

# Final report summary

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Improved bacterial identification  
and antimicrobial susceptibility testing



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## Abstract

**Effective antimicrobial treatment requires careful selection of appropriate drug(s) based, initially, on results from previous cases. Ideally, drug choice is refined, if necessary, based on identification and sensitivity test results from the individual patient. This project was conducted to improve treatment of bacterial infections of horses by documenting the identity and antimicrobial susceptibility of bacteria from horses collected over a 10-year period, in two locations, from samples collected by private practitioners and from equine referral hospital populations.**

**Poor treatment results have been associated with the development of antimicrobial resistance and this resistance is an important consideration for equine health. Because of our close contact with horses, it is also a direct threat to human health associated with exposure to resistant bacteria. Newer techniques used in medical diagnostic laboratories for more rapid and accurate identification and antimicrobial susceptibility testing of bacteria were evaluated to facilitate improved antimicrobial use in horses.**

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## Background

Antimicrobial resistance has been reported in bacteria isolated from horses since the 1970s. The extent of the problem is increasing in human and veterinary medicine, due largely to widespread and indiscriminate or inappropriate antimicrobial use, and with subsequent increased costs of treatment and adverse outcomes. Knowledge of antimicrobial susceptibility is important for effective treatment of infectious diseases of horses, and is relevant to human health due to our close contact with horses.

Bacterial identification and antimicrobial susceptibility allows for targeted treatment, has been shown to improve treatment outcomes and may

reduce the emergence of resistance. There is limited information regarding antimicrobial susceptibility of bacteria from Australian horses, and results from overseas studies are not necessarily relevant. Studies in other species have suggested that practice type (referral or primary accession, hospital or community-based) can affect the expression of resistance, and have recommended collation of available data to inform use of available antimicrobials. Improved laboratory techniques now routinely used in medical diagnostic settings might further enhance identification and antimicrobial susceptibility testing for equine bacteria.

Compared with conventional techniques that can take more than 48 hours for preliminary identification, MALDI-TOF provides rapid (within minutes) identification of bacteria from single colonies or clinical specimens. Antimicrobial susceptibility testing using broth microdilution techniques to determine the minimum inhibitory concentration (MIC) for each bacteria-antimicrobial (bug-drug) combination allows more precise determination of susceptibility and improves drug dosing for each patient.

## Objectives

The aims of this project were to improve antimicrobial treatment of horses by collating existing culture and susceptibility results to inform antimicrobial selection, to document the emergence of resistance, and to assess the impact of newer laboratory techniques for improved bacterial identification and antimicrobial susceptibility testing. Study aims were achieved by:

1. Collation of results of conventional bacterial identification and susceptibility testing from two locations (New South Wales and South Australia) and two practice types (ambulatory private practices and veterinary teaching hospitals), collected over a 10-year period.
2. Identification of bacteria using matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF), a technique that rapidly recognises bacteria based on their unique protein 'fingerprint', and determination of bacterial susceptibility to antimicrobials based on microbroth MIC techniques. Results using these advanced techniques were compared with results from conventional laboratory tests to evaluate their impact on equine veterinary care.

## Research

This project reviewed human and animal publications on antimicrobial resistance and recommendations for optimal bacterial identification and antimicrobial use. Project objectives were then addressed in three studies.

Firstly, results of conventional laboratory techniques for identification and susceptibility testing of more than 1100 bacteria from horses were collated into a spreadsheet. Results were evaluated to assess whether antimicrobial resistance increased over time, and whether differences were evident based on sample type, practice location (NSW or SA) or practice type (ambulatory or hospital-based).

In the second study, MALDI-TOF identification was compared with conventional results for approximately 300 bacteria. Differences between MALDI-TOF and conventional identification were identified as major (defined as an inconsistency at family level or a lesser difference likely to influence clinical outcome) or minor (genus/species discrepancy or difference unlikely to affect clinical outcome).

Finally, results from conventional antimicrobial susceptibility tests were compared with MIC results for 229 bacteria from horses. Drug-bacteria combinations were recorded as sensitive, intermediate or resistant based on clinical breakpoints, and differences between the two techniques were reported as minor, major or very major errors based on established definitions and potential to affect treatment outcomes (a 'very major' error is defined as a test result of 'sensitive' for a drug that is likely to be ineffective, leading to treatment failure).

## Key findings

Increased resistance was observed in *Staphylococcus* and *Pseudomonas* species isolated from samples collected in the latter part of the study (2016-2020), compared with isolates from early samples (2010-2015). Gastrointestinal bacteria (Enterobacterales isolates), *Enterococcus* and *Pseudomonas* species were more likely to demonstrate extensive resistance. Differences in the type of bacteria recovered were observed between sample types (Figure 1), as well as between locations (SA and NSW) and between hospital-based and ambulatory practices. Antimicrobial susceptibility also varied between sample types (Figure 2).



