Errors in Antibiotic Susceptibility Testing and the Association with Licensure and Certification: A Contributor to Antibiotic Resistance

A dissertation

Presented to the Faculty of the College of Health and Human Services

of Trident at American Intercontinental University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Health Sciences

by

HEATHER L. PHILLIPS

Cypress, California

2020

Defended July 31, 2020

Approved by

Office of Academic Affairs

July 31, 2020

Dean: Dr. Mickey Shachar Ph.D.

Director, Ph.D. Program: Dr. Ryan Dwight Ph.D.

Committee Chair: Dr. John Forsyth Ph.D.

Committee Member: Dr. Carlos Cardillo Ph.D.

Committee Member: Dr. Perry Scanlan Ph.D. MLS (ASCP)^{CM}

Committee Member: Dr. Bill Tawil Ph.D.

Abstract

Errors in Antibiotic Susceptibility Testing and the Association with Licensure and Certification: A Contributor to Antibiotic Resistance

Trident University 2020

Errors are a problematic theme in today's medicine. While error is associated with humans, error brings substantial increase in medical expense, morbidity, mortality, and can contribute to public health concerns, such as antibiotic resistance. Error in medicine can be explained by system's theory, where human error is attributed to system flaws. The purpose of this correlational quantitative research is to bring light to the prevalence of error that occurs with antibiotic susceptibility testing in the clinical laboratory; and to further establish the relationship that exists among dependent variable procedural knowledge and independent variables licensure and certification. A retrospective review of antibiotic susceptibility proficiency testing was conducted to indicate outliers in proficiency testing results. Descriptive statistics were performed to determine the prevalence of incorrect proficiency testing results for antibiotic susceptibility testing. A survey was distributed to five hundred and thirty-six medical laboratory microbiology professionals to determine the relationship between dependent variable procedural knowledge and independent variables licensure and certification. Survey questions were designed using Clinical Laboratory Standards Institute (CLSI) standards for performing daily routine bacterial inoculums for antibiotic susceptibility testing. A multiple regression analysis indicated a strong statistical significance between the independent variables' certification and state licensure, and dependent variable procedural knowledge. It was determined there was a strong statistically significant correlation between the lack of antibiotic susceptibility procedural knowledge and laboratory professionals who do not hold a state licensure ($p < 0.001$). There was also a strong

statistically significant correlation between the lack of antibiotic susceptibility procedural knowledge and laboratory professionals who are not certified by a nationally recognized certifying agency ($p < 0.001$). A parallel study was conducted congruently to ensure reproducibility. The parallel study results also indicated a correlation between dependent and independent variables. Laboratory professionals who are certified and/or hold a state licensure reduce medical error and contributors of antibiotic resistance. This research demonstrates the value of certification and state licensure among laboratory professionals when performing antibiotic susceptibility testing .

Preface

In the laboratory profession, it can be easy to label each patient sample as a number and forget there is a patient's life that hangs in the balance with each result reported to their treating physician. A laboratory professional is responsible for the majority of medical diagnosis, as indicated by literature. Though anyone who works as a laboratory professional, does not need literature to acknowledge their importance in medicine.

When a patient comes into the clinic with a flu like illness, the treating clinician orders lab work. It is the laboratory professional's results that determine if the illness is likely viral, bacterial, allergy, iron deficiency, leukemia, etc. It is the laboratory professional who then determines through laboratory testing which viral illness is to blame for the immune response. It is the laboratory professional who reports you have the gene for breast cancer and need a double mastectomy, the gene that can give your unborn child cystic fibrosis, the gene for Huntington's disease, Duchenne muscle dystrophy, and the list goes on and on. It is the laboratory professional's results that confirm your home pregnancy test. It is the laboratory professional who anxiously reports your abnormal leukemic cells to the physician before giving you the diagnosis of blood cancer. It is the laboratory professional's results that tell the physician you lost to much blood during surgery, with your life depending on their ability to blood type you, and then match your blood to another person's through a series of rigorous tests. It is the laboratory professional who monitors your drug therapy to make sure it is still working, who monitors your glucose level ensuring it does not get to high or too low, tests your kidney function, your liver function, makes sure you do not have immediate heart damage, looks for

signs of clotting, looks through the mess of stool for parasites, sends results diagnosing you with pancreatitis, thyroid disease, gout, and the list again goes on and on. We study every fluid pulled from your body, from the fluid your baby develops in, to the fluid your brain rests in. It is the laboratory professional who identifies urinary tract infections, identifies the bacteria growing in the urine, and then reports the list of antibiotics that can be used to treat the infection. It is the laboratory professional who explains your grandmother or asthmatic husband has COVID-19. It is the laboratory professional who provides the results for almost every diagnosis. Laboratory professionals are the unsung heroes of medicine.

Knowing the weight laboratory professionals bare, the responsibility of reporting correct results for a proper diagnosis is both exasperating and exhilarating. I chose, this dissertation subject, not because I fault any individual who performs lab testing. Not because I criticize any path chosen to get into the clinical laboratory; I wholeheartedly chose this dissertation subject because I care about the patient, the results used to diagnose that patient, the overall impact these results will have on that patient's life. The slightest incorrect decision when performing testing, can have a huge impact on a patient's results and ultimately alter their diagnosis. Making the slightest incorrect decision when testing can turn a positive result negative (false negative) or can make a negative result positive (false positive). To put this into perspective, paternity testing may prove a child is yours, even when the child really is not. Once you obtain the results, you would not have any way of knowing the results are inaccurate. For an even deeper perspective, you may be having a heart attack or you might not be, you may be pregnant or may not be, your blood type may be A or could be O, or you may have blood cancer or might not. Where would medicine be without laboratory testing accuracy? We do not talk about it, but the errors are staring us right in the face. The errors are hiding in numbers that were designed to keep us safe.

Throughout my career as a medical laboratory scientist, I have had the opportunity to work as a laboratory professional in states that require laboratory professionals be licensed and I have worked in states that do not require this licensure. I have worked alongside people with various backgrounds and routes that gained them entry into the field. I have worked beside, before, and after those with certification and those without. I have worked with those who have a long list of degrees and certifications but do little to achieve successful completion of the ever-growing schedule of laboratory tests. I have worked with those unable to successfully pass a certification exam but work harder and longer hours than anyone else without complaint, filling voids in the lab schedule that could not be filled by others.

Medical laboratory professionals are in short demand and the need grows daily. Laboratory directors and managers must make regular decisions if voids in the employee schedule should be filled by those designed to fill them, medical laboratory professionals who graduated from an accredited laboratory program and passed a certification exam, or individuals who fall into another facility approved route.

There are many variables that may contribute to one's inability to pass a certification exam. However, when an individual cannot pass, it opens the door to question if this individual possesses the fundamental knowledge necessary for result accuracy, as this is the purpose of the certification exam. Success in the knowledge and techniques in the clinical laboratory are imperative in an accredited laboratory program but may not be taught in a field other than laboratory science. Also, while some instrumentation provides limited safeguards designed to catch such behaviors, such as quality controls, testing performed outside of instrumentation would not catch these mishaps. This includes things such as sample preparation and testing that require visual acuity and visual identification. While working, this led me to question the degree

of accuracy with testing when individuals who did not graduate from an accredited laboratory program or were unable to pass a certification exam, were allowed to perform intricate testing relying on specific testing methods without instrumentation or testing relying on visual identification. In the field of medical laboratory science, a laboratory professional runs the tests, interprets the results, and then reports the results. There is a large degree of trust among clinicians that these results reported are accurate, as the physician relies on the results for patient diagnosis.

It does not matter the background, or the route taken to become a laboratory professional; we have all shed a tear for a patient we thought might make it and did not. We have all lost sleep wondering if we performed a crossmatch on a patient's blood correctly. At some point, we have all seen the cost of a test performed or reported inaccurately. Lab work is life altering, life depending, and expensive. Because of this, it does not matter the background, we can all agree everyone's results should be as accurate as possible, and this is the goal behind the years of work I have poured into this dissertation.

Acknowledgements

I would like to thank my dissertation committee: Dr. John Forsyth Ph.D., Dr. Carlos Cardillo Ph.D., Dr. Perry Scanlan Ph.D. MLS (ASCP)^{CM}, and Dr. Bill Tawil Ph.D. Thank you all for your generous guidance and mentorship. Thank you for helping me think critically through every stage of this work, for your insightful suggestions, and critical input.

Dr. John Forsyth Ph.D. I would like to say a special thank you for the immense amount of patience and for the pressure less environment you have provided, thus allowing me to be successful. I once thought, I am that person that thrives under stressful circumstances. If you were to ask anyone close to me, they would agree. You have shown me another way. Not only were you patient with me, you taught me to be patient with myself. Your overwhelming support has been incredibly motivating. With this, I learned I can be successful in environments other than the ones I had created for myself. I will prize this life lesson and I promise, "to pass the torch!" Thank you for investing in my success and patiently supporting me through each twist and turn.

I would like to thank, Dr. Ryan Dwight Ph.D. Dr. Dwight, you were the intense professor, everyone has, and never forgets! You made me push my limits and made me a better student for it. I appreciate your thorough and critical evaluations throughout this process. I also appreciate your recommendations to get the book "Getting What You Came For."

I would also like to thank Dean Mickey Shachar Ph.D. Everything you said could happen during my dissertation, did. I am grateful for all the ample advice you have provided me before this process began for my path of success.

I would like to give a special thank you to Dr. Perry Scanlan Ph.D. MLS(ASCP)^{cm}. It was in Dr. Scanlan's class in 2006, I was introduced to the world of diagnostic microbiology that I have come to love. Fresh out of graduate school, he was eager to teach and deeply passionate about the field of Medical Laboratory Science. Fourteen years later, Dr. Scanlan's games, goofy acronyms, and terrible drawings still reside in the fundamental memory bank for my diagnostic microbiology. Dr. Scanlan, I cannot thank you enough for taking a chance with me. I will forever be grateful! I appreciate your constant support and encouragement through all the runs of bad luck and never-ending work without pay. You have been an invaluable resource throughout this process. I also appreciate your availability for questions and direction. In this journey, I have not felt lost. Wherever this path leads me, before I make a judgement, I was sure to always "think about the bigger picture."

I would like to thank, Dr. Robert Robinson Ph.D. I was an academically lost sophomore that happened to land in Dr. Robert Robinson's office. I fell into his office because I needed an advisor. He was one of few professors who did not tell me what I wanted to hear. He told me exactly what I needed to hear. He gave me the drive I needed to make honor roll for classes such as, organic chemistry, biochemistry, and genetics. It was these classes that landed me a competitive position in the Medical Laboratory Science program at Austin Peay State University. I am sure it's hard to think of the impact one visit can have on a student's life when you are simply doing your job, but in that one moment, taking time from your busy day, you changed

mine. You started me on a path, that I would come to love, and I would never regret. For this, I will forever be grateful.

Thank you to all the laboratories that allowed me to invite their employees to be participants in this study. Thank you to all the supervisors and managers that moved mountains to get me in the doors. Thank you to all the medical laboratory professionals who took time out of their busy schedules to participate in this study. This would not be possible without each of you.

Finally, and most importantly, I would like to thank my parents, my family, and close friends. I appreciate my parents, Wayne and Theresa Burkhart, for their endless support and countless babysitting hours. They encouraged me in the only ways they knew how. I am indebted to you for your unconditional love, endless encouragement, and support that paved the path for me to reach high and achieve my dream.

I want to express my deepest appreciation to my husband Quintin Phillips, you have always been a constant source of support, encouragement, and affection. You were understanding and patient when others could not be. I appreciate the immense amount of sacrifices made on behalf of my education. Thank you for giving so much of yourself to support me on this journey.

To my children, Aaron, Lilly, and Emilie, you are a constant source of love and encouragement for me every day. You have made me stronger, better, and more fulfilled than I could have ever imagined. You will always be my greatest contributions to this world.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AAFP-PT	American Academy for Family Physicians
AAB	American Association of Bioanalysts
AMT	American Medical Technologist
API	American Proficiency Institute
AR	Antibiotic Resistance
ASCP	American Society of Clinical Pathology
AST	Antibiotic Susceptibility Testing
CAP	College of American Pathologists
CDC	Centers for Disease Control
CFU	Colony Forming Unit
CLIA	Clinical Laboratory Improvement Amendment
CLSI	Clinical and Laboratory Standards Institute
CMS	Centers for Medicaid and Medicare
EUCAST	European Committee on Antibiotic Susceptibility Testing
MDK	Minimum Duration of Killing
MIC	Minimum Inhibitory Concentration
MLEP	Medical Laboratory Evaluation Program
MT	Medical Technologist
MLS	Medical Laboratory Scientist
MLT	Medical Laboratory Technologist
PCR	Polymerase Chain Reaction
PT	Proficiency Testing
SPSS	Statistical Package for the Social Sciences
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
WSLH	Wisconsin State Laboratory of Hygiene

To my family

Chapter I: Introduction

Procedural lapses performed in clinical microbiology by medical laboratory professionals, prove to be a prelude for antibiotic resistance. Research that aims to elucidate causes of antibiotic resistant bacterial pathogens is important, as these pathogens pose a significant and growing risk to public health. Antibiotic resistant pathogens are onerous to treat with traditional antibiotics, resulting in severe morbidity and substantially higher mortality rates when compared with antibiotic susceptible infections (Frieri, Kumar, Boutin 2017). As multiantibiotic resistant bacteria are currently found on every continent of the world, the World Health Organization (WHO), explains medical procedures once common place and taken for granted, such as cesareans and catheterization, "could conceivably be consigned to medical limbo with repercussions almost unimaginable" (2014). More than two million people are infected with antibiotic resistant pathogens annually in the United States, with more than twenty-three thousand deaths occurring as a direct cause (Blair *et al* 2015).

