tolerance to the antimicrobial drugs (Fig. 1). Resistance genes may be located on the chromosome, or on the mobile genetic elements, i.e. on a plasmid, or on a transposon (de Carvalho, 2010).

Among chromosomal genes, *rpoB,* a gene encoding RNA polymerase β-subunit, was found to have an influence on antimicrobial resistance profile of *R. equi.* In *Mycobacterium tuberculosis* rifampicin resistance is related to

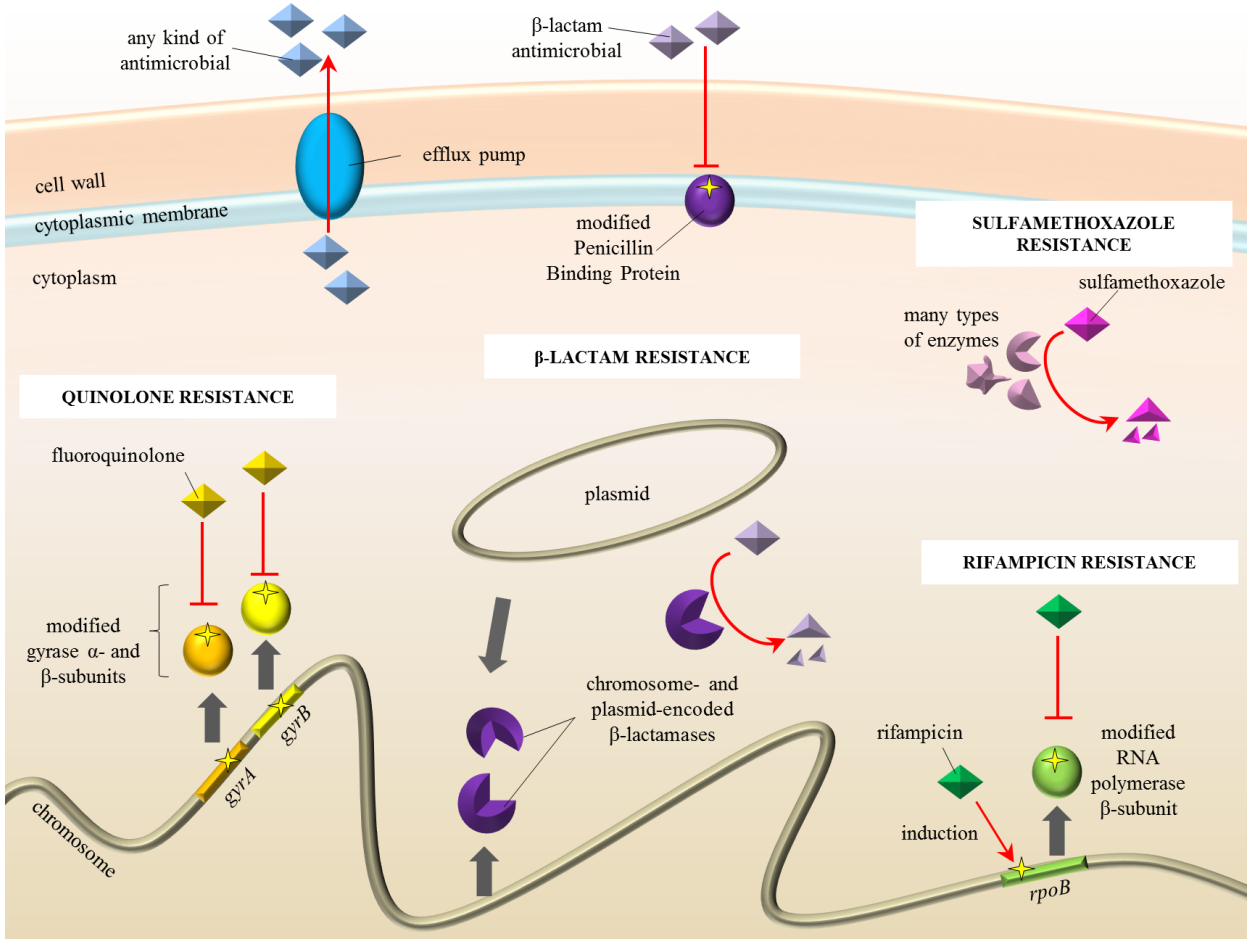

Figure 1. Molecular mechanisms of antimicrobial resistance in *R. equi*

spontaneous mutations in one of three loci in *rpoB* gene (Lambert *et al.*, 2002). Similar observations were made for *R. equi*, and it was proven that monotherapy with rifampicin induced mutations in *rpoB* gene, which resulted in occurrence of rifampicin resistance (Fines *et al.*, 2001; Asoh *et al.*, 2003; Giguère *et al.*, 2011). Spontaneous substitutions of nucleotides located in a 81 bp-long region of *rpoB* gene, i.e. region between codons 507 and 533 (*Escherichia coli*-numbering), resulted in alterations within the RNA polymerase β-subunit composition in the corresponding positions. This region of polymerase is also known to be a rifampicin target, which means that alterations in the polymerase composition and structure may result in decreased affinity of rifampicin to this enzyme (Fines *et al.*, 2001). The low-level resistant strains of *R. equi* (MIC 1–8 μg/ml) had the following substitutions: Ser509Pro, Asp516Val, His526Asn and Ser531Leu, whilst high-level resistant strains (MIC ≥ 128 μg/ml) had Ser531Trp, His526Asp, His526Tyr and His526Arg substitutions (Fines *et al.*, 2001; Asoh *et al.*, 2003; Boyen *et al.*, 2011). The His526Arg-mutant was constructed from a rifampicin-susceptible reference strain *R. equi* ATCC 33701, while the rest of mutations were observed in isolates of an equine and human origin.

Molecular mechanisms of macrolide resistance of *R. equi* are still unknown. However, bacteria resistant to one macrolide, such as erythromycin, are usually resistant to azithromycin and clarithromycin as well. Moreover, sometimes strains classified as resistant are in fact susceptible and in such case retesting is needed (Giguère *et al.*, 2011).

