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United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034429

Project Title

Enhancing veterinary diagnostic capabilities for antimicrobial susceptibility testing

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-69015-45830	**Cumulative Award Amt.** $299,922.00
Proposal No. 2024-11401	**Multistate No.** (N/A)
Project Start Date Feb 01, 2026	**Project End Date** Jan 31, 2028
Program Code [A1366] Mitigating Antimicrobial Resistance Across the Food Chain	

Project Director

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VETERINARY MICROBIOLOGY AND PREVENTIVE MEDICINE - CVM

Non Technical Summary

When faced with a patient needing treatment for a bacterial infection, both human physicians and veterinarians rely on laboratory testing for antimicrobial resistance (AMR) to help direct selection of antibiotic therapy. The ability for laboratories to rapidly respond in the face of emerging antimicrobial resistance threats to both human and animal health is also critical to enable identification and containment of such threats. Within this Seed Grant project, we will repurpose existing technology - the Hewlett Packard D300e digitaldispensing system (D300e) - to develop a reproducible and cost-effective method for on-site generation within veterinary diagnostic laboratories of custom antimicrobial susceptibility testing (AST) supplies in the form of 96-well broth microdilution plates to allow for rapid response to changing needs for veterinary AST. Following initial on-site validation, we aim tosuccessfully freeze andshipfrozen custom platesto outside labs tovalidatefor use following overnight shipment. As a proof-of-concept, we will then use these custom plates to generate critical data necessary for improving antimicrobial stewardship in poultry

production.Validation of the D300e to rapidly generate custom AST plates will allow veterinary diagnostic laboratories to incorporate this technology in their workflows to rapidly address emerging AMR threats to animal production systems in the future, thereby improving antimicrobial stewardship.

Animal Health Component

Animal Health Component: 100%

Research Effort Categories

Basic	0%
Applied	50%
Developmental	50%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
311	3999	1100	100%

Knowledge Area

[311] Animal Diseases

Subject Of Investigation

[3999] Animal research, general

Field Of Science

[1100] Bacteriology

Keywords

Antimicrobial resistance, diagnostic laboratory, susceptibility testing

Goals / Objectives

Antimicrobial susceptibility testing (AST) is an essential component of antimicrobial stewardship(AMS) in both human and animal health and is performed daily in veterinary diagnostic labs (VDLs) to assist veterinarians with clinical decision making regarding antimicrobial selection. Within this Seed Grant project, our first objectiveis to validate the use of the Hewlett Packard D300e digital dispensing system (D300e) for generation of custom 96-well plates for performing broth microdilution AST at VDLs to allow for rapid response to changing needs for veterinary AMS. Current commercially available options for custom AST plates are expensive and require a months-long process from concept to receipt, and the use of the D300e has been previously validated by the Centers for Disease Control and Prevention (CDC) for on-site/on-demand generation of custom broth microdilution AST plates for detection of antimicrobial resistance (AMR) in human laboratories. Following initial on-site VDL validation, we aim tosuccessfully freeze and shipfrozen custom platesto outside labs tovalidatefor use following overnight shipment, which has yet to be done for human or animal testing. The long-term goal of ourwork is to use the D300e to generate extended dilutionminimum inhibitory concentration (MIC) data necessary for generation of epidemiological cut offvalues (ECVs) that are missing

for major veterinary and zoonotic pathogens. As a proof-of concept, the final objective for this seed grant will be to utilize the D300e to generate custom broth microdilution AST plates that will be shipped to VDLs and used to generate data that can be used to begin to establish ECVs for poultry pathogens and commonly tested antibiotics which are currently lacking and negatively affect antimicrobial stewardship in these production systems.

