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AUSTRALIAN Pork®

# Standardisation of Antimicrobial Susceptibility Testing Methods Used for Porcine Pathogens

## Final Report
## APL Project 2016/2242

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## Executive Summary

One of the key steps in controlling and managing bacterial infections in animals is by accurate identification and antimicrobial susceptibility testing of the disease-causing pathogens. In Australia, veterinary diagnostic laboratories that service the pig industry utilise different methods for culture, identification and antimicrobial susceptibility testing of porcine bacterial pathogens. The resistance data generated by different standards and methods cannot be compared. This affects the control and management of diseases on a farm level and limits the ability to accurately measure the antimicrobial resistance of bacterial pathogens within and across production units. Standardisation of these methods will allow for benchmarking of antimicrobial resistance and facilitate the reduction of antimicrobial resistance in the pig industry.

Standardisation of antimicrobial resistance testing will also allow for evaluating the success of intervention strategies used by the pig industry to reduce antimicrobial resistance among commensal and pathogenic bacteria. It is also envisaged that standardised methods may allow for decreased test cost via the potential for automation, and the ability for bulk purchase of reagents across the diagnostic network.

To identify the key issues facing veterinarians and the main barriers for veterinary diagnostic laboratories that service the pig industry, we undertook the following activities for developing standardised bacterial isolation and antimicrobial susceptibility testing.

- A survey of veterinarians was undertaken to identify key porcine pathogens in Australia and the drugs used to treat each pathogen group. The veterinarian survey also enabled the investigating team to identify the needs for improvement in certain areas (e.g. need for MIC testing and standardisation of isolation).
- A survey of veterinary diagnostic laboratories was completed to identify methods used by laboratories for isolation, identification and antimicrobial susceptibility testing for porcine pathogens. In addition, key barriers for the standardisation of antimicrobial susceptibility testing were also identified.
- A joint meeting of veterinary diagnostic laboratories and key veterinarians was held at the Australian Centre for Antimicrobial Resistance Ecology conference in July 2018, conducted to discuss the barriers and enablers for standardising bacterial isolation and antimicrobial susceptibility testing.
- The data and feedback compiled through the above mentioned activities were then discussed by a panel of experts in first week of June 2018 in the Gold Coast. The experts included Dr. Aileen Vanderfeen (Ace Lab Services), Dr. Joanne Mollinger (QLD DPI), Prof. David Jordan (NSW DPI) and Dr. Sam Abraham (Murdoch University).

This meeting led to the following outcomes, which were also discussed with Prof. Darren Trott (University of Adelaide):

- Minimum inhibitory concentration (MIC) antimicrobial susceptibility using Clinical and Laboratory Standards Institute (CLSI) guidelines was recommended for porcine pathogens
- A standard, Australia-specific antimicrobial susceptibility testing panel was developed for manufacturing with SensititreTM (Thermo Fisher) and is currently available in Australia.

- Antimicrobial susceptibility testing method using SensititreTM (Thermo Fisher) following the clinical laboratory standards protocol was recommended
- Reporting of antimicrobial susceptibilities was also identified and recommended as part of this report
- Simplified bacterial isolation protocols for key porcine pathogens were developed and recommended.

The key recommendation arising from this project is that all veterinary diagnostic laboratories that service the Australian pig industry adhere to the protocols recommended in this report for bacterial isolation and antimicrobial susceptibility testing for obtaining consistent laboratory diagnoses and antimicrobial susceptibility data. It is reasonable to expect that laboratories would require additional training and support to implement these recommendations. As part of the ongoing efforts in combating antimicrobial resistance, reference laboratories such as the Antimicrobial Resistance and Infectious Diseases Laboratory at Murdoch University and Australian Centre for Antimicrobial Resistance Ecology at University of Adelaide will be in a position to offer support and training for laboratories requiring additional support and training.

The proposed standardisation of bacterial isolation, antimicrobial susceptibility testing and reporting will enable:

- Accurate identification of bacterial pathogens and antimicrobial resistance which will assist the veterinarians in managing bacterial infection and antimicrobial resistance.
- Facilitation of ongoing farm-level surveillance of pathogens and antimicrobial resistance monitoring for individual farms for reducing bacterial infections
- Consistency of reporting of bacterial identification and antimicrobial resistance
- Enhanced management of antimicrobial resistance in the Australian Pig Industry.

Overall the project has led to some significant outcomes, however for long term success there is a need for on-going dialogue and collaboration with both veterinarians and veterinary diagnostic laboratories that service the pig industry.