Quinolone resistance is related to mutations in the *gyrA* and *gyrB* genes (encoding DNA gyrase α- and DNA gyrase β-subunit), in particular to the quinolone resistance-determining region (QRDR) of these genes. Studies made on ciprofloxacin-exposed *R. equi* revealed that there are nine single nucleotide substitutions observed in QRDRs of *gyrA* and *gyrB* corresponding to eight amino acid alterations in both gyrases. Mutation in gyrase α-subunit resulted in higher-level resistance (MIC from 8 to >64 μg/ml) of *R. equi* than mutation in gyrase β-subunit (MIC 4 μg/ml). Moreover, substitutions of serine in position 83 to arginine or isoleucine in the gyrase α-subunit (Ser83Arg or Ser83Ile) led to higher-level resistance than other substitutions of the same enzyme, such as Asp87Asn, Asp87Gly, Asp87His, Asp87Tyr, or Gly81Cys. Such differences may result from the fact that Ser83 is of great importance, and its substitution may be related to reduction of affinity of the active site of DNA gyrases to quinolones (Niwa *et al.*, 2006; Niwa & Lasker, 2010). Therefore, in order to decrease the chance of such quinolone-resistance acquirement, quinolones should be administered together with other antibiotics.

As for β-lactam resistance, it was unclear whether it was related to penicillin-binding proteins (PBPs), enhanced antibiotic degradation, or efflux pump activity for a long period of time. Recently, Letek *et al.* (2010) have found several genes encoding β-lactamases, present both on chromosome and on a plasmid. This study revealed that 9 out of 10 β-lactamases were encoded by the chromosomal genes, which supported the idea that β-lactam-resistance is an evolutionary reminder of close relationship between *R. equi* and other rhococcocci which are naturally more resistant to antimicrobials. It was also proven that resistance to β-lactam antibiotics is sometimes related to DNA mobility genes, and horizontal gene transfer (HGT). Only one β-lactamase is plasmid-encoded and undergoes HGT (Letek *et al.*, 2010).