Antibiotic resistance has been bolstered by incorrect use of antibiotics, in both animals and humans; and unexpected bacterial exposure to antibiotics as occurs with unregulated sewage (Rodriguez-Rojas 2013). Incorrect usage of antibiotics leads to the spread of multi-antibiotic resistant pathogens with resistance accelerating dramatically, in pathogenic bacteria, over the last fifty years of antibiotic use (Friedman, Temkin, and Carmeli 2016). Antibiotic resistance is a genetic evolutionary means of survival for bacterial pathogens that has existed for billions of years. These evolutionary attributes cannot be removed from existence, according to Jessica Blair and colleagues (2015). Variables contributing to antibiotic resistance, such as procedural

laps in clinical microbiology laboratories, must be identified and monitored for corrective action if prevalence of antibiotic resistant infections is to be reduced for pipeline antibiotics.

The role that clinical laboratory errors play in the evolution of antibiotic resistance has yet to be thoroughly explored. Medical laboratory professionals test specimens for bacteria and their sensitivity or resistance to available antibiotics. Physicians use this information to choose appropriate therapeutic antibiotics and dosing. If testing or interpretation errors occur, an incorrect clinical report regarding a patient's bacterial antibiotic sensitivity, results. Jun Li and colleagues (2017) explain the procedural error inoculum effect (caused by incorrect bacterial inoculum concentration) is important in the emergence of antibacterial resistance. Aude Ferran and colleagues (2007) use an in vitro model to demonstrate the inoculum effect's cause of increased antibiotic resistance, when testing antibiotic susceptibility for bacterium *Escherichia coli*, an increase in resistance against fluoroquinolone antibiotics was demonstrated.

Practicing medical professionals, often require the passing of a board of certification and/or licensure for employment. Certification demonstrates the medical health professional exhibits foundational knowledge necessary to prove the integrity of medical care. 60% to 70% of all critical medical decisions are based on laboratory results performed by a medical laboratory scientist (Forsman 1996). While most medically related healthcare professions require certification or licensure, only eleven states and one territory, license and regulate medical laboratory professionals with degree, licensure, national certification, continuing education, training or experience (Steward and Schulze 2005). Procedural lapses occurring within the clinical microbiology, contributing to antibiotic resistance, may be remediated by the licensure of medical laboratory professionals in all states of the United States of America.

In the fight against antibiotic resistance, clinicians continually broaden antibiotic therapies, clinical laboratories continue to improve rapidity and sensitivity of results, and hospitals continue to enhance infection control practices to overcome antibiotic resistant bacterial infections (Friedman, Temkin, and Carmeli 2016). Literature has not demonstrated consideration of the accuracy of the antibiotic sensitivity results for pathogenic infections nor antibiotic resistant pathogen treatment.

This research proves and establishes a prevalence of error that exists among antibiotic susceptibility testing. Current literature does not provide descriptive statistical analysis to indicate the ubiquity of error with antibiotic susceptibility testing. This was performed by conducting a retrospective review of antibiotic susceptibility proficiency testing results. This research also determined the relationship between dependent variable procedural knowledge and independent variables licensure and certification. This is performed to fill a literary void concerning this relationship. Data concerning variables was obtained by performing a survey, distributed to medical laboratory microbiology professionals. A multiple logistic analysis determined the significance of the relationship among dependent and independent variables.

Background

Antibiotic resistance is the ability of a bacteria to genetically develop resistance against antibiotic exposures that are designed to prompt their death. Antibiotic resistance can develop in the bacterial genome intrinsically or by a horizontal gene mutation method. Before this antibiotic resistance can be genetically ingrained, bacteria must be directly exposed or indirectly through bacterial lineage. The scope of bacterial exposure to antibiotics is substantial. Zeeshan Khan and colleagues (2019) explain a 35% rise in antibiotic consumption from 2000 to 2010, and a 67%

rise until 2015. The World Health Organization (WHO) produced a report in 2016, explaining seven hundred thousand lives per year are lost due to antibiotic resistance (Brogan and Mossialos 2016). At the current rate of antibiotic resistance development, ten million people will die per year by year 2050, making antibiotic resistance the most prominent cause of death (Khan, Siddiqui, and Park 2019).

Antibiotic resistance is detected through antibiotic susceptibility testing, unless directly looking for a gene in the bacteria that encodes for the resistance. Antibiotic susceptibility testing also identifies effective antibiotics and therapeutic dosages for management of bacterial infections often deadly to the host if not properly treated. Antibiotic susceptibility testing has a long and overtly complicated history that first begins with the early dilutions used in the 1870's. The first known antibiotic susceptibility testing performed was macrodilution, performed by the early pioneers Pasteur, Lister, Koch, and Ehrlich (Rittenburg 1965).

Fleming and his pioneering contributions are what led to today's methods of antibiotic susceptibility testing. It was Fleming's gutter method, developed in the 1920's that first opened the door to the diffusion of antibiotics in an agar covered with bacterial growth, to today's antibiotic susceptibility testing methods (Fleming 1929). Fleming's method was modified by Abraham *et al* (1941), by replacing the gutter with an Oxford Cup. Simultaneously, throughout the early forties, different research groups began to impregnant filter paper with antibiotics. Hoyt, Levine, and Bondi (1947), introduced the standard 6.5mm antibiotic disc. Multiple research groups in the 1950's began to differentiate bacteria as susceptible or resistant to multiple antibiotics. Although much information had been gained regarding antibiotic susceptibility testing at this point, results were not reproducible and were considered to

inaccurate for patient diagnosis and treatment. We owe the standardization of the bacterial inoculum and antibiotic testing disc diffusion method currently used to Kirby and Bauer (1966).

Antibiotic susceptibility testing has continued to be researched and modified throughout the century. Epsilometer testing (Etest) was developed by Bolstrom and Eriksson in the 1980's (Picard 1990). By 1991, AB BIODISK began the manufacturing process of the Etest and released the products to clinical customers across the world (Picard 1990). Continued scientific updates to propel bacterial identification in clinical microbiology has occurred more rapidly with instrumental methods such as quantitative polymerase chain reaction (qPCR) and the Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS) discovered in year 2000. However, very few updated technologies and scientific methodologies have occurred in the way of antibiotic susceptibility testing.

Most recent technologies do not provide full antibiotic susceptibility results but can indicate the presence of resistant genes present for few bacteria in patient samples within one to three hours. These genes are methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus* species, and multiantibiotic (isoniazid, rifampin, streptomycin, pyrazinamide, and fluroquinolones) resistant *Mycobacterium* species. Emerging methods for antibiotic susceptibility testing may involve micro total analysis systems (uTAS) using nano microfluidics and AC electrokinetic fluid motion to sense bacterial rRNA (Khan, Siddiqui, and Park 2019). Currently, three methods currently exist to perform antibiotic susceptibility testing.

Disc Diffusion

Disk diffusion was first described by Pope in 1940, followed by J.W. Foster and H.B. Woodruff in 1943, where the microbiological aspects of penicillin were described (Foster and Woodruff 1943). In 1944, J.G. Vincet, H.W. Vincet, and J. Morton (1944) were first to describe

the modification of the Oxford Cup penicillin to filter paper disc (Vincet, Vincet, and Morton 1944). Standardization of the disk diffusion method was created by Bauer and Kirby in 1966 (Bauer et al 1966).

The disc diffusion method is phenotypic in nature, requiring detailed knowledge and technique regarding result interpretation. A bacterial inoculum is created, and a lawn streaking technique is used to inoculate the agar. A disc containing a standardized amount of antibiotic is strategically placed on the agar and is allowed to diffuse at thirty-five degrees Celsius for sixteen to twenty-four hours. This diffusion process is well standardized. Known amounts of antibiotic is diffused into the media with known quantities of antibiotic concentration at each distance diffused from the antibiotic disc. After incubation, the zone of inhibition formed around the antibiotic disc is measured. The size of the zone of inhibition is recorded and results are interpreted when compared to antibiotic breakpoints established by the European Committee on Antibiotic Susceptibility Testing (EUCAST) and Clinical Laboratory Standards Institute (CLSI).

Minimum Inhibitory Concentration (MIC) of Macro and Microdilution

In 1942, Rammelkamp and Mason introduced the standardized tube dilution method used today for minimum inhibitory concentration (MIC), performed on a ninety-six well microtiter plate. Each well contains a standardized amount of dried antibiotic. A bacterial inoculum is created from the bacterial colony antibiotic susceptibility testing needs to be performed on. A small volume of bacterial inoculum is added to each well, containing a final volume no greater than 0.1 mL. The ninety-six well panels are incubated at thirty-five degrees for sixteen to twenty-four hours. Results are usually interpreted by using an instrument designed to indicate the presence of turbidity, indicating bacterial growth, for each well.

Epsilometer Testing (E-test)

The epsilometer test (Etest) was developed by Bolstrom and Eriksson in the 1980's and began the manufacturing and product distribution in 1991 (Picard 1990). The E-test is a small plastic strip, with visible numerical grading used to visual identify pre-defined antibiotic concentrations found on the strip. A bacterial inoculum is created, and a lawn streak is used to inoculate an agar plate. An E-strip is placed on the agar and incubated at thirty-five degrees Celsius for sixteen to twenty-four hours. Elliptical zones of inhibition are seen around the plastic strips. The numerical grading allows for the visual interpretation of the minimum inhibitory concentration for each E-test.

Creation of a Bacterial Inoculum

All current methods require the creation of a bacterial inoculum. The bacterial inoculum is a solution containing a diluent of inoculum water or broth and the suspected bacterial colony in a homogenous mixture. A patient's sample infected with pathogenic or opportunistic bacteria is cultured onto microbiological media and incubated for twenty-four hours. The media is reviewed and at least three morphologically similar colonies are pulled. It is important each colony is well isolated to prevent contamination. If more than one pathogenic or opportunistic colony is present, an antibiotic susceptibility test should be set up for each bacterial isolate.

Bacteria are added to the inoculum. According to CLSI standards (document M07-A9), bacteria inoculum concentrations must contain 1.5 x 10^8 of bacteria, comparable to a 0.5 McFarland Standard, when performing antibiotic susceptibility testing on human bacterial pathogens. According to these same standards, final antibiotic well suspensions must be a final concentration of 5.0 x 10^5 CFU/mL when minimum inhibitory concentrations are performed.

When performing disc diffusion or E-test antibiotic susceptibility testing, the bacterial inoculum is used to create a lawn on the microbiological agar. When performing macro or microdilution inhibitory concentration (MIC) methods, the bacterial inoculum is deposited into each tube (macrodilution) or each well of a (microdilution).

Medical Laboratory Science the Unknown Profession

The general public is not aware of who laboratory professionals are, how laboratory professionals are involved in their healthcare, and how these professionals are involved in their diagnosis. Consistent evidence suggests medical laboratory professionals are responsible for 70% of healthcare decisions, often undenounced to patients (Hallsworth, 2011). While newer literature does not exist to dictate the involvement of the laboratory in diagnosis for year 2020, most literature suggests this value is now higher than 70% with the dependence of high complexity testing, such as testing utilizing genomic and qPCR methods. Laboratorians are the science behind the diagnosis. To complicate matters, medical laboratory professionals with doctorate in philosophy degrees are limited among the field. For this reason, limited published literature exists in all aspects of medical laboratory science.

Results from the Medical Laboratory

Antibiotic susceptibility testing results can be difficult to understand and require specialized knowledge for result interpretation. Ifeoma Perkins (2016) explains in Error Disclosure in Pathology and Laboratory Medicine: A Review of the Literature, “some anatomic and laboratory diagnostics information can be technical, complex, and conceptually challenging to lay people.” Attempting to understand diagnostic results and their implications can be difficult for an individual who has not received medical or pathology training. Perkins (2016) further

points out that factors such as literacy level and the "situational context of disclosures" can influence both a treating physician and a lay person's capacity to understand the presenting error. Patients do not understand the errors as they relate to antibiotic susceptibility testing, nor do they understand the impact to themselves or repercussions to public health when inaccuracies occur. In the research study, Anatomic Pathologists' and Laboratory Medical Directors' Attitudes and Experiences conducted by S. Dintzis and colleagues (2011), it is explained that almost 50% of one hundred sixty-nine surveyed anatomic pathologists and laboratory medical directors indicated "the patient would not understand what he or she was being told." Perkins (2016) further explains, that there is fear among laboratorians that treating clinicians might not be able to adequately explain an error that occurs with a patient as this is not the treating physician's specialty. It is best understood that treating clinicians are the message delivery system for diagnostic results. These reasons further perpetuate the lack of discussion regarding error in the clinical laboratory, when error in medicine is a current topic of contention.