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## 1. Background to Research

The Australian pork industry is currently undertaking a surveillance program on antimicrobial resistance (AMR) in pigs with support from the Commonwealth Government. An antibiotic usage tool has been developed (through the Pork CRC) and adoption of this tool by producers, in association with their veterinarians, to provide information on the quantity of antibiotics (and number of treatments) administered per standard pig unit is imperative to enable industry to demonstrate practice change in antibiotic use. However, producer understanding of the risks associated with AMR remains relatively low (relative to antimicrobial residues that are monitored through NRS).

One of the key steps in controlling and managing bacterial infections in animals is by accurate identification and antimicrobial susceptibility testing of the disease causing pathogens. In Australia, veterinary diagnostic laboratories that service the pig industry utilise different methods for culture, identification and antimicrobial susceptibility testing of porcine bacterial pathogens. For example veterinary diagnostic laboratories use a number of different standards (Clinical Laboratory Standards Institute (CLSI) or Calibrated Dichotomous Sensitivity (CDS)) and methods (minimum inhibitory concentration (MIC) testing or disc diffusion) for antimicrobial susceptibility testing. The resistance data generated by different standards and methods cannot be compared. This affects the control and management of diseases on a farm level and limits the ability to accurately measure the AMR of bacterial pathogens within and across production units. Standardisation of these methods will allow for benchmarking of AMR and facilitate the reduction of AMR in the pig industry. Standardisation of AMR testing will also allow for evaluating the success of intervention strategies used by the pig industry to reduce AMR among commensal and pathogenic bacteria. It is also envisaged that standardised methods may allow for decreased test cost via the potential for automation, and the ability for bulk purchase of reagents across the diagnostic network. This demonstrates the need for the standardisation of culture, identification and susceptibility testing of bacterial pathogens.

## 2. Objectives of the Research Project

1. Standardisation of antibiotic sensitivity testing methodologies used, including standard operating procedures for culturing different microbial species, in Australian veterinary laboratories that service the pig industry to ensure consistency in reporting.

2. Identify potential for sensitivity testing methodologies to be automated to manage cost issues.

3. Conduct a survey of diagnostic laboratories that service the pig industry to understand methodologies being used for antibiotic sensitivity testing.

4. Conduct a joint meeting involving key pig veterinarians and key laboratory personnel to discuss methodologies being used, to ensure consistency in antibiotic sensitivity reporting between laboratories. This includes identifying the requirements for timely collation of data for providing national summaries to industry and veterinarians.

## 3. Results

Based on the information provided in the surveys and meetings with expert microbiologists, best-practice procedures for the isolation and culturing of porcine bacterial pathogens were discussed and agreed upon. Summarized below in Table 1, are details pertaining to the media and conditions specific to each pathogen that should be used for their isolation and culturing.

*Table 1 Culture conditions and agar used for the isolation of major Australia porcine pathogens*

| **Porcine pathogens** | **Isolations Agar and conditions** |
|---|---|
| *E. coli* | Sheep blood agar/ MacConkey agar cultured in aerobic |
| *Salmonella* (for clinical infections) | Sheep blood agar/ MacConkey agar/ XLD Agar or other *Salmonella* selective agar cultured in aerobic |
| *Pasteurella multocida* | Sheep blood agar/ MacConkey agar cultured in aerobic |
| *Staphylococci* | Sheep blood agar/ MacConkey agar cultured in aerobic |
| *Clostridium* (*perfringens/ septicum/ botulinum*) | Sheep blood agar cultured in Anaerobic conditions |
| Respiratory Pathogens<br>*Actinobacillius pleuropnuemoniae, Streptococcus suis, Haemophilus parasuis, Truperella pyogenes* | Sheep blood agar with *Staphylococcus aureus* streak/stab (Nurse Culture) in $CO_2$ and MacConkey agar cultured in aerobic |
| *Fusobacterium necrophorum* | Sheep blood agar cultured in Anaerobic conditions |
| *Brachyspira* spp. | Direct platting on to VSSR Agar cultured in Anaerobic conditions at 42°C |
| *Listeria* spp. | Direct platting on to Sheep blood agar cultured in cultured in aerobic |
| *Erysipelothrix rhusiopathiae* | Sheep blood agar cultured at in $CO_2$ and MacConkey agar |
| *Yersinia* | Sheep blood agar cultured in aerobic and Cefsulodin-Irgasan-Novobiocin (CIN) agar cultured at 30°C in aerobic |
| *Brucella* | Sheep blood agar and BSA cultured in $CO_2$ and MacConkey agar cultured in aerobic |
| *Actinobaculum suis* | Sheep blood agar/ MacConkey agar in aerobic conditions and sheep blood agar cultured in anaerobic conditions |
| *Bordetella bronchoseptica* | Sheep blood agar/ MacConkey agar cultured in aerobic |
| *Mycoplasma (hyosynoviae/ hyorhinis)* | Fris Modified Broth (enrichment) and culture on to Mycoplasma Agar |

Additionally, the meeting of experts and survey results allowed the determination of the most relevant and important antimicrobials for treating porcine bacterial pathogens. An antimicrobial susceptibility testing panel was subsequently developed for these antimicrobials, taking into consideration the necessary concentration ranges required to classify pathogens as susceptible or resistant (Table 2).