Of course, different molecular mechanisms of resistance to β-lactam antibiotics, such as PBP alterations (resulting in a target site changes for the antimicrobials), and efflux pump activity are valid, especially since it has been reported that several strains resistant to β-lactam antibiotics were lacking β-lactamases (McNeil & Brown, 1992; Nordman *et al.*, 1993; Nordman *et al.*, 1994; Linder *et al.*, 1997). Surprisingly, Meroueh *et al.* (2003) and Martinez (2009) suggested that plasmid-encoded β-lactamases, may have been originally the PBPs, and their activity against β-lactams might be a side effect of their original function.

The β-lactamases are not the only enzymes produced by *R. equi* in order to protect it from antimicrobials. Study made on strains exposed to sulfamethoxazole revealed that *R. equi* had the greatest ability to metabolize sulfamethoxazole amongst other rhodococci. Enzymes proposed for sulfamethoxazole degradation included the arylamine N-acetyltransferase, an amidase that degraded lysergamide to lysergic acid, an urethanase which hydrolyzes anilides, and the N-acetyl-phenylethylamine hydrolase which hydrolizes N-acetylated compounds (Larcher & Yargeau, 2011).

Cell envelope is the main defense barrier of many bacteria. Cell wall of *R. equi* slightly resembles cell envelope properties of the Gram-negative bacteria. It is highly hydrophobic because of the presence of mycolic acid and glycolipids. These compounds are responsible for increased cellular tolerance to hydrophilic antimicrobials and organic solvents, which cannot diffuse across this hydrophobic layer, and are thought to use the porin channels, which are present in *R. equi* (de Carvahlo, 2010; Kuyukina & Ivshina, 2010; Sutcliffe *et al.*, 2010).

Resistance to hydrophobic antibiotics, such as rifampicin and quinolones, depends upon the effective efflux pump systems. This mechanism is commonly found in microorganisms, including mycobacteria and *Rhodococcus* species (de Carvahlo, 2010). Genes encoding exporters and transporters are located both on chromosome and on mobile genetic elements. The chromosome-encoded pump systems are responsible for multidrug resistance, whilst plasmid ones remove only specific groups of antimicrobials, which is usually related to the acquired drug resistance to macrolides, lincosamides, tetracyclines, rifampicin and chloramphenicol. This specific efflux system responsible for chloramphenicol resistance have been well-described for *Rhodococcus fascians* and *Rhodococcus rhodochrous*, for which twelve membrane spanning domains were detected as a consequence of *cmr* and *cmrA* gene expression, respectively. The *cmr* gene was found on a conjugative plasmid pRF2, while *cmrA* gene is a component of a transposon Tn5561 (Butaye *et al.*, 2003). In *R. equi*, chloramphenicol-resistance has been observed as well (Vázquez-Boland *et al.*, 2010; Silva *et al.*, 2012), which only leads to an assumption, that this species may possess similar efflux pump systems, which are not described yet.

Apart from the mechanisms responsible for an increase in antimicrobial resistance of *R. equi*, there is one of an opposite relevance. Surprisingly, antimicrobial susceptibility of *R. equi* depends on presence of the virulence-associated plasmids (VAPs) as well. These plasmids contain *lsr2* gene alongside with the virulence genes *vap*. The Lsr2 protein is homologous to the one that in *Mycobacterium tuberculosis* serves as a regulator of the antibiotic-induced responses, phage infections, and plays a role in modifications of the mycolic acid (Arora *et al.*, 2008; Colangeli *et al.*, 2007; Vázquez-Boland *et al.*, 2010). In *M. tuberculosis* Lsr2 nonspecifically binds to AT-rich sequences,

including those that seem to be antibiotic-induced genes, changes the way that DNA is shaped, and prevents from the antibiotic-induced responses in mycobacteria (Colangeli *et al*., 2007). Similar mode of action is predicted for *R. equi* (Vázquez-Boland *et al*., 2010). This unspecific reaction is responsible for repression of the resistance genes expression, and increases rhodococcal susceptibility to the antimicrobials (Arora *et al*., 2008).

