Resistance studies of erythromycin and rifampin for *Rhodococcus equi* over a 10-year period

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Abstract

This study sought to determine whether an increase in resistance of *Rhodococcus equi* to the antibiotics rifampin and erythromycin occurred over a 10-year period. This was carried out by the use of E test strips for rifampin and erythromycin to determine the MIC (minimum inhibitory concentration) values of *Rhodococcus equi* to this combination of antibiotics.

The findings of this study indicated that there was an increase in resistance of *Rhodococcus equi* to rifampin and erythromycin over the 10-year period. The MIC for rifampin increased from 0.081µg/ml in 1996 to 0.187µg/ml in 2006 and from 0.258µg/ml for erythromycin during the years prior to 2000 to 0.583µg/ml in 2006.

This finding suggests that there may be a problem in the treatment of *Rhodococcus equi* infections in foals in the future, particularly as the number of drugs available for treatment of *Rhodococcus equi* infection is limited because of the intracellular capabilities of this bacterium. Antibiotics used in its treatment have to be able to penetrate the polysaccharide cell wall of *Rhodococcus equi* as well as the alveolar macrophages in which the bacterium is capable of surviving.

Key words: antibiotic, erythromycin, foals, *Rhodococcus equi*, rifampin, resistance

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Introduction

Rhodococcus equi is a facultative, intracellular, non-motile, non-spore-forming, Gram-positive cocco-bacillus (Kedlaya and Ing, 2007). The bacterium has the name *Rhodococcus* because of its ability to form a red (salmon coloured) pigment. *Rhodococcus equi* was previously called *Corynebacterium equi* and currently is grouped with the aerobic actinomycetes (Kedlaya and Ing, 2007). *Rhodococcus equi* organisms most often induce chronic bronchopneumonia in young foals (Bertone *et al.*, 1998). *Rhodococcus equi* can also cause other clinical conditions such as intestinal disease, non-specific synovitis and sporadic abscesses, but these syndromes are not as common as the pneumonic syndrome. *Rhodococcus equi*-associated bronchopneumonia of young foals was originally identified in 1923 (Bertone *et al.*, 1998). *Rhodococcus equi* is largely a soil organism with simple growth requirements, which appear to be met perfectly by herbivore manure and summer temperatures in temperate climates (Prescott, 1991). Farms used for foal breeding over many years may thus become particularly dangerous for foals (Prescott, 1987).

Rhodococcus equi, although not the most common cause of pneumonia in foals from one to five months of age, has significant economic consequences due to mortality, prolonged treatment, surveillance programmes for early detection and relatively expensive prophylactic strategies (Prescott and Baggott, 1993). The susceptibility of foals under six months of age to respiratory infections may be due to a number of factors, including: immunodeficiency; overcrowding; heavy parasite burden; poor nutritional status; and heat stress (Prescott, 1993). There may also be a genetic basis for foal susceptibility to *Rhodococcus equi* infection based on the type of transferrin, an iron-binding protein that has bacteriostatic properties, in the blood (Mousel *et al.,* 2003). Also, only certain types of *Rhodococcus equi* are pathogenic: those that contain virulence-associated protein A -Vap A. (Giguere *et al.,* 1999). *Rhodococcus equi* pneumonia can progress to severe and extensive lung involvement prior to the development of clear clinical signs (Bertone *et al.,* 1998). Of the many bacterial and viral agents that can cause foal pneumonia, *Rhodococcus equi* is one of the most difficult to treat but has been shown