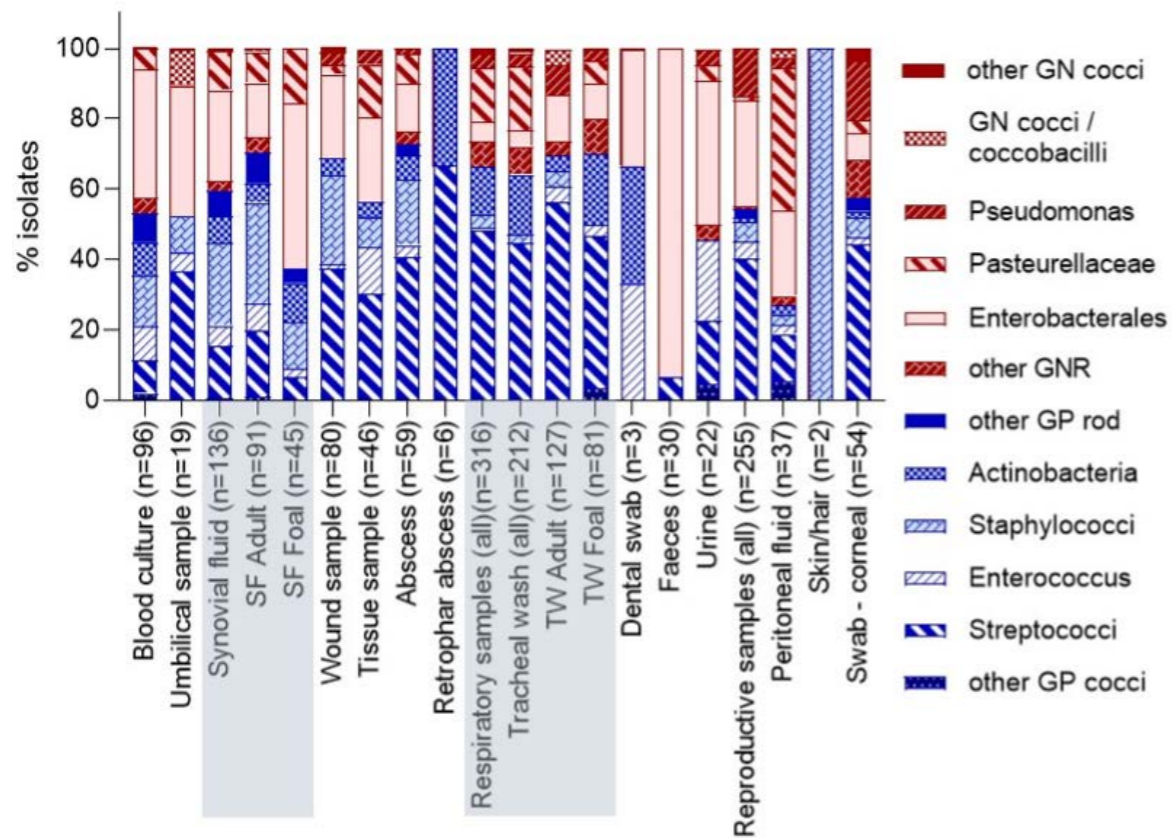


Figure 1. Bacterial isolates from different sample types.