CONCLUSIONS

Treatment of rhodococcosis usually consists of a combination of at least two antibiotics to which the agent is susceptible. These include macrolides, rifampicin, fluoroquinolones, aminoglycosides, glycopeptides and carbapenems, although the increase of rifampicin and erythromycin resistance is progressing. Other macrolides, such as azithromycin, demonstrate good inhibitory activity, however cross-resistance among macrolides is common. Susceptibility to other antibiotics is variable, and depends upon various resistance mechanisms, characteristic for each group of antimicrobials. The genetic potential for antimicrobial resistance revealed in the *R. equi* genome, indicates that we may be at a critical junction in effective antimicrobial treatment of rhodococcal infection.

Acknowledgements

The work was supported by a grant from the National Science Centre in years 2010-2013 as a research project No. N N308 131638.

REFERENCES

Arora K, Whiteford DC, Lau-Bonilla D, Davitt CM, Dahl JL (2008). Inactivation of *lsr2* results in a hypermotile phenotype in *Mycobacterium smegmatis*. *J Bacteriol* **190**: 4291–4300.

Asoh N, Watanabe H, Fines-Guyon M, Watanabe K, Oishi K, Kositsakulchai W, Sanchai T, Kunsuikmengrai K, Kahintapong S, Khantawa B, Tharavichitkul P, Sirisanthana T, Nagatake T (2003) Emergence of rifampin-resistant *Rhodococcus equi* with several types of mutations in the *rpoB* gene among AIDS patients in northern Thailand. *J Clin Microbiol* **41**: 2337–2340.

Bowersock TL, Salmon SS, Portis ES, Prescott JF, Robison DA, Ford CW, Watts JL (2000) MICs of Oxazolidinones for *Rhodococcus equi* Strains Isolated from Humans and Animals. *Antimicrob Agents Chemother* **44**: 1367–1369.

Boyen F, Pasmans F, Haesebrouck F (2011) Acquired antimicrobial resistance in equine *Rhodococcus equi* isolates. *Vet Rec* **168**: 101.

Buckley T, McManamon E, Stanbridge S (2007) Resistance studies of erythromycin and rifampin for *Rhodococcus equi* over a 10-year period. *Ir Vet J* **60**: 728–731.

Butaye P, Cloeckaert A, Schwarz S (2003) Mobile genes coding for efflux-mediated antimicrobial resistance in Gram-positive and Gram-negative bacteria. *Int J Antimicrob Agents* **22**: 205–210.

Chaffin MK, Fajt V, Martens RJ, Arnold CE, Cohen ND, O'Conor M, Taylor RJ, Bernstein LR (2010) Pharmacokinetics of an orally administered methylcellulose formulation of gallium maltolate in neonatal foals. *J Vet Pharmacol Ther* **33**: 376–382.

Cisek AA, Rzewuska M, Witkowski L, Binek M (2013) Antimicrobial susceptibility of *Rhodococcus equi* isolated from wild boars. *Post Mikrobiol* **52** (Suppl 1): 102.

Colangeli R, Helb D, Vilchèze C, Hazbón MH, Lee CG, Safi H, Sayers B, Sardone I, Jones MB, Fleischmann RD, Peterson SN, Jacobs WR Jr, Alland D (2007) Transcriptional regulation of multi-drug tolerance and antibiotic-induced responses by the histone-like protein Lsr2 in *M. tuberculosis*. *PLoS Pathog* **3**: e87.

Coleman M, Kuskie K, Liu M, Chaffin K, Libal M, Giguère S, Bernstein L, Cohen N (2010) *In vitro* antimicrobial activity of gallium maltolate against virulent *Rhodococcus equi*. *Vet Microbiol* **146**: 175–178.

de Bruijn M, Boschloo H, Fink-Gremmels J (2013) Clinical report: gamithromycin treatment for *Rhodococcus equi* pneumonia in foals. *Proceedings of the European Veterinary Conference Voorjaarsdagen 2013*.

de Carvahlo CCCR (2010) Adaptation of *Rhodococcus* to organic solvents. In *Biology of Rhodococcus* Alvarez HM, ed, pp 109–132. Springer-Verlag Berlin Heidelberg

Fines M, Pronost S, Maillard K, Taouji S, Leclercq R (2001) Characterization of mutations in the *rpoB* gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. *J Clin Microbiol* **39**: 2784–277.