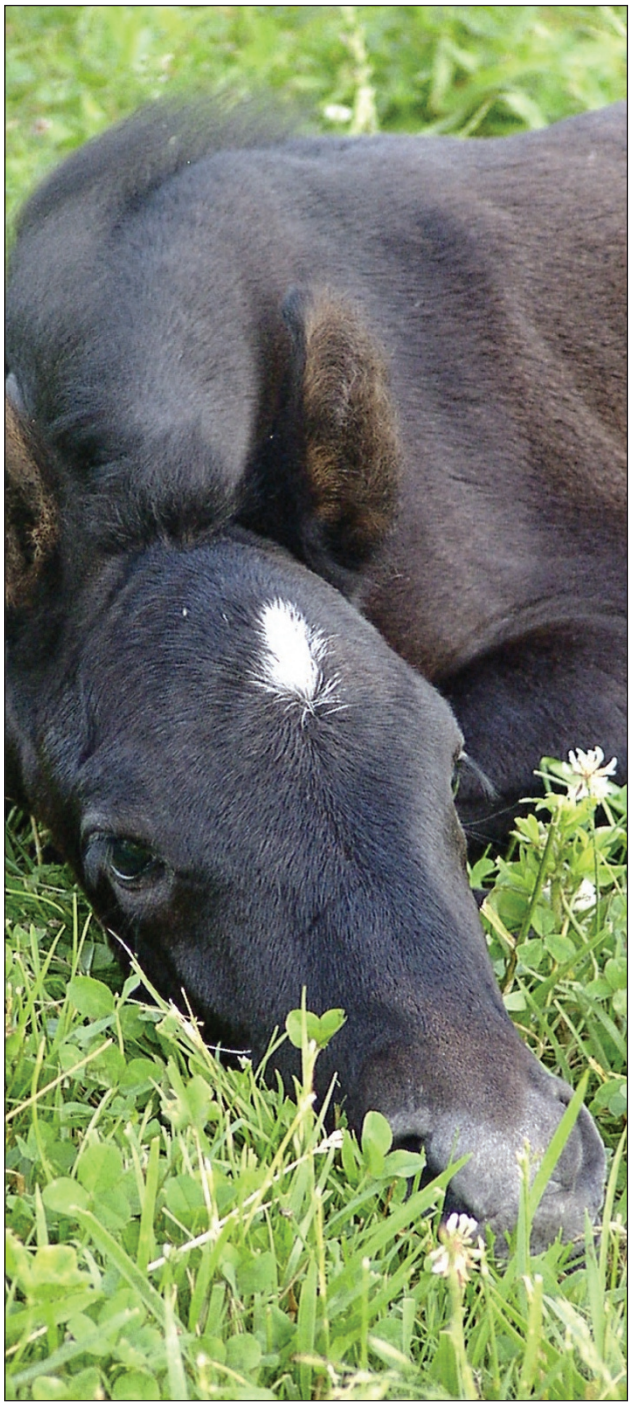

Figure 1: *Rhodococcus equi* organisms most often induce chronic bronchopneumonia in young foals.

to leave no residual lung damage following recovery (Ainsworth *et al*., 1993) and have no lasting affect on future racing performance (Ainsworth *et al*., 1998).

Rhodococcus equi was previously thought to be exclusively an equine pathogen, but in recent years *Rhodococcus equi* infection is occurring with increasing frequency in humans (Prescott, 1991). Infection in a human was first reported in 1967 in a 29-year-old man with plasma cell hepatitis receiving immunosuppressant medications (Kedlaya and Ing, 2007). It is found exclusively in immunocompromised individuals, such as those with AIDS, small cell carcinoma of the lung, malignant lymphoma, or recipients of kidney or bone marrow transplants (Todar, 2002).
The organism is particularly susceptible to erythromycin and clindamycin; the aminoglycosides, amikacin, gentamycin, neomycin and tobromycin, rifampin and vancomycin (Prescott, 1991). It is moderately susceptible to penicillin G, ampicillin and tetracyclines, and is usually moderately susceptible or resistant to first and second generation cephalosporins (Prescott, 1991).