*Table 2 Proposed AMR panel for Australian Livestock with breakpoints*

| **Antimicrobial** | **Sensititre™ Concentration Range (µg/mL)** |
|---|---|
| Ampicillin | 0.25-16 |
| Apramycin | 4-32 |
| Ceftiofur | 0.25-8 |
| Colistin | 0.12-8 |
| Florfenicol | 0.25-8 |
| Lincomycin | 2-8 |
| Neomycin | 4-32 |
| Penicillin | 0.12-8 |
| Spectinomycin | 8-128 |
| Tetracyclines | 0.5-16 |
| Tiamulin | 8-32 |
| Tilmicosin | 2-32 |
| Trimethoprim/sulfamethoxazole | 0.25/4.75 – 8/152 |
| Tulathromycin | 8-64 |
| Erythromycin | 0.12-16 |
| Ciprofloxacin | 0.0156-4 |
| Cefoxitin | 0.5-32 |

### *3.1 Custom Porcine Sensitire MIC Panel*

Based on the recommendation from this project Sensititre™ has developed an Australia-specific porcine panel. This panel now available to order from ThermoFisher Australia and is stocked in Australia. The panel is suitable for most Gram negative and positive porcine pathogens.The panels is shown below and the can be ordered using the following details:

**Product code:** **TRKAUSMUPIG**

**Product Description:** **Custom Porcine Panel AU**

MIC Plate Layout:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CIP | CIP | CIP | CIP | CIP | CIP | CIP | CIP | TUL | TUL | TUL | TUL |
| | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 |
| B | TIA | TIA | TIA | NEO | NEO | NEO | NEO | TIL | TIL | TIL | TIL | TIL |
| | 8 | 16 | 32 | 4 | 8 | 16 | 32 | 2 | 4 | 8 | 16 | 32 |
| C | COL | COL | COL | COL | COL | COL | SXT | SXT | SXT | SXT | SXT | SXT |
| | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 0.25/4.75 | 0.5/9.5 | 1/19 | 2/38 | 4/76 | 8/152 |
| D | XNL | XNL | XNL | XNL | XNL | XNL | FFN | FFN | FFN | FFN | FFN | FFN |
| | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
| E | PEN | PEN | PEN | PEN | PEN | PEN | PEN | SPE | SPE | SPE | SPE | SPE |
| | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 8 | 16 | 32 | 64 | 128 |
| F | ERY | ERY | ERY | ERY | ERY | ERY | ERY | ERY | APR | APR | APR | APR |
| | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 4 | 8 | 16 | 32 |
| G | FOX | FOX | FOX | FOX | FOX | FOX | FOX | TET | TET | TET | TET | TET |
| | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 0.5 | 1 | 2 | 4 | 8 |
| H | AMP | AMP | AMP | AMP | AMP | AMP | AMP | LIN | LIN | LIN | TET | POS |
| | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 2 | 4 | 8 | 16 | |

| ANTIMICROBICS | |
|---|---|
| CIP | Ciprofloxacin |
| TIA | Tiamulin |
| COL | Colistin |
| XNL | Ceftiofur |
| PEN | Penicillin |
| ERY | Erythromycin |
| FOX | Cefoxitin |
| AMP | Ampicillin |
| NEO | Neomycin |
| SXT | Trimethoprim / sulfamethoxazole |
| FFN | Florfenicol |
| TIL | Tilmicosin |
| SPE | Spectinomycin |
| TET | Tetracycline |
| LIN | Lincomycin |
| TUL | Tulathromycin |
| APR | Apramycin |
| POS | Positive Control |

## 4. Discussion

Antimicrobial resistance is a vast and complex issue that must be addressed by many stakeholders in the pork industry and at many levels of understanding. The first step in ensuring accurate and meaningful communication between parties about AMR is to ensure observations on resistance are made in the most precise way possible. The manner of collection of observations must also allow for comparisons to be made between the different entities involved (isolates, animals, herds, etc.) and through time (so to judge changes in response to efforts on antimicrobial stewardship). For this reason "standardisation" of the technical approach to measuring and reporting AMR in the pork industry is essential. With standardisation in place it is much more likely that constructive dialogue about AMR can occur and result in profitable changes in the management of pigs, farm environments and the food supply.