Giguère S, Cohen ND, Chaffin MK, Slovis NM, Hondalus MK, Hines SA, Prescott JF (2011) Diagnosis, treatment, control, and prevention of infections caused by *Rhodococcus equi* in foals. *J Vet Intern Med* **25**: 1209–1220.

Giguère S, Lee E, Williams E, Cohen ND, Chaffin MK, Halbert N, Martens RJ, Franklin RP, Clark CC, Slovis NM (2010) Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R equi*. *J Am Vet Med* Assoc **237**: 74–81.

Giguère S, Lee EA, Guldbech KM, Berghaus LJ (2012) *In vitro* synergy, pharmacodynamics, and postantibiotic effect of 11 antimicrobial agents against *Rhodococcus equi*. *Vet Microbiol* **160**: 207–213.

Gilbert DN, Moellering RC Jr., Eliopoulos GM, Chambers HF, Saag MS, editors., eds (2010) Table 2. In *The Sanford Guide to Antimicrobial Therapy 2010*. 40th edn, pp 62–64. Antimicrobial Therapy, Inc., Sperryville, VA.

Girardini LK, Gressler LT, da Costa MM, de Avila Botton S, da Cruz Payão Pellegrini D, de Vargas AV (2013) Susceptibility profile of Brazilian *Rhodococcus equi* isolates to different antimicrobial classes and the presence of vapA gene. *Pesq Vet Bras* **33**: 735–740.

Hsueh PR, Hung CC, Teng LJ, Yu MC, Chen YC, Wang HK, Luh KT (1998) Report of invasive *Rhodococcus equi* infections in Taiwan, with an emphasis on the emergence of multidrug-resistant strains. *Clin Infect Dis* **27**: 370–375.

Jacks SS, Giguére S, Nguyen A (2003) *In vitro* susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin, and 20 other antimicrobials. *Antimicrob Agents Chemother* **47**: 1742–1745.

Kuyukina MS, Ivshina IB (2010) Application of *Rhodococcus* in bioremediation of contaminated environments. In *Biology of Rhodococcus,* Alvarez HM, ed, pp 231–262. Springer-Verlag Berlin Heidelberg.

Larcher S, Yargeau V (2011) Biodegradation of sulfamethoxazole by individual and mixed bacteria. *Appl Microbiol Biotechnol* **91**: 211–218.

Letek M, González P, MacArthur I, Rodríguez H, Freeman TC, Valero-Rello A, Blanco M, Buckley T, Cherevach I, Fahey R, Hapeshi A, Holdstock J, Leadon D, Navas J, Ocampo A, Quail M.A, Sanders M, Scortti MM, Prescott JF, Fogarty U, Meijer WG, Parkhill J, Bentley SD, Vázquez-Boland JA (2010) The Genome of a Pathogenic *Rhodococcus*: Cooptive Virulence Underpinned by Key Gene Acquisitions. *PLoS Genet* **6**: 1–17.

Linder R, Bernheimer AW (1997) Oxidation of macrophage membrane cholesterol by intracellular *Rhodococcus equi*. *Vet Microbiol* **56**: 269–276.

Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc Biol Sci* **276**: 2521–2530.

McNeil MM, Brown JM (1992) Distribution and antimicrobial susceptibility of *Rhodococcus equi* from clinical specimens. *Eur J Epidemiol* **8**: 437–443.

Meroueh SO, Minasov, G, Lee W, Shoichet BK, Mobashery S (2003) Structural aspects for evolution of beta-lactamases from penicillin-binding proteins. *J Am Chem Soc* **125**: 9612–9618.

Muscatello G (2012) *Rhodococcus equi* pneumonia in the foal — part 2: diagnostics, treatment and disease management. *Vet J* **192**: 27–33.