As determined by FIC (fractional inhibitory concentration) indices, four combinations were synergistic: rifampin–erythromycin, rifampin–minocycline, erythromycin–minocycline and imipenem–amikacin (Nordmam and Roncon, 1992). Standard therapy involves the use of a combination of erythromycin and rifampin (Bertone *et al*., 1998). In order to treat *Rhodococcus equi* infection successfully, it is necessary to select an antibiotic which is not only effective against the organism *in vitro*, but which also has good distribution and activity in the lungs, with the ability to penetrate and sterilise caseous abscess cavities and to kill *Rhodococcus equi* organisms within neutrophils and macrophages (Hillidge, 1987). By using the combination of erythromycin and rifampin, not only are these drugs highly effective *in vitro*, they also penetrate macrophages well and have been shown to have additive and often synergistic activity *in vitro* (Prescott, 1991).

Erythromycin is a member of the macrolide group of antibiotics; it selectively inhibits protein synthesis in a broad range of bacteria by binding to the 50S subunit of the bacterial ribosome (Todar, 2002). Resistance to erythromycin can occur by methylation of different bases within the same region of the 23S rRNA (Todar, 2002). Erythromycin also blocks translation.

Rifampin is the most important synthetically modified member of the family of the rifamycins, antibiotic products of *Streptomyces mediterranei* (Prescott and Baggot, 1993). It is always combined with other antibiotics because of the ready development of resistance to the drug (Prescott and Baggot, 1993). Rifampin has the unique action among antibiotics of inhibiting RNA polymerase, the enzyme that catalyses the transcription of DNA to RNA (Prescott and Baggot, 1993). Rifampin binds to the beta subunit of the enzyme and causes abortive initiation of RNA synthesis (Prescott and Baggot, 1993). Rifampin is a bactericidal antibiotic with a wide spectrum of antimicrobial activity including activity against Gram-positive bacteria and anaerobes and some antiviral and antifungal activity (Prescott and Baggot, 1993). An attractive feature of rifampin is its ability to kill intracellular bacteria (Prescott and Baggot, 1993).

Dosages for the erythromycin–rifampin combination range from 5-10 mg/kg orally one- to two-times daily for rifampin to 10-37.5 mg/kg orally two- to four-times daily for erythromycin (Bertone *et al.,* 1998).

The length of treatment depends on the clinical response, the return of the complete blood count and fibrinogen concentrations to normal and the resolution of radiographic and ultrasonographic abnormalities (Bertone *et al.,* 1998). Treatment can last anything from 30 to 60 days, depending on the recovery of the foal.

Although the combination of rifampin and erythromycin has been used in the treatment of *R. equi* infection of foals for many years, adverse effects to treatment can occur, including hyperthermia, tachypnoea and, in rare circumstances, severe diarrhoea. In addition, occasional reports of resistance of *R. equi* to rifampin and erythromycin have been published over the last number of years (Kenney *et al.,* 1994; Takai *et al.,* 1997; Fines *et al*., 2001).

This study sought to determine whether an increase in resistance of *Rhodococcus equi* to the antibiotics rifampin and erythromycin occurred over a 10-year period.

Materials and methods

Ninety-four *Rhodococcus equi* isolates, each isolated from clinical cases of *Rhodococcus equi* pneumonia over a period of 10 years (1996-2006), were stored in cooked meat medium at -80°C. The isolates of *Rhodococcus equi* were analysed for their MIC values to rifampin and erythromycin over a 10-year period using E test strips (PDM Epsilometer; AB Biodisk, Solna, Sweden) of each of the two antibiotics. The range of the strips used was 0.002-32µg/ml for rifampin and 0.016-256µg/ml for erythromycin. The zones of inhibition showed by each of the isolates of *Rhodococcus equi* were clear and easily readable. ATCC (American Type Culture Collection) control strains were used throughout the study. These showed sensitivity at a low concentration.

Results

A total of 94 samples of *Rhodococcus equi* were analysed for their MIC values to rifampin and erythromycin over a 10-year period using E test strips of each of the two antibiotics. The samples were grouped according to the year in which they were detected and isolated within the Irish Equine Centre. The range of each of the antibiotic strips was 0.002-32µg/ml for rifampin and 0.016-256µg/ml for erythromycin.