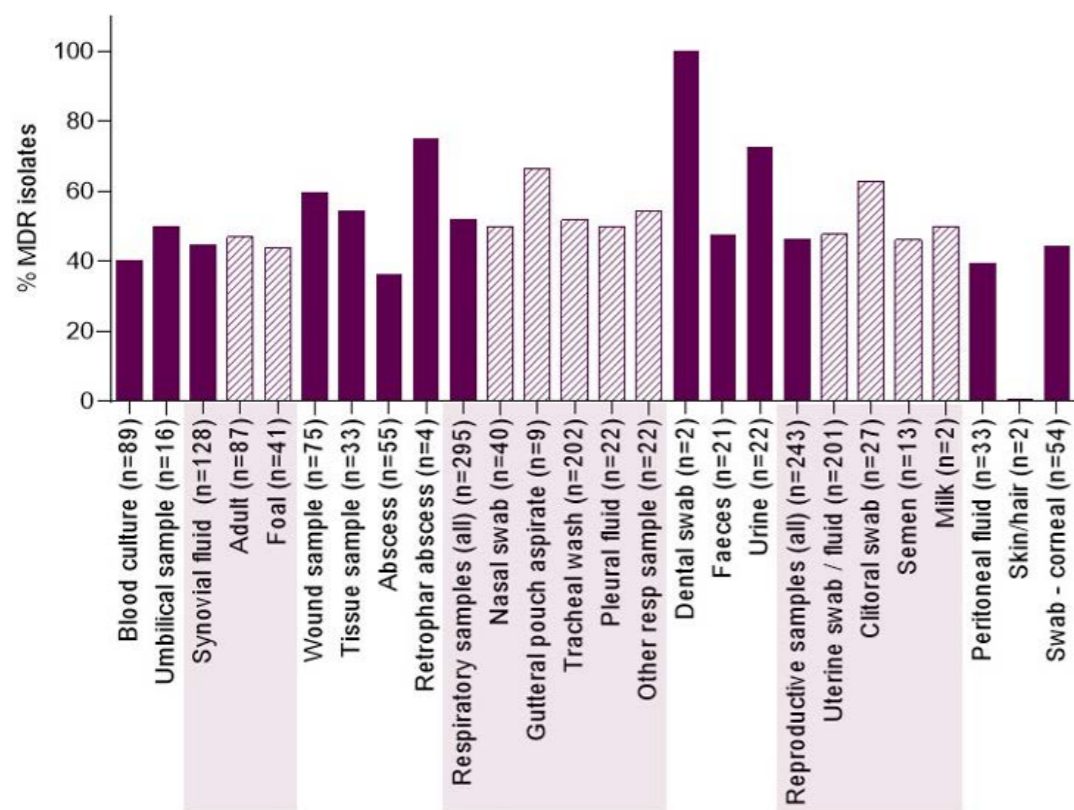


Figure 2. Effect of sample type on percentage of isolates exhibiting multiple drug resistance (MDR). The number of isolates tested is shown in brackets.

MALDI-TOF identification was more precise than conventional techniques, identifying 309 of 327 (94.5%) available isolates to species level, compared with 77.7% for conventional approaches. MALDI-TOF identification was identical to conventional results at species level for 77.2% (241/312) of isolates, but disagreement between MALDI-TOF and conventional results was evident on 78 occasions, including major disagreement (likely to affect clinical outcome) for 28 isolates (8.9%).

MIC results were available for 229 isolates tested against 20 antimicrobials (amikacin, gentamicin, penicillin, ampicillin, ticarcillin, ticarcillin-clavulanate, oxacillin, cefazolin, ceftazidime, ceftiofur, imipenem, tetracycline, doxycycline, chloramphenicol, enrofloxacin, erythromycin, azithromycin, clarithromycin, rifampin and trimethoprim-sulphonamide). Multiple drug resistance was observed in 79.5% of isolates, compared with 51.7% based on conventional results. Conventional results overestimated susceptibility for gentamicin and amikacin, but underestimated susceptibility to other drugs tested. Very major errors were observed for 76 drug-bacteria combinations (3.9%).

### Implications for industry

This project has demonstrated differences in bacterial species identified and antimicrobial susceptibility of isolates between regional locations in Australia, and between hospital-based and ambulatory practice. Observed increases in antimicrobial resistance are consistent with findings in other veterinary species and internationally, emphasising the need for careful use of available drugs. Bacterial culture and susceptibility results are important to inform empirical selection of antimicrobials for future patients, and to monitor for the emergence of AMR within local practice populations.

Data has been presented as a filterable, or stratified, antibiogram to facilitate antimicrobial selection. Guidelines for the ongoing collation of antimicrobial susceptibility data, and for the selection and judicious use of antimicrobials in horses, have been developed. The use of newer laboratory techniques – MALDI-TOF for bacterial identification and MIC testing for characterisation of bacterial susceptibility – proved fast and effective, and provided greater accuracy than conventional techniques, suggesting they are processes likely to improve patient outcomes.

The results of this work promise economic benefits to equine industries through more astute and appropriate use of antimicrobial drugs, ensuring optimal treatment of each individual animal. At an industry level, measures to delay or prevent the emergence of AMR will ensure that our limited antimicrobial arsenal remains effective.

### Acknowledgements

The contributions of laboratory staff at the Veterinary Diagnostic Laboratory (VDL) at Charles Sturt University (NSW) and at the Australian Centre of Antimicrobial Resistance Ecology (ACARE) at The University of Adelaide (SA) in identifying and susceptibility testing clinical samples submitted over the duration of this project are gratefully acknowledged.

