

# Review of: "International spread of emerging multidrug-resistant *Rhodococcus equi*"

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Val-Calvo and collaborators describe for the first time the detection of MDR-*R. equi* 2287 clone outside the US, linked to the movement of horses. The manuscript is clear and well written. The figures are similarly well presented. The addition of more metadata will help determining the real scope of this finding (see comments below). It seems that Dr. Huber and Dr. Alvarez-Narvaez have been publishing quite a bit about MDR *R. equi* epidemiology and molecular mechanisms recently. I was surprised to find that Dr. Vazquez Boland did not reference some of their most recent work in this manuscript (see comments below).

Specific comments to be addressed:

1. "Systematically applied since the 1980's, no significant resistance was detected until the early 2000's following mass prophylactic application of the combination therapy at endemic farms in the United States (US) (5,6)". **Reference 6 is not suitable as it refers to a study conducted at a single farm in Kentucky. Please replace with Giguère et al. 2010 (<https://doi.org/10.2460/javma.237.1.74>) and Alvarez-Narvaez et al. 2021 (<https://journals.asm.org/doi/10.1128/MMBR.00011-21>)**
- "MDR-RE 2287 arose by co- acquisition of the conjugative plasmid pRErm46 and a specific rpoBS531F (TCGàTTC) mutation conferring high-level rifampin resistance (7, 8)". **Reference 7 is not suitable as the work of Anastasi et al. does not describe neither pRErm46, nor the specific *rpoB* mutation. Please remove citation.**
- "pRErm46 specifies resistance to macrolides, lincosamides and streptogramins via the *erm*(46) gene carried on TnRErm46, a highly mobile transposon, and to sulfonamides, streptomycin, spectinomycin, tetracycline and doxycycline via a class 1 integron (C1I) and associated *tetRA* determinant (9)." **This work is also covered in Alvarez-Narvaez et al. 2021 ([https://wwwnc.cdc.gov/eid/article/27/2/20-3030\\_article](https://wwwnc.cdc.gov/eid/article/27/2/20-3030_article)). Please add citation.**
- "Only detected so far in the US, MDR-RE was predicted to disseminate to other countries with the movement of equines (10)." **A more comprehensive epidemiology work is covered in Alvarez-Narvaez et al. 2021 (<https://journals.asm.org/doi/10.1128/MMBR.00011-21>). Please, add citation.**

- “Two equine clinical strains from necropsied foals in Ireland met the criterion: PAM 2528 recovered in 2016 and PAM 2578 in 2021.” **Please specify the animals’ primary cause of death. Was it *R. equi* pneumonia?**
- **Please, add corresponding citation and versions of FastTree and ParSNP programs.**
- **I am intrigued by the differences found with the different SNP-calling methods. Have the authors tried to construct a clone tree with ParSNP? Are there significant differences in tree topology between the two trees? How many SNP differences have been found with SNIPPY compared to ParSNP? I consider unfair that the authors blame ParSNP for introducing genome alignment errors while enhancing SNIPPY as a better tool, especially in the absence of supporting data. Both programs follow different approaches in the alignment process (contig versus read alignment), that could justify the potential differences in the outcomes too. Have the authors considered that SNIPPY may be too conservative (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6562250/>) and that it can be masking some of the real genetic variability?**
- **As a side note, two different lineages can be distinguished in the ParSNP tree too.**
- **Did the authors resequence the MDR-RE 2287 clonal strains? Otherwise, I assume the authors used the assemblies included in Alvarez-Narvaez et al. 2021 ([https://wwwnc.cdc.gov/eid/article/27/2/203030\\_article](https://wwwnc.cdc.gov/eid/article/27/2/203030_article))? If the latter is the case, some of the isolates were sequenced with short read technology (older strains) and some with long read technology (non-Irish younger strains). The expected sequencing errors and read length of these technologies are different. Do the authors consider that this may be affecting the clustering in the clone tree? Especially when using SNIPPY that aligns reads (and not contigs) to a reference genome? Please add a justification to the manuscript**
- **“This is taking place at a much slower pace with MDR-RE, likely because of the lesser opportunities for transmission afforded by horse trade and inter-horse contacts compared to human interactions and travel.” What about the fitness cost associated with the expression of AMR? Do the authors consider that a constant antimicrobial selective pressure is required for this clone to succeed against susceptible strains? Would this affect the expansion rate of the clone? Are the treatment/prophylactic strategies followed in Ireland and the other countries similar to the ones applied in the US? Please discuss this points in the manuscript.**
- **I also consider that to have a better idea of the scope of this finding, the authors should include some data regarding the number of *R. equi* isolates identified per country per year. It is not the same if only two *R. equi* isolates have been identified in the last 10 years in Ireland and both are resistant versus 100 isolates identified and only 2 are resistant.**

- “Pointing to an MDR 2287 subpopulation established in a farm(s) in New York State as the likely source of the isolates recovered in Ireland.” **Any information from the Irish farm that can back this finding up?**
- “The positioning of the Irish isolates in two separate sub-branches of the “New York” radiation (Figure 2) may indicate they represent independent, temporally distinct import events that took place around 2016 and 2021 involving different subclones”. **This is a very long shot, especially considering that your analysis does not have many strains and that they differ in just a few SNPs that may or may not be influenced by sequencing errors. It could also be caused by natural evolution from a common ancestor clone that arrived to the Irish farm in 2013, right? Please discuss this option in the manuscript.**
- “This suggests a transmission history in which a relatively recent MDR-RE 2287 subclone which acquired at some point a *ÄC1I-tetRA* deletion”. **Acquire a deletion is confusing. Please rephrase.**
- “... ,possibly originating from Kentucky (where MDR- RE emerged and is prevalent) (8, 9).”**These two citations are not suitable because they do not describe the epidemiology of *R. equi* in Kentucky. Please replace with the work of Dr. Huber (<https://doi.org/10.1128/AAC.01714-18>,<https://doi.org/10.2460/javma.258.6.648>, DOI: 10.1016/j.vetmic.2019.07.010).**
- “... the affected farm in Ireland received horses from America, Europe, UK and other Irish farms on a regular basis”. **See comment above. Any information about NY in particular?**