Theoretically, the knowledge that error exists in medicine is well established and the knowledge that error exists in the medical laboratory has been well studied in limited areas with supporting documentation. Current studies continue to focus on areas of improvement among the clinical laboratory and many of these studies use proficiency testing as a quality indicator. Dintzis (2011) describes the published recommendations regarding error mitigation for specialty associations in the field of laboratory medicine, such as the College of American Pathologists (CAP) and the Association of Directors of Anatomic and Surgical Pathology (ADASP). She also discusses the vast majority of pathologists and laboratory medical directors have had experience with errors but are not discussed as she further explains, "relatively few have experience disclosing errors." While literature points to areas of improvement in the laboratory, studies that

focus on error in antibiotic susceptibility testing in the clinical laboratory is limited. Studies that use the quality indicator proficiency testing to indicate the presence of error for antibiotic susceptibility testing is nonexistent.

Targeting areas that rely on procedural knowledge, visual acuity, visual identification, subject knowledge, theoretical concepts, and principles without instrumentation is important in identifying a problem among laboratory professionals. This avoids a laboratory professional relying on the checks and balances of manufactured instrumentation. There are multiple areas of the clinical laboratory that rely on correct preparation to obtain and interpret correct patient results.

Retrospective reviews of proficiency data have been used and statistically analyzed in previous studies, regarding other context in the clinical laboratory, such as coagulation and clinical chemistry. Retrospective reviews of proficiency testing data have not statistically analyzed antibiotic susceptibility testing. A prevalence of incorrect antibiotic susceptibility proficiency testing has not been conducted in previous studies. Providing descriptive statistics on the prevalence of inaccuracies for antibiotic susceptibility proficiency testing can indicate the significance within the field of medical laboratory science. Previous studies, such as those performed by Delost et al (2009), were performed using similar context, but only focused on proficiency data from one manufacturer. This study uses cross-sectional data from five Centers for Medicaid and Medicare (CMS) approved proficiency manufacturers.

Unsuccessful proficiency testing failures have been monitored through Clinical Laboratory Improvement Amendment (CLIA) sanction and remediation for individual laboratories. Unsuccessful proficiency testing data has not been compared against CMS sanctions over a large scope in previous studies. This information will prove valuable to the field

as a prevalent quantity of proficiency testing inaccuracies for antibiotic susceptibility testing may be present, but CMS sanction for inaccuracies may be lower than expected which can be described using data collected for this study.

Though literature does exist that describes deficiencies leading to inaccurate antibiotic susceptibility testing results, interviews have not been conducted for supporting evidence as to why inaccurate antibiotic susceptibilities are prevalent.

Demographic collection is a common component of questionnaires. Current laboratory professionals have not been surveyed with up-to-date demographics regarding state of employment and level of education as it relates to their employment as a laboratory professional, and national certification. Research has not been performed to determine if there is an association between the variable licensure and likelihood of procedural error when a lack of knowledge regarding antibiotic susceptibility testing is exhibited in the developed questionnaire.

The clinical microbiology laboratory has not been attributed as a source for elucidating antibiotic resistance in past literature. If a prevalence of unsuccessful proficiency testing exists for antibiotic susceptibility testing and likelihood of procedural error is demonstrated on questionnaires due to a lack of knowledge in the subject for antibiotic susceptibility testing, the clinical microbiology laboratory may be construed as a possible contributing factor for antibiotic resistance. An association between procedural errors and demographics discussed previously, may contribute to a means of remediating possible contributing factors.

Problem Statement

The system designed to prevent laboratory errors is faulty. Laboratory professionals should perform antibiotic susceptibility testing on patient samples using recommended Clinical

Laboratory and Standards Institute (CLSI) guidelines and established hospital protocols, in order to ensure proper antibiotic therapy and treatment for patients with bacterial infections. Analytical errors occur during patient testing leading to inaccuracies in antibiotic susceptibility testing and result interpretation for patient samples. The lack of licensure requirements in thirty-nine states and practicing laboratory professionals without certification are both contributing factors for these errors.

The intent of this quantitative observational study is to determine prevalence of antibiotic susceptibility testing errors and to indicate a correlation between antibiotic susceptibility testing procedural knowledge and variables, state licensure and certification. Translational methods correlate specific procedural errors associated with bacterial inoculum, known to contribute to the elucidation of antibiotic resistance in microbiology, in the clinical laboratory. A questionnaire and retrospective review of external control proficiency testing in CLIA licensed clinical microbiology laboratories, was used to demonstrate the relationship between licensed and/or certified laboratory personnel and the lack of procedural knowledge This provided an avenue for remediation of antibacterial resistance, caused by clinical microbiology laboratories.

Foundational and technical knowledge necessary to perform accurate bacterial pathogenic antibiotic susceptibility testing, is demonstrated through certification standardized testing. If thirty- nine of the fifty United States that who currently do not require medical laboratory licensure, made licensure a requirement, laboratory personnel would be obligated to maintain licensure for professional employment by demonstrating the necessary microbiologic knowledge to prevent procedural inaccuracies with bacterial antibiotic susceptibility testing.

Many analytical errors can be attributed to inaccuracies of result interpretation for antibiotic susceptibility when translational microbiology literature is considered. Testing

standards documented by Clinical Laboratories Standard Institute (CLSI) demonstrate specific procedural standards for antibiotic susceptibility testing. According to CLSI standards (document M07-A9), bacteria inoculum concentrations must contain 1.5 x 10^8 of bacteria, comparable to a 0.5 McFarland Standard, when performing antibiotic susceptibility testing on human bacterial pathogens. According to these same standards, final antibiotic well suspensions must be a final concentration of 5.0 x 10^5 CFU/Ml when minimum inhibitory concentrations are performed. Inoculum effect (Brooks 1989) describes inaccuracies in inoculum concentrations ultimately yielding in incorrect antibiotic susceptibility result interpretation. If too much bacterial organism is added, the bacterial organism would incorrectly be interpreted as antibiotic resistant. If too little organism is added, the bacterial organism would incorrectly be reported as antibiotic susceptible. Tested bacterial pathogens appear as antibiotic resistant organisms when inoculum effect occurs. When procedurally correct inoculum concentrations are used, antibiotic sensitivity result interpretation would appear as sensitive for these same pathogens, causing a misrepresentation of bacterial antibiotic susceptibility results. According to CLSI standards, homogeneity of bacterial concentration throughout the bacteria inoculum is also of importance. A bacterial inoculum lacking homogeneity throughout the solution, results in antibiotic wells of a sensitivity panel with unknown and unequally distributed bacterial concentrations, ultimately resolved by vigorously shaking the inoculum and performing bacterial antibiotic susceptibility testing immediately.

Today's microbiologic research describes the importance of inoculum concentration. As Li and colleagues (2017) explain, inoculum concentration is important in the elucidation of antibacterial resistance. Li and colleagues (2017), who also describe inoculum effect errors, further explain antibiotic resistance development through sub-inhibitory concentrations, by

describing pathogenic treatment through suboptimal dosing therapy. Aude Ferran and colleagues (2005), demonstrate inoculum effect in an in vitro model and its contribution to antibiotic resistance. Laboratory professionals using incorrect inoculum concentrations for antibiotic susceptibility testing, may experience inoculum effect, producing incorrect results and interpretation. This leads to incorrect patient diagnosis and treatment, preluding to antibiotic resistance. Three specific aims are detailed for this study:

Aim I: To determine the prevalence of procedural errors occurring with antibiotic susceptibility testing in the clinical microbiology laboratory among patient samples.

Aim II: To investigate the relationship between procedural knowledge and non-licensure requiring states for practicing laboratory professionals.

Aim III: To determine if there is a relationship between the certification of laboratory professionals and procedural knowledge in clinical antibiotic susceptibility testing.

Purpose of Study

The purpose of this quantitative, observational, collaborative study is to determine if there is a relationship between the dependent variable antibiotic susceptibility testing procedural knowledge and independent variables certification and licensure. A retrospective review was conducted of proficiency testing to identify the prevalence of error that occurs with antibiotic susceptibility proficiency testing. An electronic questionnaire was taken by five hundred and twenty-nine laboratory professionals to identify a lack of procedural knowledge with antibiotic susceptibility testing and to obtain professional demographics.

Determining a relationship exists between certification, licensure, and procedural knowledge for antibiotic susceptibility testing would prove a course to remediate medical error among results reported to clinicians for patients. This would also provide a means to reduce the elucidation of antibiotic resistance among bacteria.

Research Questions

Research question (RQ) one is a descriptive statistic to determine the prevalence of error among retrospectively collected data. Current literature does not provide descriptive statistical analysis to indicate the ubiquity of error with antibiotic susceptibility testing. Results indicated by research question one, were used to provide supporting evidence for this research, but will not be used in the multivariate analysis for the correlation study between the dependent variables and independent variables used in research questions two and three.

Research questions two and three are designed to determine the relationship between the dependent variable bacterial inoculum procedural knowledge and independent variables licensure and certification. A correlational quantitative study was conducted using surveys to obtain data for independent dichotomous categorical variables, licensure and certification. The survey was designed to also obtain data for the dependent variable and to access the knowledge of each laboratory professional. Specific research questions are demonstrated below.

RQ I: What is the prevalence of procedural errors among antibiotic susceptibility testing in the clinical microbiology laboratory?

RQ II: Is there a relationship between laboratory personnel's lack of aerobic bacterial inoculum procedural knowledge and those laboratory professionals who do not hold a state licensure?

RQ III: Is there a relationship between a laboratory professional who is not registered with a certifying agency and laboratory personnel's lack of aerobic bacterial inoculum procedural knowledge?

Significance of Study

This study is significant because it contributes to society and increase the body of scientific knowledge. Contribution to society are performed by identifying precursors that lead to medical error, erroneous errors among patient results can be reduced. Errors in medicine are expensive and have proven to cause increased and unnecessary morbidity and mortalities. It can also identify precursors that elucidate antibiotic resistance. Prescribing incorrect antibiotics because of incorrect diagnostic results can propel antibiotic resistance in the community. Incorrect usage of antibiotics leads to the spread of multi-antibiotic resistant pathogens, accelerating dramatically over the last fifty years of antibiotic use (Friedman, Temkin, and Carmeli 2016). While many areas have been identified by literature as variables in the propelling antibiotic resistance, none have identified the clinical laboratory as a potential variable.

While this is a heavily debated topic in medical laboratory science, prior to this research, there existed no literature to describes the significance of licensure or certification on results reported from the clinical laboratory. This research brings the topic to light and describes an area of great concern that stems beyond the focus of antibiotic susceptibility testing. Currently, Tennessee, the first state to require licensure for laboratory professionals, has temporarily suspended licensure and certification requirements under executive orders 15, 20, 24, 28, and 32. This allows anyone with a science degree to perform CLIA high complexity medical testing in the clinical laboratory without meeting academic requirements, licensure, and certification requirements. States who temporarily suspended licensure and certification requirements for

laboratory testing in light of COVID-19 have left many questioning the accuracy of results reported. There are many other variables contributing to this inaccuracy in SARS-COV-2 testing beyond the scope of this study. Had previous literature existed which proved the positive benefits of licensure and certification, this may not have happened.

Key Terms

Antibiotic susceptibility/sensitivity testing: In vitro testing to determine which marketed antibiotic and antibiotic concentration causes a specific bacterial organism's cell death or inhibits the growth of the targeted bacterial cells. Information gained from antibiotic susceptibility testing is used by the treating physician to therapeutically medicate patients infected with opportunistic and pathogenic bacteria.

Bacterial inoculum: A pure bacterial suspension, obtained by removing opportunistic or pathogenic bacteria grown from patient samples on media and autoclaved deionized water or nutrient broth. The inoculum is then vigorously mixed to create a homogeneous mixture. The suspension is then standardized by comparing the turbidity to a 0.5 McFarland Standard. This standardized pure bacterial suspension is then used for each antibiotic susceptibility testing method. The main purpose of this suspension is to standardize the collection of bacteria for antibiotic susceptibility testing. While many antibiotic susceptibility testing methods exist, one uniformity exists among all manufacturers and methods, the creation of a bacterial inoculum. The Clinical Laboratory Standards Institute (CLSI) has created a series of standards followed by laboratorians in creating bacterial inoculums. Bacterial inoculum is also referred to as standardization suspension in some texts.