Table 1: Means and ranges of the MIC concentration of *Rhodococcus equi* to rifampin and erythromycin for each year (µg/ml)

Year of sample (No. of samples tested)	Rifampin (range)	Erythromycin (range)
2006 (12)	0.187 (0.12 – 1.00)	0.583 (0.50 – 0.75)
2005 (10)	0.181 (0.12 – 0.64)	0.496 (0.25 – 0.75)
2004 (10)	0.142 (0.12 – 0.25)	0.333 (0.25 – 0.50)
2003 (10)	0.137 (0.03 – 0.25)	0.348 (0.25 – 0.50)
2002 (11)	0.132 (0.03 – 0.25)	0.400 (0.25 – 0.38)
2001 (11)	0.125 (0.12 – 0.12)	0.265 (0.12 – 0.38)
2000 (10)	0.114 (0.04 – 0.19)	0.233 (0.19 – 0.38)
Pre-2000 (20)	0.081 (0.03 – 0.12)	0.258 (0.12 – 0.38)

As can be seen in **Table 1**, it is clear that there was a marked increase in the MIC concentration of *Rhodococcus equi* to the antibiotic rifampin. This is shown as an increase of the MIC from 0.081µg/ml pre-2000 to 0.187 µg/ml in 2006. This is a major increase in MIC concentration for any bacterium, especially *Rhodococcus equi*, because of the difficulties in its treatment. There is also a notable increase in the MIC concentration of *Rhodococcus equi* to erythromycin. This increase is not as extreme as for rifampin but its increase nevertheless is noteworthy; it increased from 0.258µg/ml from pre-2000 to 0.583µg/ml in 2006.

Discussion

The MIC concentrations of rifampin to *Rhodococcus equi* increased from 0.081µg/ml pre-2000 to 0.187µg/ml in 2006. This is a marked increase in MIC concentration for any bacterial pathogen, including *Rhodococcus equi*. If the trend of increasing resistance continues at the same rate, the bacterium may soon be completely resistant to this antibiotic and new methods of treatment may have to be used. Low levels of resistance are 2-8µg/ml and high levels of resistance are >128µg/ml (Fines *et al*., 2001).

The MIC concentrations of erythromycin to *Rhodococcus equi* increased from 0.258µg/ml pre-2000 to 0.583µg/ml in 2006. This is also a large increase in MIC concentration representing an increase of 0.238µg/ml over a 10-year period. No resistant strains or samples of *Rhodococcus equi* to erythromycin were detected during this experiment and all of the samples were within the range reported by Prescott (1991). There is, however, a steadily increasing trend of resistance occurring in *Rhodococcus equi* to erythromycin and this increase may cause serious future problems in the treatment of *Rhodococcus equi* foal pneumonia. There is also evidence of resistance in the area of human medicine to these antibiotics (Asoh *et al.,* 2003).

The consequence of these findings is that there is an inevitable resistance occurring in *Rhodococcus equi* to rifampin and erythromycin and that, in the future, these antibiotics may be less effective in the treatment of foal pneumonia caused by *Rhodococcus equi*. Because of this, the antibiotics azithromycin and clarithromycin have been proposed as alternatives to erythromycin and rifampin for the treatment of *Rhodococcus equi* infections in foals. They are more chemically stable, have a greater bioavailability and achieve higher concentrations in phagocytic cells and tissues than erythromycin (Jacks *et al*., 2003). Also, persistence of high concentrations of azithromycin in bronchoalveolar cells after discontinuation of administration suggests that a shorter dosage regime may be applied (Jacks *et al*., 2001).

Conclusion

The overall conclusion is that there is an increase in resistance occurring in *Rhodococcus equi* to the antibiotics commonly used in its treatment; rifampin

and erythromycin. MIC concentrations of each of these antibiotics to the bacterium were determined over a 10-year period and a steady increase recorded for both antimicrobial agents.

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