Project Methods

Objective 1: Validate use of the HP digital dispenser D300e for generating broth microdilution plates for veterinary AST. Task 1.1: Onboarding and validation of D300e generated AST plates in the ISU VDL. Initial validation of the D300e unit will be done at the ISU VDL and will follow previously published validations (Smith and Kirby, 2016) as well as CLSI guidelines (CLSI, VET01). All antibiotic stock solutions will be stored as aliquots at -80°C and discarded after use. Reference strains of bacteria corresponding to appropriate QC strains according to CLSI will be purchased from the American Type Culture Collection (ATCC), stored at -80°C, and passaged according to CLSI guidelines (CLSI, VET01). The following QC organisms will be initially targeted for validation: E. coli, Staphylococcus aureus, and Mannheimia haemolytica. The D300e will be programmed to generate serial 2-fold dilutions of antimicrobials of interest in 96 well plates using cation adjusted Mueller-Hinton broth (CAMHB). As a comparator, commercially available AST testing plates (Sensititre™; ThermoFisher) currently utilized in VDL AST workflows will initially be run side-by-side with the D300e generated plates to ensure that the QC organisms are resulting in equivalent MIC values between plates. CLSI guidelines will be followed for inoculum preparation and plate incubation. Both uninoculated control wells (to test for contamination during plate setup) and positive growth control wells will be included on all plates. Plates will be initially read both visually and using commercially available broth microdilution testing and analysis platforms (Sensititre™ Complete Automated AST System, ThermoFisher; BIOMIC V3, Giles Scientific). Precision and accuracy tests will be performed using a minimum of three technical replicates performed on a minimum of three separate days (biological replicates) as previously described (Smith and Kirby, 2016). Following verification using the ATCC QC strains, 20 clinical isolates matching the QC organisms will be utilized as a side-by-side comparison to commercially available test plates to ensure repeatable results. Based on the methods developed, an SOP manual will be generated and used to train both ISU VDL staff as well as outside VDL staff (Task 1.3) in appropriate creation and use of D300e generated AST plates.Task 1.2: Validation of stored and shipped HP D300e-generated AST plates. Validated plates generated using the D300e at ISU will be frozen at -80°C and later shipped overnight using dry ice to outside labs. Labs will be asked to test half of the frozen plates upon arrival and half following additional storage for 1 month and 6 months at -80°C to determine the repeatability of test results. For all testing, labs will be asked to run AST testing side-by-side with both the ATCC QC strains listed above and commercial plates. When complete, an additional SOP will be generated and used to inform VDL staff from outside labs in appropriate use of frozen and shipped D300e-generated plates.Objective 2: Utilize D300e created AST plates to generate data for ECVs for poultry pathogens. Task 2.1: Generate 250 AST plates using the D300e for testing of Pasteurella multocida against commonly used antibiotics in poultry. Communication with key stakeholders has revealed that 1) the lack of poultry clinical breakpoints negatively impacts AMS in this key livestock sector, 2) development of clinical breakpoints for poultry is unlikely given that differences in pharmacokinetics exist between poultry species, and 3) the use of ECVs as developed for aquaculture (CLSI, VET04) may represent a viable approach to improving AMS in poultry. An informal survey of poultry diagnosticians has been performed, and P. multocida was highlighted as a key pathogen for which AST interpretations would be beneficial. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) database was surveyed, and the following antibiotics present on the Sensititre™ AVIAN1F AST plate were identified as missing ECV data: clindamycin, novobiocin, penicillin, streptomycin, sulfadimethoxine, sulfathiazole, and tylosin. Therefore, utilizing the SOPs generated under Objective 1, extended dilution AST plates will be designed and generated at ISU to test clinical isolates using the listed antibiotics. QA/QC will be performed on the plates at the ISU VDL prior to shipment.Task 2.2: Utilize the NIAMRRE laboratory network to perform AST testing to generate missing ECVs for P. multocida in poultry. Utilizing our existing network of NIAMRRE laboratories, frozen D300e generated plates will be shipped to VDLs for testing. To meet EUCAST requirements (EUCAST, 2021), a minimum of 5 laboratories will be used to test a target of 250 P. multocida isolates on the D300e generated plates. Data will be compiled, MIC distributions generated, and ECVs

calculated using ECOFF Finder (Turnidge et al., 2006). The final data will be shared with EUCAST and CLSI to help promote the use of ECVs in poultry.