Board of Certification: Certification of laboratory personnel, by one of three accrediting agencies, American Society of Pathology (ASCP), American Medical Technologists (AMT), American Society for Bioanalysts (AAB). Entry to sit for certifying standardized testing, ensures graduation from an accredited laboratory program, assures ethics and moral conduct, and passing of background check. Passing of this test indicates the laboratory professional possess the minimum foundational knowledge for successful job performance without causing patient harm.

Centers for Medicare and Medicaid Services (CMS): Federal umbrella agency who regulates all human medical laboratory testing (excluding research) under the Clinical Laboratory Improvement Amendment (CLIA).

Clinical Laboratory Improvement Amendments (CLIA): Implemented in 1988, the Clinical Laboratory Improvement Amendment (CLIA) is responsible for regulating all clinical facilities who perform human testing, such as laboratories who contain clinical microbiology. All facilities who perform human medical laboratory testing must maintain CLIA licensure and maintain greater than eighty percent in proficiency testing using a Centers for Medicare and Medicaid (CMS) approved proficiency manufacturer.

CLIA Certificate of Compliance (COC): Certificate issued by the Clinical Laboratory Improvement Amendment (CLIA) to a medical laboratory once the State Department of Health has conducted an inspection and determines the certifying laboratory is compliant with non-waived testing standards of moderate to high testing complexity.

CLIA Certificate of Accreditation (COA): Certificate issued by Clinical Laboratory Improvement Amendment (CLIA) to a clinical laboratory whose is accredited by a Centers for Medicare and Medicaid (CMS) approved agency and performs nonwaived moderate or high complexity testing. The CMS approved accrediting agencies are AABB, American Osteopathic Association (AOA), American Society of Histocompatibility and Immunogenetics (ASHI), COLA, College of American Pathologists (CAP), and Joint Commission on Accreditation of Healthcare Organizations (JCAHO).

Clinical Laboratory Science Institute (CLSI): A not for profit organization designed to develop standards and guidelines in clinical laboratory science, with the goal of bettering

healthcare worldwide. More than two-thousand volunteers comprise nine consensus committees, who are leading experts in the field.

Clinical/medical laboratory professional: An actively practicing clinical laboratory professional who performs medical testing on patient specimens in areas of clinical chemistry, immunology and serology, genetics, diagnostic microbiology, immunohematology, urinalysis, bodily fluids, and hematology. Interpreted results are relayed to physicians who ordered testing for treatment or diagnostic purposes. Personnel performing antibiotic susceptibility testing in licensure requiring states are graduates from an accredited laboratory program and may possess an associates, bachelorette, graduate level, or terminal degree. These individuals will also possess certification from a recognized certifying agency. Personnel performing antibiotic susceptibility testing in a non-licensure requiring state, can possess those some credentials as listed for personnel employed in licensure states. However, laboratory personnel may also be employed who have varying non-associated degrees, are not required to obtain certification from a recognized certifying agency, and do not have requirements to graduate from a laboratory program.

Clinical microbiology: A subsection of the clinical laboratory, responsible for identifying the presence of opportunistic and pathogenic bacterial infections among natural commensals, in human samples. After bacterial identification, bacteria are tested against antibiotics and available antibiotic concentrations approved for treatment of the specific infection. This information is relayed to treating physicians, where treatment decisions are made based on laboratory results for the patient.

Colony Forming Unit (CFU): A unit of measurement commonly used in microbiology, to determine the number of viable bacterial cells in one milliliter of fluid.

Disc diffusion Method: Also known as Kirby-Bauer testing, paper discs impregnated with known concentrations of specific antibiotics are distributed among the surface of a Mueller-Hinton agar plate. The entire surface of the Mueller-Hinton agar plate has been swabbed with prepared bacterial inoculum. After antibiotics have been added to the surface of the Mueller-Hinton agar plate as well and following a twenty-four-hour incubation, zones lacking bacterial growth caused by antibiotic inhibition are measured. The zone of inhibition measurements are compared against established antibiotic zone of inhibition ranges to determine susceptibility or resistance for testing antibiotics.

European Committee on Antimicrobial Susceptibility Testing (EUCAST): A scientific community formed in 1977, who provide free online guidelines to interpret antibiotic susceptible testing. www.eucast.org

Genotypic: genetic components that make up an organism.

Gradient Method: Also known as the Epsilometer Test or E-test, a strip of plastic in which a manufacturer applies a gradient amount of antibiotic to one surface and labelled concentrations of the antibiotic on the opposite surface. The E-test strip is placed on a Mueller-Hinton agar plate whose entire surface has been swabbed with prepared bacterial inoculum. Following a twenty-four-hour incubation, zones lacking bacterial growth caused by antibiotic inhibition are measured. The zone of inhibition measurements is compared against established antibiotic zone of inhibition ranges to determine susceptibility or resistance to testing antibiotics.

High complexity testing: The Clinical Laboratory Improvement Amendment explains this type of testing is the hardest and is subject to the most error.

Intermediate antibiotic susceptibility: Bacterial organism's antibiotic susceptibility falls into a range where susceptibility of the tested antibiotic's minimum inhibitory concentration (MIC) or zone of inhibition is approaching a point where the antibiotic will not produce a positive clinical therapeutic response as a susceptible strain of the same bacteria. Susceptibility to the antibiotic exists therefore it is not considered antibiotic resistant.

Macrodilution antibiotic susceptibility testing: Minimum inhibitory concentration (MIC) is performed using test tubes. A standard bacterial inoculum is created to inoculate each tube containing antibiotic in a two-fold dilution.

McFarland Standard: A reference used to standardize bacterial concentration when bacteria are suspended in solution. The concentration of bacteria in a prepared bacterial inoculum is visually or spectrophotometrically determined by comparing the inoculum, to be used for antibiotic susceptibility testing, to a McFarland Standard of 0.5. This turbidity is comparable to a bacterial suspension containing 1.5×10^8 CFU/mL.

Microdilution antibiotic susceptibility testing panel: A panel created by various manufacturers, used to test bacteria against a panel of antibiotics to determine the minimum inhibitory concentration (MIC) for each antibiotic tested on the panel. A list of antibiotics that may be found on the panel are demonstrated in Appendix A. The panel is often designed similar to a ninety-six well plate or card, depending on the manufacturer. Each section of the plate or card contains a different antibiotic with serial dilutions increasing in two-fold concentrations. The manufacturer individually dilutes each well containing antibiotics with Mueller-Hinton broth and dehydrates the panel for transport to the clinical microbiology laboratory. When each panel is used to perform antibiotic susceptibility testing, each antibiotic well was further diluted with a

standardized amount of prepared bacterial inoculum and then incubated. Growth is determined by indicating the presence of a button or increased turbidity of the suspension found in each well.

Minimum Inhibitory Concentration (MIC) Method: The minimum concentration of antibiotic that inhibits the growth of bacteria being tested. This is performed by creating a standardized bacterial inoculum and inoculating each well of an antibiotic susceptibility panel (microdilution method) or each labelled test tube (macrodilution method). After incubation, growth of a bacterial organism can be determined by indicating the presence of turbidity or a bacterial pellet. The first well indicating no obvious bacterial growth for each antibiotic tested is the antibiotic concentration reported as the minimum inhibitory concentration (MIC). It is this value that physicians use to therapeutically treat patients infected with opportunistic and pathogenic bacteria.

Phenotypic: Visualized expression caused by an organism's genotype

Proficiency testing (PT): An external quality control sample distributed by one of six Centers for Medicare and Medicaid (CMS) approved manufacturers. Participation in a proficiency testing program is required by Clinical Laboratory Improvement Amendment (CLIA). Successful performance in proficiency testing is also required as participating medical laboratories are obligated to maintain a minimum of eight percent proficiency in all patient testing subject areas. CLIA licensed laboratories participate in three proficiency testing events for each rolling twelve-months.

Unsatisfactory proficiency testing: A proficiency event scoring less than 80% in any one testing area or analyte. An unsatisfactory event requires internal remediation only. One unsatisfactory proficiency does not meet the requirements for an unsuccessful proficiency event

and does not warrant a Clinical Laboratory Improvement Amendment (CLIA) investigation or sanction.

Unsuccessful proficiency testing: Two consecutive unsatisfactory proficiency tests or two of three proficiency testing events for the same analyte (in this case antibiotic), indicates an unsuccessful proficiency. Antibiotics flagged as deficient (incorrect result) count against the overall microbiology score of the obligatory minimum of 80% proficiency. Maintaining less than eighty percent of the established threshold indicated by the government regulating agency Clinical Laboratory Improvement Amendment (CLIA). CLIA imposes a sanction or partial license removal for subject area with unsuccessful proficiency tests.

Zone of Inhibition: The area surrounding an antibiotic disc where no bacteria have grown. This type of zone of no growth appears when performing disc diffusion and E-test antibiotic susceptibility methods.

Chapter II: Literature Review

Antibiotic resistance is a genetic mechanism developed by bacteria, billions of years ago, through an evolutionary means for survival (Cox and Wright 2013). In the natural environment, antibacterial molecular components are produced by organisms such as fungi, as an evolutionary means to self-eradicate a bacterial pathogen (Rodriguez-Rojas, Rodriguez-Beltran, Couce, and Blazquez 2013). In 1928, Alexander Fleming discovered what is today recognized as pharmaceutical antibiotics (Aminov, R. 2010). In the pre-antibiotic era, most bacterial infections could be correlated with a loss of life (Friedman, Temkin, and Carmeli 2016). According to Friedman and colleagues (2015), analysis of pathogens and epidemiological data, suggest the evolvement and spread of multi-antibiotic resistant pathogens has accelerated dramatically over the last fifty years. This time coincides with both antibiotic discovery and their widespread medical applications (Friedman 2015).

Today, antibiotic resistance is a pandemic evolving public health crisis. Global organizations such as the World Health Organizations (WHO), federal agencies such as the Centers for Disease Control (CDC), Agency for Healthcare Related Quality (AHRQ), and local hospital policies and departments, such as infection control, are attempting to reduce the rapid spread and slow progression of antibiotic resistance, while new pipeline antibiotics are discovered and researched. A vital component of reducing the progression of antibiotic resistance (AR), is identifying each contributing factor. Then, create a mechanism to prevent this factor from further contributing to the elucidation of antibiotic resistance. Many researchers such as Bush and colleagues (2011) and Berendonk and colleagues (2015), consistently reiterate the importance of contributing factors, such as antibiotic distribution control and public education.

Other researchers, such as Murrell and Harrington (2016) and Li and colleagues (2017), further identify unique contributing factors, as requested by authorities such as WHO, who indicate, without control, would continually contribute to the resistance of antibiotics.

Tackling Antibiotic Resistance

Bush, Courvalin, Dantas, and colleagues (2011), explain the economic and human cost of antibiotic resistance, as well as address research questions, and recommend urgent actions to combat the growing problem of antibiotic resistance. A group of thirty scientists from academia and industry qualitatively explored the problem of antibiotic resistance. These researchers explained in 2007, over four hundred thousand multi-drug resistant infections occurred with twenty-five thousand attributable deaths, with two and a half million extra hospital days, and costing one and half billion pounds (USD $1.9 billion) each year. Researchers provide societal expenditures in the United States for 2007 as US$35 billion per year, eight million additional hospital days, and explain the United States spends US$20 billion in excess healthcare costs. Researchers further explain antibiotic resistance is not preventable but is best controlled.

What is important about this literature, is its explanation of research priorities to control resistance. Researchers note the lack of basic information that is required to direct strategic efforts towards the control antibiotic resistance. Key questions recommended for researchers to address, are how can modern diagnostic technology be improved to facilitate more accurate and efficient decision making in individual point-of-care settings? The questions also asked how surveillance can be established and maintained to ensure prescription of the most appropriate

antibiotic and treatment of infected people? These academic researchers explain the importance of the priority in preventing the development of antibiotic resistance worldwide.

The recommended urgent actions for tackling antibiotic resistance include, public education, increasing sanitation and quality of life, the creation of new antibiotics, the discarding and repurposing of old antibiotics, controlling proper antibiotic usage, investigating non-antibiotic novel approaches, and collaborating with agencies for the creation of new antibiotics. From researcher's discussions emerged a priority of urgent actions, though they failed to correlate laboratory medicine with the sensitivities that are produced clinically and how medications are prescribed. This is a very important aspect of antimicrobial resistance and concerns in public health.

Thomas Berendonk, Manaia, Merlin, Fatta-Kassinos, Cytryn, Walsh, et al (2015), explain antibiotic resistance is a threat to humans and animals worldwide. Researchers contribute qualitatively measures to reduce antibiotic resistance, which include risk assessment procedures, preventing environmental contamination, and implement reliable surveillance of antibiotic resistance. Berendonk and colleagues (2015) express the importance of standardization of resistance testing. They explain antibiotic susceptibility is determined by using minimum inhibitory concentrations (MIC) or polymerase chain reaction (PCR) methods. Researchers further explain, different methodologies are used to identify human and environmental pathogens as well as antibiotic sensitivities and results obtained should not be compared.