In particular, the work of Lyn Matthews and Tania Veltman was invaluable during completion of this project, and Ken Lee and Natalie Allison also provided laboratory expertise in completing preliminary and early phases of this project. Dr Patrick Shearer assisted with retrieval of submission information for samples submitted to the VDL, and Madeline O'Brien and Dr Danielle Crosby with collation of results from the VDL.

The excellent work of research students from the University of Adelaide – Jess Ellis, Hollie Kerr, Charlotte Camail-Pearce and Jennifer Connolly – contributed to study outcomes, as did the prior work of CSU students (now graduates) Amanda-Lee Charman and James Mizzi.

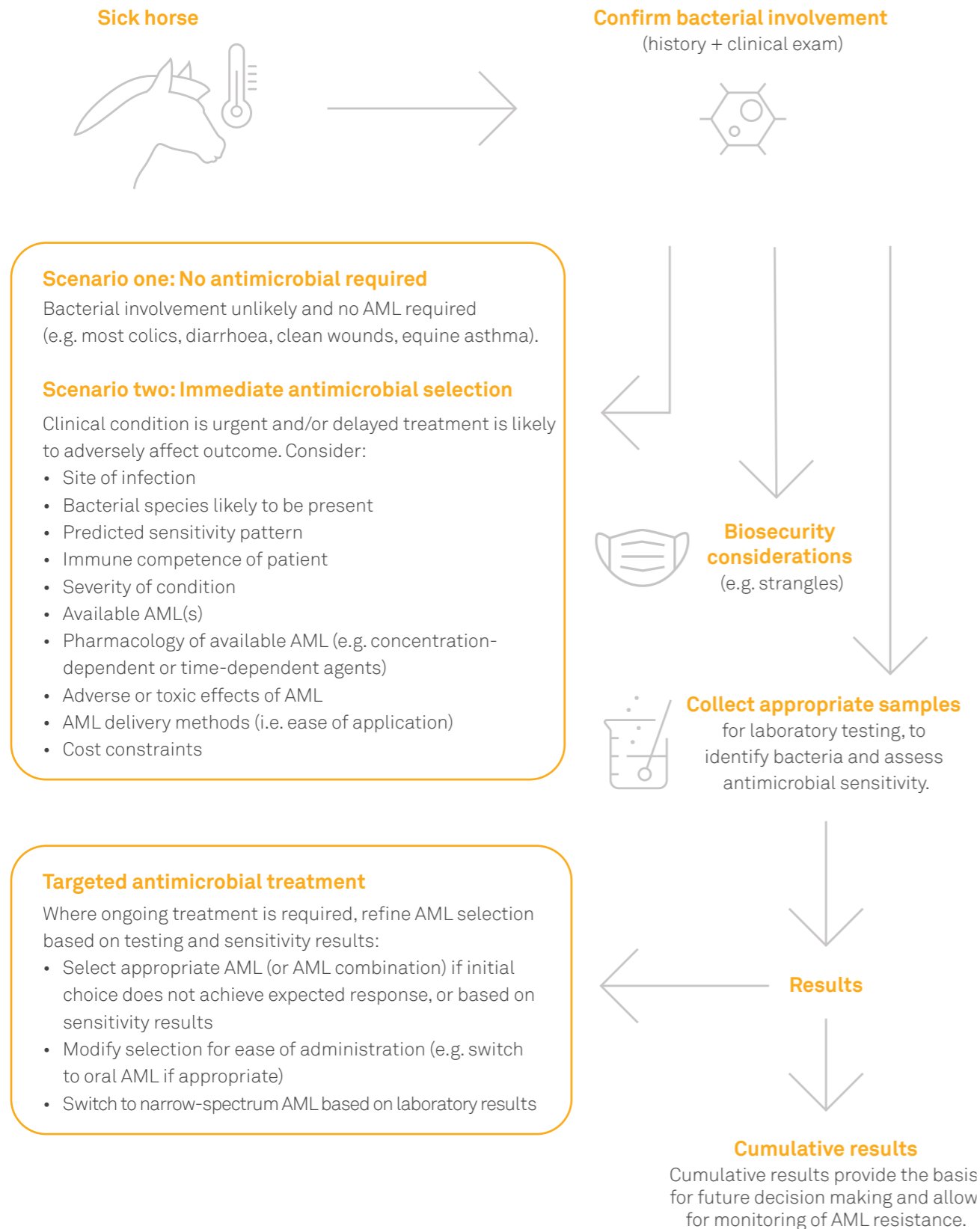
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## Best practice antimicrobial (AML) treatment of horses



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AgriFutures Australia Publication No. 22-056  
AgriFutures Australia Project No. PRJ-011234

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