Niwa H, Hobo S, Anzai T (2006) A nucleotide mutation associated with fluoroquinolone resistance observed in *gyrA* of *in vitro* obtained *Rhodococcus equi* mutants. *Vet Microbiol* **115**: 264–268.

Niwa H, Hobo S, Higuchi T (2005) Antimicrobial susceptibility of 616 *Rhodococcus equi* strains isolated from tracheobronchial aspirates of foals suffering from respiratory disease in Japan. *J Equine Sci* **16**: 99–104.

Niwa H, Lasker BA (2010) Mutant selection window and characterization of allelic diversity for ciprofloxacin-resistant mutants of *Rhodococcus equi*. *Antimicrob Agents Chemother* **54**: 3520–3523.

Nordmann P, Keller M, Espinasse F, Ronco E (1994) Correlation between antibiotic resistance, phage-like particle presence, and virulence in *Rhodococcus equi* human isolates. *J Clin Microbiol* **32**: 377–383.

Nordmann P, Nicolas MH, Gutmann L (1993) Penicillin-binding proteins of *Rhodococcus equi:* potential role in resistance to imipenem. *Antimicrob Agents Chemother* **37**: 1406–1409.

Nordmann P, Ronco E (1992) *In-vitro* antimicrobial susceptibility of *Rhodococcus equi*. *J Antimicrob Chemother* **29**: 383–393.

Ribeiro MG, Paes AC, Listoni FJP (2006) Minimal inhibitory concentration of azithromycin in *Rhodococcus equi* strains isolated from foals. *Arq Bras Med Vet Zootec* **58**: 1244–1246.

Silva Pd, Miyata M, Sato DN, Santos AC, Mendes NH, Leite CQ (2010) *Rhodococcus equi* isolation from sputum of patients with suspected tuberculosis. *Mem Inst Oswaldo Cruz* **105**: 199–202.

Soriano F, Zapardiel J, Nieto E (1995) Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming gram-positive bacilli to 18 antimicrobial agents. *Antimicrob Agents Chemother* **39**: 208–214.

Sutcliffe IC, Brown AK Dover LG (2010) The rhodococcal cell envelope: composition, organisation and biosynthesis. In *Biology of Rhodococcus* Alvarez HM, ed, pp 29–71. Springer-Verlag Berlin Heidelberg.

Tomlin P, Sand C, Rennie RP (2001) Evaluation of E test, disk diffusion and broth microdilution to establish tentative quality control limits and review susceptibility breakpoints for two aerobic actinomycetes. *Diagn Microbiol Infect Dis* **40**: 179–186.

Vázquez-Boland JA, Letek, M, Valero-Rello A, González P, Scortti M, Fogarty U (2010) *Rhodococcus equi* and its pathogenic mechanisms. In *Biology of Rhodococcus* Alvarez HM, ed, pp 331–359. Springer-Verlag Berlin Heidelberg.

Venner M, Credner N, Lämmer M, Giguère S (2013) Comparison of tulathromycin, azithromycin and azithromycin-rifampin for the treatment of mild pneumonia associated with *Rhodococcus equi*. *Vet Rec* **173**: 397.

Villarino N, Martín-Jiménez T (2013) Pharmacokinetics of macrolides in foals. *J Vet Pharmacol Ther* **36**: 1–13.

Weinstock DM, Brown AE (2002) *Rhodococcus equi*: an emerging pathogen. *Clin Infect Dis* **34**: 1379–1385.

Wilson WD (2001) Rational selection of antimicrobials for use in horses. *Proceedings of the Annual Convention of the AAEP 2001* **47**: 75–93

Witkowski L, Rzewuska M, Rzewuska D, Kizerwetter-Świda M, Frymus T, Kita J (2011) *Rhodococcus equi* infections in animals and humans. *Wiad Lek* **64**: 306–309.

Woolcock JB, Mutimer MD (1980) *Corynebacterium equi*: *In vitro* susceptibility to twenty-six antimicrobial agents. *Antimicrob Agents Chemother* **18**: 976–977.

Yamshchikov AV, Schuetz A, Lyon GM (2010) *Rhodococcus equi* infection. *Lancet Infect Dis* **10**: 350–359.