Minimum inhibitory concentrations (MIC) antibiotic sensitivity methods are discussed in the literature. It also explains antibiotic sensitivity methods should not be cross compared with environmental sciences because of the lack of standardization in field results. The lack in

accuracy in results is not discussed, but implications are assumed as harmonized guidelines between human antibiotic sensitivity testing and environmental testing is recommended.

Rodriguez-Rojas, Rodriguez- Beltran, Couce, and Blazquez, (2013), explains that antibiotic resistance is a multifactorial problem that must be addressed from different disciplines, such as medicine, microbiology, epidemiology, and evolutionary science. Researchers explain that it is clear, antibiotics act as true promoters of antibiotic resistance and certain antibiotics can fuel mutagenesis, recombination, and horizontal gene transfer, which are all key processes for evolutionary emergence and spread of antibiotic resistance (Rodriguez-Rojas, Rodriguez-Beltran, Couce, and Blazquez, 2013).

Surveillance of Antibiotic Resistance

Alan Johnson (2015) uses quantitative surveillance data, collected in Europe, to draw conclusions to the antibiotic resistance crisis. Johnson (2015) describes a paradox where treating critical patients with present medical advancement has made patients more susceptible to opportunistic pathogens. He explains as an added complication, these patients are clustered together in health facilities, and often serve as cross contamination from one to another. For these reasons both therapeutic and prophylactic antibiotics are used in medical care to reduce the morbidity and mortality from opportunistic healthcare acquired infections. These complications add complexity to the growing public health concern, as they demonstrate a need in prevention of multidrug antibiotic resistance and testing accuracy, but also demonstrates a facilitation for AR in the treatment of patients with AR infections. These problems are further discussed without delivering a solution. Johnson (2015) does well to collectively demonstrate the problem at hand and his concern, though he does little to give potential alternatives as a solution to the problem.

This literature provides evidence of increased susceptibility for opportunistic infections among hospitalized patients. This researcher also provides literature to support the value of clinical microbiology antibiotic sensitivity testing, as he recommends this information as a useful surveillance tool to monitor antibiotic resistance.

Role of Clinical Laboratory in Antibiotic Susceptibility Testing

Jun Li, Xie, Ahmed, Wang, Gu, Zhang, et al (2017) qualitatively explain influencing factors for antibiotic resistance among human pathogens. This literature is valuable, and is the sentinel study for this dissertation, as it describes current methods in determining antibiotic sensitivities, defines clinical resistance from a clinical laboratory perspective, and explains how inaccuracies in reporting MIC provides an avenue for further antibiotic resistance.

Researchers explain pathogenic antibiotic sensitivity are measured by determining the minimum inhibitory concentration (MIC). They define this value as the point in which antibiotics inhibit the bacterial growth. It is also explained how this value is determined, as each known volume of pathogens, are exposed to a series of increasing concentrations of antibiotics. Researchers explain that tested pathogens are phenotypically recognized as susceptible and resistant according to the epidemiological cut-off (ECOFF) value or breakpoint. Clinical resistance is defined as, "a condition in which the clinical criteria of cure was not reached, when a sufficient antibiotic dosage and administration timetable are applied for a specific infection," (Li *et al* 2017). This clinical resistance is determined by the clinical breakpoint achieved. Clinical breakpoints are usually defined by the criteria established by the Clinical and Laboratory

Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (Li *et al* 2017).

Other bacterial contributors to antibiotic resistance are defined, such as tolerance, which is "the capacity of a bacteria to stay alive in a fleeting exposure to antibiotics (bactericidal antibiotics)" (Li *et al* 2017). Researchers explain that longer exposures to antibiotics rather than higher concentrations of antibiotic exposure, is what is necessary to have the same effective level of killing in tolerant pathogenic strains, as would occur with susceptible stains, *which would likely not be indicated in MIC testing.* Minimum duration of killing (MDK) is the amount of time required to kill a known fraction of bacterial pathogens at a known antibiotic concentration over the MIC. Time-kill curves are quantitative measures of this tolerance (Li *et al* 2017).

Other factors that contribute to antibiotic tolerance are slow growth by certain species of bacteria (Mycobacteria) and providing poor growth conditions (location of biofilm) (Li *et al* 2017). These factors cause, what is referred to as the lag phase. Lag phase is a lag in bacterial growth, when unfavorable conditions are present. By removing the inhibiting conditions, exponential growth occurs with the pathogen. This information is valuable to this study as, it demonstrates the importance of MIC values reported by the clinical laboratory. Incorrectly decreased MIC values reported, would demonstrate a lag phase in pathogenic bacterial growth within the host, both giving an impression of an infection with an antibiotic resistant pathogen and promoting antibiotic resistance through plasmid development in pathogenic infections.

Marion Bayot and Bradley Bragg (2020) discuss antibiotic susceptibility testing performed in the clinical laboratory. They explain the importance of specimen collection techniques and performance to obtain well isolated colonies. Bayot and Bragg further discuss the steps to correctly produce a bacterial inoculum, microinhibitory concentration (MIC), and disc

diffusion methods. This article is one of few that explain the bacterial inoculum must be used within fifteen minutes. One of the errors possibly occurring while making the bacterial inoculum are the bacterial inoculums are not used within fifteen minutes because laboratory professionals are busy performing multiple other tests while performing antibiotic susceptibility testing. One of the questions on the questionnaire, ask participants if they are able to focus on antibiotic susceptibility testing or if they perform multiple other tests while trying to perform antibiotic susceptibility testing.

Zeeshan Khan, Mohd Siddiqui, and Seungkyung Park (2019), discuss methods of antibiotic susceptibility testing currently used in the clinical laboratory. They also discuss emerging methods and their implications on testing. Khan and colleagues discuss the history of antibiotic susceptibility testing. Antibiotic susceptibility testing has a long and overtly complicated history that first begins with the early dilutions used in the 1870's. The first known antibiotic susceptibility testing performed was macrodilution. Macrodilution was performed by the early pioneers Pasteur, Lister, Koch, and Ehrlich (Rittenburg 1965).

Fleming and his pioneering contributions are what led to today's methods for susceptibility testing. It was Flemings gutter method, developed in the 1920's that first opened the door to the diffusing of antibiotics in an agar covered in bacterial growth, to today's antibiotic susceptibility testing methods (Fleming 1929). Fleming's method was modified by Abraham *et al* (1941), when the gutter was removed and was replaced with an Oxford Cup. Through the early forties, nearly simultaneously, different research groups began to impregnant filter paper with antibiotics. Hoyt, Levine, and Bondi (1947), introduced the standard 6.5mm antibiotic disc. Multiple research groups in the 1950's began to differentiate a bacteria as susceptible or resistant to multiple antibiotics. Although much information had been gained

regarding antibiotic susceptibility testing at this point, results were not reproducible and were considered to inaccurate for patient diagnosis and treatment. We owe the standardization of the bacterial inoculum and antibiotic testing disc diffusion method currently used to Kirby and Bauer (1966).

Antibiotic susceptibility testing has continued to be researched and modifffied throughout the century. Epsilometer testing (Etest) was developed by Bolstrom and Eriksson in the 1980's (Picard 1990). By 1991, AB BIODISK began the manufacturing process of the Etest and released them to clinical customers across the world (Picard 1990). Continued scientific updates to propel bacterial identification has occurred more rapidly in the field with instrumental methods such as, the Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry (MALDI-TOF MS) that were introduced in 2000.

Khan and colleagues (2019) also discuss the benefits and disadvantages of current antibiotic susceptibility testing methods, such as disk diffusion, macrodilution, and microdilution methods. They explain disc diffusion methods are simple and cost-effective. The noted disadvantages are limited semi-automation is available, poor performance with slow growing and fastidious bacteria. Another disadvantage is there is insufficient data available for many bacteria, such as stains of *Pseudomonas*, *Bacillus*, and *Cornyebacterium* (Khan, Siddiqui, Park 2019).

Procedure Errors in the Clinical Laboratory

Daniel Murrell and Amanda Harrington (2016) question the potential impact of the limited incubation time and antimicrobial sensitivity reporting and optimal antibiotic therapy in their article, "Impact of Limited Incubation of Bacterial Growth-Positive Cultures and Antibiotic

Sensitivity Testing." Murrell and Harrington (2016) use a qualitative research method to collect three thousand six hundred and eighty-two patient samples for antimicrobial testing in monobactermia infections and report the findings as very major, major, or minor criteria. The findings indicated that limited incubation of antimicrobial sensitivity testing did not influence the antimicrobial sensitivities reported. Testing was performed on subcultures and implications on highly resistant organisms, such as Vancomycin Resistant Enterococci (VRE), was not considered.

Appold (2013) explains the role of clinical microbiology in a hospital setting in determining antibiotic resistant pathogens. She explains, clinical laboratories are responsible for both identifying the infectious pathogen in clinical samples and testing organisms for the presence of antibiotic resistant mechanisms. Molecular methods are available for the detection of genes that determine specific resistant mechanisms in pathogens, though these systems are not widely used or distributed. Appold (2013) attributes antibiotic resistance to the overuse of antibiotics with the main concern being the lack of new pipeline antibiotics.

Appold (2013) explains it is challenging to detect antibiotic resistance with automated systems but fails to identify any of these challenges. She explains traditional antibiotic sensitivity detection methods, such as Kirby Bauer, should be used to backup questionable results, though it is manual and time consuming.

This literature is important as it contributes information regarding the role of the clinical laboratory in the detection of antibiotic resistance. This research fails to recognize contributing factors of the clinical laboratory and explains the presence of challenges in determining antibiotic resistance using automated systems.

Jun Li, Xie, Ahmed, Wang, Gu, Zhang, et al (2017) describe the importance of bacterial inoculum size when determining antibiotic susceptibilities. The inoculum effect, as described by Brooks (1989), occurs when bacteria appear as susceptible when inoculums are standard, but resistant if the inoculum size is increased. As described by Li et al (2017), inoculum size is also important in the emergence of antibacterial resistance. Ferran (2007) describes the appearance of E. coli mutants as resistant to marbofloxacin, were more frequent when the initial size of bacterial inoculum was increased. Lee (2010) explains, pathogens in small numbers, that demonstrate antibiotic resistance, can provide protection to other pathogens by producing the signaling molecule indol, causing other non-resistant pathogens to turn on drug efflux pumps and oxidative-stress protective mechanisms, enhancing the survival capacity of the overall pathogenic population. Jun Li and colleagues (2017) describe antibiotic resistance development through sub-inhibitory concentrations, by describing pathogenic treatment through suboptimal dosing therapy.

Daniel Edson and Laura Massey (2007) explain in Proficiency Testing Performance in Physician's Office, Clinic, and Small Hospital Laboratories their research suggests for most analytes, PT performance has improved in laboratories since the implementation of CLIA '88, but problems remain in microbiology. Edson and Massey (2007) state, "Under CMS criteria, a laboratory that consistently scores 80% on PT events is judged successful in proficiency testing. This implies that, at least theoretically, up to 20% of a laboratory's patient test results could be unreliable, and PT would not detect this problem."

Jun Li, Shuy Xie, Saeed Ahmed, Funan Wang, Yufeng Gu and colleagues (2017) describes the inoculum effect and its impact on MIC results. The inoculum effect was first described by Brook in 1989, who described a proportional increase in MIC with increasing bacterial inoculum size. Researchers explain, "if there is an inoculum effect, bacteria might appear as susceptible when the inoculum is standard or decreased, but resistant if the inoculum size is increased (Li *et al* 2017)." They further explain that inoculum size is also important in the emergence of antibacterial resistance (Li *et al* 2017).

Aude Ferran, Veronique Dupouy, Pierre-Louis Toutain, and Alain Bousquet-Melou (2007) best describe the inoculum effect's relationship with antibiotic resistance, when researchers presented an in vitro model demonstrating *E. coli* bacteria mutants were shown to be resistant to fluoroquinolone when the initial size of bacterial population was increased.

P. Morency-Potvin, D. Schwartz, and R. Weinstein (2017) explain in Antimicrobial Stewardship: How the Clinical Microbiology Can Right the Ship, the emergence of antibiotic resistance among organisms is due to the suboptimal use of antimicrobials both inside and outside the clinical setting. Researchers further explain "suboptimal antimicrobial usage often stems from inappropriate interpretation or use of microbiological test results: lack of a microbiologically confirmed diagnosis, laboratory test errors, failure to submit appropriate specimens for culture, misuse of microbiology resources, and a general overreliance on empirical antimicrobial therapy with attendant disregard of microbiological results." The Centers for Disease Control and Prevention (2014) estimated antibiotics incorrected prescribed in 30 to 50% of all prescriptions.

Medical Laboratory Professional Licensure

Tony Badrick and Andrew StJohn (2014) explain the benefits and resilience to medical laboratory professional licensure or certification in Australia, in the article, "Does Medical Science in the Workforce Deliver a Competent Profession." They look at each nation around the world, including the United States, countries of United Kingdom, European Union, and New Zealand, that requires either medical laboratory professional registration, certification, or both. Badrick and StJohn (2014), explain through a qualitative research study that registration as a medical scientist, is seen as the Holy Grail for those who are unregistered, bringing with it notoriety, increased pay, and better job opportunities. However, Badrick and St John question nations who make these registrations and certifications a requirement.