This work has succeeded in delivering a standardised approach to the assessment of the AMR status of bacterial pathogens from pigs. The outcomes are derived from a broad-based consultation with parties comprised of "users" of AMR information (e.g. veterinarians), "makers" of AMR information (e.g. the laboratories involved in testing) and "experts" in AMR information. The advantage of this comprehensive approach is that the outcomes have applicability at many practical levels of interest, including herds, laboratories and across-industry so that the needs ranging from individual diagnosticians to future surveillance needs for the industry as a whole can all be met. The major elements of standardisation achieved were:

- Identification of authoritative and nationally-applicable methods for isolation of bacterial pathogens of pigs in veterinary laboratories.
- Confirmation of the optimal laboratory approach for measurement of MIC being the broth microdilution assay as defined by CLSI documentation.
- Derivation of a standard panel of drugs for assessment of the MIC of pathogens which will meet both the diagnosticians' needs for choosing amongst treatment options and form a basis for monitoring resistance in a herd over time as part of antimicrobial stewardship.

With standardised procedures now available for the veterinary laboratories servicing the pork industry, advantages can be realised, including:

- Increased consistency in the interpretation of AMR diagnostic assays.
- Providing herd owners and veterinarians with greater confidence in AMR testing.
- Enhanced ability for laboratory results to be collated at the herd-level to inform antimicrobial stewardship.
- An improved capacity to meet customer and trade driven demand for surveillance data.
- Expansion of the amount and quality of expertise on AMR available to veterinary laboratories.

## 5. Implications & Recommendations

1. All laboratories that service the pig industry should follow the bacterial isolation procedures listed in Table 1.
2. Use of MALDI-TOF for the identification of common bacterial pathogens is the preferred method due to its consistency in identifying common bacterial pathogens. However, use of conventional biochemical tests is also acceptable.
3. MIC testing should be performed as part of routine antimicrobial susceptibility testing using the custom made Antimicrobial Susceptibility Test (AST) panels specific for Australian porcine pathogens described in Table 2.
4. Use of Sensititre™ panels with the antimicrobials shown in table 2 is now available through Thermo Fisher. The use of these panels is recommended due to the strict quality control process for manufacturing pre-prepared plates and their ease of use in routine diagnostic testing. Please note: Sensititre™ is the only system that can prepare custom panels for AST testing.
5. The most up-to-date CLSI recommended methodology is to be used for MIC testing using the most appropriate and up-to-date breakpoints.
6. For antimicrobials with no clinical breakpoints (Eg; Apramycin), an Australia specific Epidemiological Cut off (ECOFF) value should be calculated and used for reporting wild-type and non-wild-type categories.
7. An annual review of the custom Australia specific AST panel is recommended for optimising treatment options for porcine bacterial pathogens and on-farm AMR monitoring.
8. Semi-automated (Sensititre™) MIC plate preparation is preferred for undertaking MIC work for consistency and accuracy. However, this can be done manually as per instructions from the manufacturer.
9. Veterinary Diagnostic Laboratories should selectively report AST results for registered veterinary drugs only for each category of pathogens (Gram Positive Vs Gram negative). Diagnostic reports including S, I or R interpretation, MIC values and MIC breakpoints to be provided where available.
10. Reporting of critically important antimicrobials (not registered for use in pigs) is not recommended for routine reporting.
11. Each farm should be able to request a consolidated AMR report every six months for all antimicrobials including critically important antimicrobials (CIA) not registered for use in livestock, for on farm monitoring of AMR in porcine pathogens.
12. It is recommended that for all isolates that are resistant to CIAs, veterinary diagnostic laboratories undertake additional genetic testing (e.g. PCR for CIA resistance genes and/ or whole genome sequencing) to provide further information on the origin and public health significance of CIA-resistant bacterial pathogens. If such testing is not available, the isolates can be sent to university reference labs such as Australian Centre for Antimicrobial Resistance Ecology (ACARE, University of Adelaide) and Antimicrobial Resistance and Infectious Disease Laboratory (AMRID, Murdoch University)

## 6. Technical Summary

### Standardisation of Antimicrobial Testing of Porcine Bacterial Pathogens

**Purpose:**

- Standardisation of isolation, identification and antimicrobial susceptibility testing of porcine bacterial pathogens.

**Take home messages:**

- An Australia specific antimicrobial susceptibility testing panel was developed for manufacturing with Sensititre™
- Antimicrobial susceptibility testing method using Sensititre™ (Thermofisher) and following clinical laboratory standards protocol was recommended
- Simplified bacterial isolation protocols for key porcine pathogens were developed and recommended
- The standardisation of these protocols will enable accurate identification of bacterial pathogens and antimicrobial resistance, which will assist the veterinarians in managing bacterial infections and antimicrobial resistance
- National industry guidelines for achieving consistency in reporting of bacterial identification and antimicrobial susceptibility of porcine pathogens were developed.
- Ongoing farm-level surveillance of pathogens and antimicrobial resistance monitoring for individual farms for reducing bacterial infection