It is apparent that when this article was published, Australia was looking at many registration or certification options as requirements for medical scientists and other clinical and ancillary staff. Badrick and StJohn (2014) discuss the benefits and potential downfalls of each certification. Upon conclusion, Badrick and StJohn (2014) do recommend a registration procedure for medical laboratory professionals, given the nature of the job. They explain that registration ensures that staff have undergone the required training for the fundamental basis of clinical laboratory work. They further explain, continuing education credit requirements should be maintained with licensure, to ensure that individuals stay up to date in the field.

While this information does prove valuable to the research question, the method section does not cover the specific questions asked to the participants in the questionnaire. This would be an important attribute to the article, as the way questions are asked, can give input as to why the participant gave the response that supports or denies the research question.

The article, “Licensure in the Era of Genomic Medicine” is also important for this research, as it describes the complexities in genomic testing from a physician’s point of view, which is a view that is not often documented in laboratory medicine. Researchers Jason Park, Stanley Leung, and Jason Wang (2016), explain how science in some areas, such as genomic medicine, are extremely technical and result implications can alter diagnosis for patients. Researchers also add that we are at a point in many aspects of laboratory medicine where state licensure should be required to ensure that these technicalities in the laboratory are performed correctly. This article hits on the complexity of clinical laboratory testing, as well as the need for licensure to perform accurate laboratory testing when qualitative methods are warranted in genomic medicine.

Jason Park, Stanley Leung, and Jason Wang (2016) explain in their article Licensure in the Era of Genomic Medicine that science has progressed to a stage of such complexity that highly technical components of the laboratory should be performed by those who are licensed. Park and colleagues explain that laboratorians, “perform expert interpretation of laboratory results within each of these disciplines [microbiology, cytogenetics, chemistry, etc.…].” The physicians also explain that, “The proper interpretation of genomic analysis involves a sophisticated understanding of clinical medicine, genetics, informatics, and complex analytic technologies.”

Park and colleagues (2016) also emphasize that while passing boards and obtaining a place on a registry (certification) does show a high academic achievement and shows that a laboratorian has the foundational knowledge required for job performance, but most states do not require licensure. Without a state licensure requirement, many laboratorians are working without certification because there is not a regulating authority. While this is true, researchers also

explain, “Applying this strict definition could lead to limitations of workforce and expertise, as many current professionals who interpret clinical genomic tests (whole genome sequencing [WGS], exome sequencing, and/or gene panels) are not licensed to practice medicine.” While these researchers have many valid points, they also suggest requirements for a medical doctor (MD) licensure to effectively operate parts of the clinical laboratory, such as genomics.

In 2005 the American Society of Clinical Pathology, the leading board of certification in the field of medical laboratory science, conducted a survey to determine if employed laboratory professionals, felt as though all working in the field, should be state licensed. C. Steward and F. Schultz (2005) describe a research in which a questionnaire was taken by laboratory professionals both in management and on the bench. Questionnaire results were interpreted quantitatively, and results indicated that support was high for state licensure. 71.6% of respondents indicated they supported licensing laboratory personnel; 18.2% were opposed; and 10.1% had no opinion (Steward and F. Schultz 2005).

Participants were asked about their opinion in the correlation between state licensure and the laboratory testing quality as well as patient safety. 62.1% of the total sample indicated they believed licensure of laboratory personnel improved overall testing quality (Steward and F. Schultz 2005). 60.9% of the total sample linked licensure of laboratory personnel with improved patient safety. Reasons for not wanting state licensure varied among position held within the lab (Steward and F. Schultz 2005). For example, laboratory directors who voted against state licensure felt that licensure will reduce the flexibility of the laboratory director in hiring laboratory staff.

The 2005 article that discusses the American Society of Clinical Pathology (ASCP)’s survey, regarding state licensure is very important to this research. While an older document, it is

the only survey of the type that has been conducted regarding medical professional licensure. This paper gives real time perspectives on how those laboratorians in the field feel about licensure, as well as discussing result quality, patient safety, and those issues involved with state licensure. I did not see any gaps in the research other than the potential bias that may have taken place from the questionnaire questions. This is a possibility, as those researchers employed through ASCP were conducting the study.

The article, "State Licensure Update: Giving Voice to the Value and Vision," written by researchers Kathy Hansen and Don Lavanty (2005). While this is also an older article, it is valuable to my research questions as it indicates the importance of state licensure (MLS) that can be difficult to find for my field. Kathy Hansen and Don Lavanty (2005) discuss the importance of state licensure of the medical laboratorian. They explain that state licensure would ensure that laboratory personnel would accurately possess the correct and accurate training required. This would also ensure that MLS would pass competency-based exams and participate in continued education programs throughout their career.

This article describes governing agencies responsible for regulation of clinical laboratories such as, Clinical Laboratory Improvement Amendment (CLIA). While this is pointed out by researchers, CLIA monitors the laboratory and not the laboratorians performing the work. CLIA is also quiet regarding certification requirements and continuing education. More specifically, this article points out that many governing agencies explain the Clinical Laboratory Improvement Amendment (CLIA) exists to regulate laboratories. This article qualitatively implores CLIA setbacks within the field of Medical Laboratory Science. These setbacks are described as things CLIA does that is not beneficial for the field of MLS. The setbacks stated by K. Hansen and D. Lavanty (2005) explain scheduled CLIA visits, better CLIA

follow-ups for discrepancies, and the lack of support for certification of MLS. While this article gives valuable information regarding CLIA regulations for the clinical laboratory from veterans in the field, little documentation to support these ideas exist.

Proficiency Testing Performance as a Quality Indicator for Laboratory Research

Maria Delost, Greg Miller, G. Andy Chang, Willaim Korzun, and Teresa Nadder (2009) conducted research to determine if credentials of laboratory professionals effected performance of proficiency testing. The variables included for the credentials of the study were degree, college major, years of clinical experience, and if they had a national certification. Successful and unsuccessful proficiency testing was used as a quality indicator for this research. The research conducted by Delost and colleagues (2009) has valuable elements and is similar in context to this study. Researchers do not use employment in a licensure state as a variable for proficiency success. Researchers also do not measure the success of antibiotic susceptibility as a proficiency marker. Researchers also focus on two board certifying agencies, American Society of Clinical Pathology (ASCP) and another board certifying agency merged into ASCP, National Credentialing Agency for Laboratory Personnel. Today, three board certifying agencies exist. This study only includes one of those. However, researchers do use proficiency testing as a quality indicator against many other subject areas in the clinical laboratory. The results of this study indicated certification nor level of education as statistically significant predictors for the success of proficiency testing performance. This study covered entire proficiency testing subject areas, (ie Hematology) instead of individual testing methods. Perhaps the statistics may have shown varying results if the scope of the study were more focused. This research also focused on local hospitals, which can attribute to higher levels of certified laboratory personnel. Location is

an attributing factor for laboratory personnel board certification as many states do not require licensure nor regulate certification.

Theoretical Orientation and Conceptual Framework

The theoretical orientation of this research is based on error in diagnostic medicine. Medical errors were brought to the forefront when the Institute of Medicine released "To Err is Human" in 2000 (Kohn, Corrigan, and Donaldson 2000). Many research projects were initiated following the report to monitor and reduce error in medical practice and diagnosis. Kohn and colleagues (2000) explained in his report something that has been well understood throughout many aspects of medicine, medical errors cause human suffering, cause loss of life, are very expensive, and in this case contribute to public health issues.

Kohn and colleagues (2000) explain that medical error can be defined "as the failure of a planned action to be completed as intended or the use of a wrong plan to achieve an aim." Lucian Leape (1994) explained in "Error in Medicine," that error is inevitable when humans are involved, "it is an error of the human condition even among conscientious professionals with high standards." He further explains that flaws must be accepted as system flaws and not character flaws of the individual. This idea is best understood when systems theory is considered.

Systems theory was proposed by Ludwig van Bertalanffy in 1968, where he described the concept that "systems cannot be reduced to a series of parts functioning in isolation, but that, in order to understand the whole, one must understand the interrelations between these parts (Anderson 2016). Systems are open, meaning they often utilize resources outside of the system or have input from components external to the system. A large system may also contain many smaller systems operating within it. Application of this theory understands an assumption that

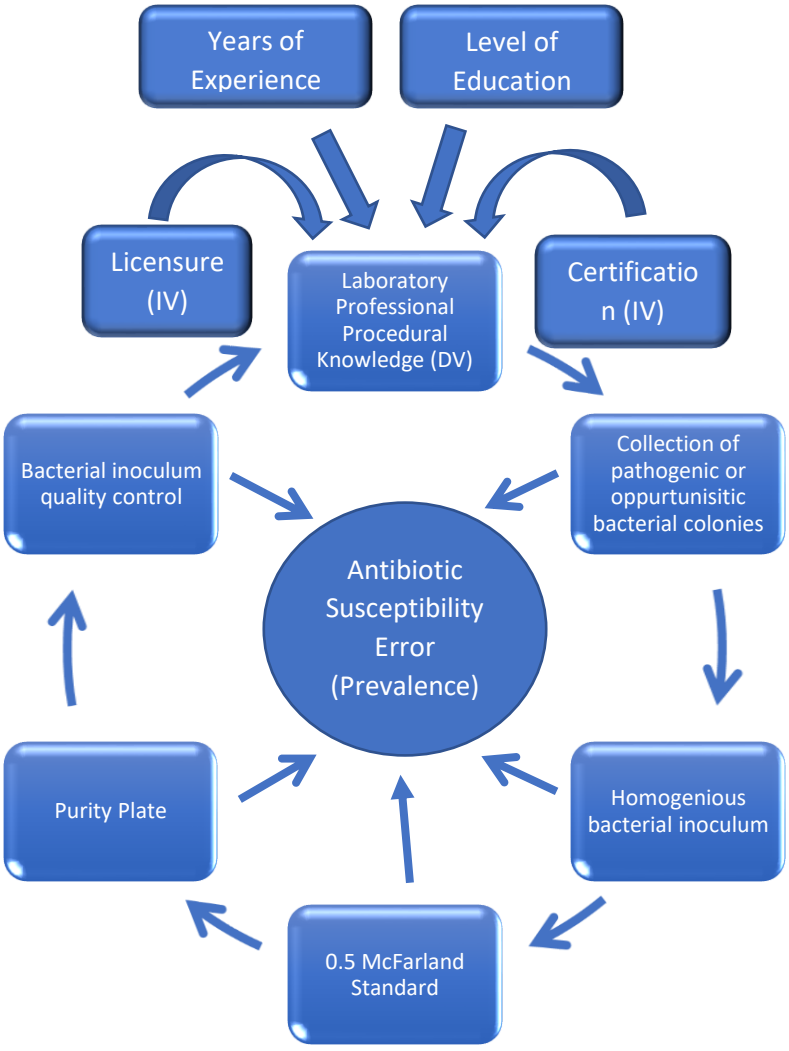
most individuals will strive to do good work, but that individuals in the system are acted upon by diverse influences that are accounted for in functional systems (Anderson 2016).

The system focused on in this research involves the professional laboratorian performing antibiotic susceptibility testing. Variables contributing to the success for optimal procedural knowledge for antibiotic susceptibility testing of the laboratory professional were tested in this study. State licensure and certification are independent variables tested by research questions two and three respectively. A correlation may exist between independent variables and the dependent variable, procedural knowledge for aerobic bacterial inoculum. Covariates in the study, years of experience and level of education may affect the dependent variable and were tested and corrected for in this study. Procedural knowledge provides the gateway for performance of each component in the procedure for creating a bacterial inoculum. These components are correctly identifying a pathogenic or opportunistic aerobic bacterial organism, creating a homogenous bacterial suspension, creating the correct concentration of the bacterial suspension (0.5 McFarland Standard), correctly setting up and observing purity plates, and performing and correctly interpreting quality control measures for bacterial inoculums. An incorrectly performed component at any stage contributes to an inaccurate antibiotic susceptibility result and incorrect result interpretation. The prevalence of these inaccuracies were determined using retrospective proficiency testing data. This system is demonstrated in figure 1. Directionality of Study, seen on the next page.

Brett Anderson (2016) further explains that individual accountability and systems theory are not mutually exclusive. According to Anderson (2016), “One can hold individuals responsible where appropriate, while simultaneously looking holistically at the system to identify weakness that allow for, or even enable, adverse events.”

Leape (1994) highlighted areas of error prevention in his article "Error in Medicine." Three of the four areas listed can be used as the focus for this research. The first area of prevention is "error proofing," meaning when possible, critical tasks should be structured so that errors cannot be made. Currently, the Clinical Laboratory Improvement Amendment (CLIA) requires procedures approved by the resident or on staff pathologist. "Standardization," an area Leape (1994) explains is one of the most effective means of reducing error should be used when possible. The second most effective means of reducing error, "standardization" is accomplished by the Clinical Laboratory Standardization Institute (CLSI). All guidelines and standards in the clinical laboratory are researched and further implemented by CLSI. Manufacturers use these standards in creation of procedures for laboratory equipment, while laboratory professionals use these standards for performance of laboratory procedures. The laboratory professional's knowledge of procedure for correctly performing antibiotic susceptibility testing found in the questionnaire was derived from these CLSI guidelines. The third area of prevention is "training." Leape (1994) states, "instruction… in procedures or problem solving should include greater emphasis on possible errors and how to prevent them. For example, many interns need more rigorous instruction and supervision than is currently provided when they are learning new procedures.

Figure 1. Directionality of Study



Young physicians need to be taught that safe practice is as important as effective practice." The third area of prevention described by Leape (1994) is implemented in the clinical laboratory through graduation of an accredited medical laboratory science program, certification, and continuing education. State licensure ensures all three of these components are met, a fete only accomplished by eleven United States.

This study acknowledges this behavior and understands that error occurs with human involvement. This study used proficiency testing and the laboratory professional to identify areas

with statistically significantly analytical errors. Then, this study addresses system flaws using a multivariate analysis to interpret the relationship between these statistically significant analytical errors and a lack of credentials and state licensures.

Another important theory considered for this study is academic knowledge theory, described by Michelle Buehl and Patricia Alexander (2001). This theory explains the body of knowledge obtained academically and is described by Buehl and Alexander (2001) to vary from that of professional knowledge. It is important to consider this theory when describing the covariate education level of the questionnaire participant. The theory explains the level of academic degree is directly proportional to the body of knowledge obtained by the individual. As the education level for the laboratory professional increases, so will academic knowledge in the subject. Many qualified laboratorians with varying levels of education can perform antibiotic susceptibility testing in the clinical microbiology laboratory. Level of education is not a tested variable by the research questions but is a variable of concern for this study.

Another important theory considered for this study is negative knowledge theory. Negative knowledge is the knowledge obtained from performing tasks incorrectly, exhibition of metacognitive behavior by the laboratorian, and using this gained knowledge to improve performance in the future. This knowledge can be seen as a professional advantage with "experienced" laboratory professionals, as "avoiding serious errors is an important quality of professional expertise," according to Martin Gartmeier, Johannes Bauer, Hans Gruber, and Helmut Heid (2008). Negative knowledge can be described as "non-viable knowledge that is heuristically valuable" and "directly influences performance by allowing professionals to identify and correct inadequate ways to proceed and thus increases their efficiency of problem solving" (Gartmeier et al 2008). To apply learned negative knowledge means the laboratorian

will avoid a suboptimal route. There is a possibility the "experienced" laboratory professional will use negative knowledge to avoid errors in antibiotic susceptibility testing and errors will not be due to a lack of academic knowledge, licensure, or certification, but self-corrected errors that have occurred over their professional experience. Laboratory professionals will make errors and corrections throughout their career. In this experience, laboratorians have contributed to their professional negative knowledge. The amount of negative knowledge obtained by the laboratory professional cannot be measured as a confounding variable by this research, but the amount of experience in the clinical microbiology laboratory can be measured by the questionnaire. This variable was controlled in this experiment. Further method elaboration can be found in the variables section under methodology.

Hypothesis

RQ1. What is the prevalence of procedural errors among antibiotic susceptibility testing in the clinical microbiology laboratory?

Research question one is a descriptive statistic and does not require a hypothesis.

RQ2. Is there a relationship between state licensure of laboratory professionals and a lack of procedural knowledge of antibiotic susceptibility testing?

H2. There is an association between state licensure of laboratory professionals and a lack of procedural knowledge of antibiotic susceptibility testing.

$H2_0$. There is not an association between state licensure of laboratory professionals and a lack of procedural knowledge of antibiotic susceptibility testing.

RQ3. Is there a relationship between laboratory professionals' registration with a board of certification and a lack of procedural knowledge in antibiotic susceptibility testing?

H3. There is an association between laboratory professionals' registration with a board of certification and a lack of procedural knowledge in antibiotic susceptibility testing.

$H3_0$. There is not an association between laboratory professionals' registration with a board of certification and a lack of procedural knowledge in antibiotic susceptibility testing.

Chapter III: Methodology

This is an observational study as variables were not manipulated and will rely on observations to determine a correlation between variables. This research is a correlational quantitative study initiated by obtaining descriptive statistics used to describe the prevalence of antibiotic susceptibility errors in proficiency testing. Both bivariate and a multivariate analysis was conducted to describe the relationship between the dependent variable, procedural knowledge of antibiotic susceptibility testing, and independent variables, licensure and certification. A questionnaire was distributed electronically to laboratory professionals who are employed in United States of America. A parallel study was conducted congruently by electronically distributing questionnaires to laboratory professionals in states Kentucky and Tennessee. The questionnaire assesses the knowledge of the laboratory professional who performs and interprets antibiotic susceptibly testing. An overview of study variables can be seen in table 1 and table 2.

Table 1. Study Variables Defined

Variable	Variable Type	Level of Measurement	Parametric (P) Non-parametric (N)	Operationalization
Proficiency Test Antibiotic Results	-	Dichotomous Categorical Nominal	P	-
Procedural Knowledge of Aerobic Bacterial Inoculum	Dependent Variable	Continuous Variable	N	1.Collection of pathogenic or opportunistic bacterial colonies. 2.Homogenous bacterial inoculum 3.0.5 McFarland Standard 4.Purity Plate 5.Bacterial inoculum quality control
State Licensure	Independent Variable	Categorical Nominal	N	California Florida Georgia Hawaii Louisiana Montana Nevada New York North Dakota Tennessee West Virginia
Certification	Independent Variable	Dichotomous Categorical Nominal	N	ASCP AMT AAB
Years of Experience	Confounding Variable	Interval	N	1-100 years
Level of Education	Confounding Variable	Nominal	N	Highschool or equivalent Associates degree Bachelor's degree Master's degree Doctorate/MD

* Dummy codes for each variable can be found in Appendix F

Table 2. RQ, Variables, and Statistical Analysis

Research Questions	Dependent Variable	Independent Variable	Confounding Variable	Covariate Variable	Statistical Test
RQ1: What is the prevalence of procedural errors among antibiotic susceptibility testing in the clinical microbiology laboratory?					Prevalence
RQ2: Is there a relationship between procedural knowledge and state licensure among laboratory professionals?	AST procedural knowledge	licensure	Experience	Education	Bivariate: ANOVA Multivariate analysis: Multiple linear regression
RQ3: Is there a relationship between clinical professionals' registration with a board of certification and between procedural knowledge of clinical antibiotic susceptibility testing?	AST procedural knowledge	certification	Experience	Education	Bivariate: ANOVA Multivariate analysis: Multiple linear regression

Prevalence of Incorrect Antibiotic Susceptibility Testing

The prevalence of incorrect antibiotic susceptibility testing was conducted, as no descriptive data exists in current literature. Obtaining descriptive statistics was important to indicate the presence and frequency of antibiotic susceptibility testing inaccuracies. This data analysis was conducted by retrospectively reviewing antibiotic susceptibility proficiency testing data from clinical microbiology laboratories. Proficiency testing data is public knowledge and was obtained from five proficiency testing manufacturers approved by the Centers for Medicare and Medicaid Services. An example of public proficiency testing results can be seen in appendix

C. A comprehensive list of CMS approved proficiency testing manufacturers being used in this study, can be seen in table 3.

Table 3. CMS Approved Antibiotic Susceptibility Proficiency Testing Manufacturers

CMS approved Proficiency Testing Manufactures	Proficiency Results Publicly available or Upon Request	Proficiency Results Used in Study	Location of Proficiency Results
American Academy for Family Physicians (AAFP-PT)	Publicly available	Use data	https://www.aafp.org/practice-management/labs/pt-central.html
American Association of Bioanalysts (AAB)	Publicly available	Use data	http://www.aab-pts.org/statistical-summaries/2018-statistical-summaries
American Proficiency Institute (API)	Publicly available	Use data	https://www.api-pt.com/pds.aspx
Medical Laboratory Evaluation Program (MLE)	Publicly available	Use data	https://www.acponline.org/practice-resources/business-resources/laboratory-proficiency-testing-program/mle-participant-summary
Wisconsin State Laboratory of Hygiene (WSLH)	Available upon request	Use data	Available upon request

International Business Machines Incorporation's (IBM) Statistical Package for the Social Sciences (SPSS) was utilized to conduct the descriptive analysis, prevalence. Proficiency testing results are dichotomous categorical variables that were dummy coded in SPSS. Proficiency testing antibiotics whose sensitivities are reported correctly were dummy coded as one. Proficiency testing antibiotics whose sensitivities were reported incorrectly were dummy coded as zero. Prevalence results indicated the overall frequency of proficiency testing result accuracies and inaccuracies. The prevalence for each antibiotic was further indicated giving possible insight for future research opportunities.

The Questionnaire

Data addressing research questions two and three of the study were obtained by conducting a cross-sectional electronic questionnaire using the web survey host, SurveyMonkey. This portion of the study is observational, as variables will not be manipulated, and correlations between variables were determined. Data collected from the questionnaire was used to conduct a bivariate and multivariate analysis to determine the relationship between the dependent variable procedural knowledge and the independent variables licensure and certification.

Conducting a survey to determine the adequacy of knowledge for a specific subject is commonly conducted. Current publications model survey questions from procedural guidelines, developed by governing institutions for use as a standard of practice among the industry. This can be demonstrated in peer reviewed articles M. Prous and M. Ponto's (2016) and Eunice Aguda's (2016) for which survey questions were modelled to ensure content validation. Further information regarding use of survey instrumentation for the determination of appropriate subject knowledge can be found in the literature review section of this document.

Electronic Component

An electronic questionnaire was used to provide easy one step dissemination to designated clinical facilities with anonymity in responses. The questionnaire was delivered through a web browser electronically and respondents will reply through this delivery method. IP web addresses will not be traceable back to respondents from the questionnaire web host, ensuring anonymity. The questionnaire was created using hypertext mark-up language (HTML) and sent to respondents using web host, Survey Monkey. Survey data was cross-sectional, with result collection occurring over the same two-week time frame from qualified participants.

Questionnaire participants will receive an electronic consent form with the questionnaire. This consent form will explain the purpose of the study, the objective of the questionnaire, anonymity of participant information, risk and benefits, who the researcher is, and who to contact if questions or concerns arise. The consent form can be found in appendix D of this document.

Target population and sample size

The required questionnaire sample size for this research is three hundred and sixty-nine participants. This sample size was derived using the Qualtrics Sample Size Calculator (2018). The confidence level was set to 95%. 95% confidence level is commonly used in survey research and is acceptable that the calculated mean will fall within the confidence interval. The margin of error was set to 5%, allowing plus or minus five percent error in results. The parallel study, focusing on states Kentucky and Tennessee, requires two hundred participants. The sample size, estimated by the power analysis, to ensure the proper rejection of the null hypothesis was achieved.

As no data exists indicating the amount of laboratory professionals working in the clinical microbiology department of the clinical laboratory, samples sizes derivations must be explained. The Bureau of Labor Statistics (2018) provides the total number of laboratory professionals by each state. For the parallel study, the state of Tennessee contains ten thousand three hundred and ten laboratory professionals. The state of Kentucky contains four thousand one hundred and sixty laboratory professionals. These values include all the laboratory professionals employed from each section, and does not list individual sections, such as microbiology. All the laboratories in the states of Tennessee and Kentucky were contacted by phone or visited in person. Detailed numbers for clinical microbiology staff were supplied at each facility. This data was compiled. It was determined 2.87% of laboratory professionals are employed in the

microbiology section. Tennessee has an estimated two hundred and ninety-six clinical microbiology professionals and Kentucky has one hundred and nineteen clinical microbiology professionals. Two hundred participants are the ideal sample size to use for the parallel study.

For the primary study, statistics involving the total population of laboratory professionals for each state were reviewed. 2.87% of laboratory professionals employed in the United States of America are nine thousand two hundred and fifteen. Using the methods described previously, the ideal sample size was determined to be three hundred and sixty-nine participants.

If the response rate for the questionnaire is low and the ideal sample size was not obtained, surveys would have been sent to additional adjoining licensure requiring state West Virginia and non-licensure requiring state, Virginia. A list of West Virginia and Virginia state hospitals can be found in appendix J. If the sample size continues to fail, a frameless sampling frame was adopted so most of the target population will have an equal opportunity to participate in the questionnaire.

Questionnaire Sampling

Stratified random sampling was used to select at least three hundred and sixty-nine random questionnaires for data analysis for the primary study. Stratified random sampling is important for this study as this method allows for random sampling while ensuring groups that may have low response rates have equal opportunities for participation. Stratification will ensure equal distribution of surveys among varying levels of education and among both licensure and non-licensure requiring states. Questionnaires are stratified into two groups based on licensure state requirements. Random numbers were assigned to qualified questionnaires in each stratum.

In the parallel study, stratum one are questionnaires from non-licensure state Kentucky. Stratum two are questionnaires from licensure state Tennessee. Random numbers from each stratum were selected using Google's Random Number Generator. Hospitals with assigned corresponding numbers drawn by Google's Random Number Generator were contacted, after Internal Review Board (IRB) approval, to disseminate the electronic questionnaire link to target participants of the study.

The primary study will also contain two strata of which two hundred and seventy-six questionnaires were required. Strata one is questionnaires from non-licensure states. Strata two are questionnaires from licensure states.

The ideal volume of target participants was not met. A frameless sampling strategy was employed. This ensured most or all of the target population would have an equal opportunity of being sampled. Participants were collected from locked social media sites, specifically designed for laboratory professionals. Entry to the website is by application only. While IP addresses were not collected, the electronic survey host, only allowed one participant from each IP address to take the survey one time.

Questionnaire Construct Validity

Conducting a survey to determine the adequacy of knowledge for a specific subject is commonly conducted. Current publications model survey questions from procedural guidelines, developed by governing institutions for use as a standard of practice among the industry. The Clinical and Laboratory Standards Institute (CLSI) is a standards development organization who provides the standards and guidelines for laboratorians and instrumentation in the field of medical laboratory science. Procedures and guidelines for creating aerobic bacterial inoculum is found in M07-A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that

Grow Aerobically; Approved Standard – Tenth Addition. Questionnaire procedural questions and responses were developed directly from procedural guidelines demonstrated in the M07-A10 procedural guide.

To ensure construct validity, the survey design is modeled from two surveys. M. Prous and M. Ponto's (2016) was the first survey, Testing Knowledge of Eye Donation: a survey of intensive care nurses. The aim of Prous and Ponto's survey was to access ICU nurses' knowledge regarding ICU patient eye donations. The objective of the researchers' project was to evaluate ICU nurses understanding of the procedure for identifying potential eye donors. The construct of this survey was successfully measured, and valuable information was obtained, as researchers state, "The results of this survey highlight the majority of nurses had a limited knowledge regarding donor suitability criteria and referral process. The perceived lack of knowledge was reported by some participants, suggesting the need for training" (Prous and Ponto 2016). Questions were designed to determine the knowledge of currently employed nursing staff working in the ICU department regarding procedures to select eye donors. Questions were also designed to determine the level of education nurses had regarding eye donation, as well as survey participants opinions regarding their own eye donation knowledge. Participants answered survey questions on a Likert scale.

The survey's knowledge questions, and possible answer responses were modelled after the second survey designed by Eunice Aguda's (2016) in Hand Off Communication: A Survey Study of What Anesthesia Providers Need to Know. The questions of this online survey were developed to determine CRNA's knowledge and awareness of current standards of practice regarding the handoff process in nursing. According to researchers, "questions were meant to elicit CRNAs understanding of the current handoff practices, identify information thought to be

crucial to communicate to enhance efficient handoff from one provider to another, and assess for knowledge gaps among the CRNAs in handoff processes" (Aguda 2016). Questions in this survey are asked based on known acceptable current standards applicable for handoff procedures. One answer to each question, corresponds with current hand off procedures. Other possible answers deviate from current applicable hand off standards.

Assessing Target Population and Associated Questions on the Questionnaire

Participants of interests are clinical laboratory professionals, actively employed in clinical microbiology departments who perform and interpret aerobic antibiotic susceptibility testing and result interpretation in a clinical laboratory setting. The questionnaire is initiated by asking questions designed to ensure participants meet the specified target population qualifications. Answers to questions one, three, and four must be "Yes," for survey results to be included in this study. Surveys that answer "No" to any of the questions one, three, or four, will not be included in study data, as these individuals are not model survey participants.

Questionnaire Demographics

Questions are designed in closed ended format. Specific questions and answer responses can be found in Appendix E. After the participant was deemed qualified by the initial questions, specific questions asking participant demographics are needed to obtain data for independent variables in aims two and three.

Question two, indicates the location of the state in which the laboratory professional is employed. This information will identify if the laboratory professional is employed in one of eleven licensure requiring states or one of thirty-nine non-licensure requiring states. Question

five, indicates the level of education the laboratory professional has obtained for employment. Licensure states have stringent requirements regarding the level of education required to apply for licensure and are also are very specific about the quantity of hours and level of class obtained for each subject area. Non-licensure states do not have specific degree requirements for laboratory professionals. As long as CLIA requirements are followed, this is determined by the hiring manager. Questions six and seven ask if the questionnaire participant is registered with a board agency and if so, which board they are registered with. This also has important implications as licensure states require registry with a board, non-licensure states do not. However, individual medical facilities operating within non-licensure states can require board of certification, which could impact testing.

Question nine determines the methodology used by the laboratory professional to preform AST testing. Disc diffusion or minimum inhibitory concentration (MIC) methods are currently available for AST testing. Most clinical laboratories utilize MIC testing, while few laboratories continue to utilize older disc diffusion methodologies. Many variations exist between manufacturers with each method, but one commonality exists between all manufacturers and methods, the creation of a bacterial inoculum. Each procedure involves plating a human specimen allegedly infected with pathogenic or opportunistic bacterium on primary and selective media. Once the presence of bacteria is indicated, specific CLSI guidelines are followed to create a bacterial inoculum. These CLSI guidelines can be viewed in appendix D. Bacterial inoculums are often created without instrumentation. A great amount of microbiologic knowledge and technical skills are involved. This inoculum is used for both disc diffusion and MIC methods, even with great manufacturer diversity in testing methods. For these reasons, the questionnaire will focus on bacterial inoculum procedures.

Questionnaire Knowledge of Bacterial Inoculum Questions

Questions fifteen through twenty-four are closed ended procedural questions based on CLSI guidelines, designed to interpret if the questionnaire participant performs antibiotic susceptibility testing within recommended CLSI guidelines. CLSI guidelines for antibiotic susceptibility testing can be viewed in appendix D. It is possible that questionnaire participants may be performing procedural errors without knowing they have done so. For this reason, questions were designed to have neither a correct or incorrect answer but contains at least one answer that falls within CLSI guidelines for antibiotic susceptibility panel procedures for bacterial inoculums. Results that fall within CLSI guidelines were marked as "1". All other answers are intentional deviations from CLSI guidelines and were coded as "2", as these results are outliers. Answers to questions nine through fifteen will also include "easy out" options that may be quite viable in some clinical laboratories such as, "I am not aware of what this question is referring to," "the laboratory I am employed and perform AST testing does not require I perform such testing," and "I was not trained or advised to perform such procedures when performing AST testing."

Questionnaires Qualifications for Study

Questionnaires that meet the following requirements were qualified for participation in the study.

- Questionnaires whose participants meet target population qualifications as defined in the questionnaire target population section
- Questionnaires whose participant demographic qualifying questions, ensuring a target participant, are filled out in entirety before submitting

- Surveys whose participants have less than twenty years of clinical microbiology experience using one antibiotic susceptibility testing technology consecutively, as defined in the confounding variable section

Questionnaires qualified for participation were assigned a unique identifying number for stratification random sampling. Data obtained from the questionnaire were used in the following data analysis.

Variables

The dependent variable in this study is knowledge regarding aerobic bacterial inoculum. Knowledge was determined by performing a procedural question analysis from the survey question results. Research demonstrates, all deviations from CLSI established guidelines for testing using a bacterial inoculum result in incorrect antibiotic susceptibility testing results. For this reason, any procedural answers that deviate from CLSI guidelines will result in a lack of procedural knowledge.

The theory to Err is Human, described by this research, became profoundly apparent when analyzing the dependent variable, procedural knowledge in antibiotic susceptibility testing. Initially, the dependent variable was to be dummy coded 1 for participants who exhibited complete procedural knowledge and dummy coded 2 for any participant who lacked procedural knowledge to any extent. This was a dichotomous nominal variable, allowing for a logistic regression analysis to determine the significance of the relationship between dependent and independent variables. While this provided valuable information, the hypothesis previously described, explained all laboratory professionals have errors and lack of knowledge to some degree, but those professionals without certification and licensure will have a statistically significant amount of error or lack of procedural knowledge. After accessing data, it was clear

this hypothesis was true, all laboratory professionals exhibit some degree of error and lack some degree of procedural knowledge, after all to Error is Human. Out of five hundred and twenty-nine total participants, all but a few exhibited some degree of procedural knowledge. After reviewing the data, it was decided to convert the dependent variable to a continuous variable. How many procedural questions did participants get correct? The response is a numerical continuous variable. This provided more insight for the research but changed the multivariate analysis to a multiple linear regression.

The independent variable state licensure is a categorical variable. All participants who hold a state licensure reported the state for which licensure is held in the demographics of the questionnaire. Each licensure was dummy coded. This coding can be found in appendix F.

The independent variable registered with a board of certification is a dichotomous categorical variable. Survey participants who indicate they have a nationally recognized board of certification for at least one of three possible board of certification agencies are dummy coded as one. Survey participants who are not registered with a board of certification were dummy coded as two.

The amount of years actively practicing as a laboratory professional and education level are confounding variables. Known confounding variable may contribute to the outcome of the bivariate and multivariate analysis and could damage the internal validity of the experiment. Failing to control for the following variable could indicate a false correlation between procedural knowledge, licensure, and certification, leading to incorrectly rejecting the null hypothesis.

The amount of years actively practicing as a laboratory professional could affect the outcome of this study. This is due to negative knowledge obtained on the job, as described in the

theoretical concept. Laboratory professionals will make errors and corrections throughout their career. In this experience, laboratorians have contributed to their professional negative knowledge. Negative knowledge cannot be measured by this research, but experience in the clinical laboratory can be measured by the questionnaire.

This variable was controlled in the study. Each study participant was asked in the demographic section of the questionnaire (question thirteen). Participants who have more than twenty years of experience utilizing the same antibiotic susceptibility testing technologies and methodologies were removed from the study to control for this confounding variable caused by negative knowledge theory.

The second confounding variable is the level of education the laboratory professional has earned. As described by academic knowledge theory in the theoretical concept, a large body of knowledge results from collective academic inquiry. Data was collected in the questionnaire to measure the level of education obtained by the laboratory professional, as it relates to laboratory medicine.

Random stratified sampling will aid in accounting for unknown confounding variables. Multiple linear regression results were further analyzed for possible additional unknown confounding variables.

Multivariate Analysis

A multiple linear regression was conducted to determine the relationship between the dependent variable aerobic bacterial inoculum procedural knowledge and independent variables, holding a state licensure and certification. Multiple linear regression was used as the correlational data analysis between variables. Assumptions for this data analysis method and

numerical data are required. The dependent variable is a continuous variable and both independent variables are categorical.

Assumptions of the data were tested before performing the multivariate analysis. Scatter matrixes with best fit lines were performed on the collected survey data using SPSS software, to ensure linearity among the dependent and independent variables. Multicollinearity among independent variables were tested using SPSS to ensure relationships do not exist between independent variables and all variable relationships are accounted for in the data analysis. Each independent variable, licensure and certification, were tested for homoscedasticity using SPSS to ensure each variable has the same degree of impact on the dependent variable, bacterial inoculum procedural knowledge. Then, the independent relationships among the variables were tested to ensure we can say that there is independence in observations when describing the data.

The multiple logistic regression was conducted in IBM's SPSS. The adjusted R square will explain the measured proportion of total variability of the knowledge of aerobic bacterial inoculum and how much is explained by the licensure and certification. The intent is to explain that a known percent of variance is explained by the independent variables, licensure and certification. Evidence to accept or reject the null hypothesis was determined by the F and significance values. A p-value of less than five hundredths will indicate rejection of the null hypothesis, indicating a relationship exists between a lack of knowledge of aerobic bacterial inoculums and independent variables state licensure and board of certification.

The coefficient table in the analysis was used to determine the effects of each licensure and certification on bacterial inoculum procedural knowledge. The significance of licensure and board of certification were determined independently with implications to accept or reject the

null hypothesis for each independent variable. System theory and Error in Medicine was used to justify by the results of Unstandard Coefficient B results.

Confidentiality and Privacy

The prevalence of antibiotic susceptibility testing errors calculated because of research question one is calculated using anonymous retrospective data. This data is obtained from proficiency testing databases found publicly on proficiency testing manufacturers websites. This data is striped of identifying information before it is made publicly available by the PT manufacturers. Data will not be obtained from manufacturers for the purposes of determining prevalence in error, with identifying information.

Clinical laboratories that have an internal microbiology department were contacted after internal review board (IRB) approval, to disseminate questionnaire link to those laboratory professionals who work in the microbiology department of the clinical laboratory. Those professionals who choose to participate will respond to the questionnaire electronically through a survey host. Participants will not be notified or made aware during the questionnaire that knowledge questions asked are presumed correct or incorrect. This will limit risk to the participant. This survey host was informed not to record IP addresses of the host. This is to ensure that all responses are left anonymous. No data will exist connecting the participant to questionnaire results. This will ensure any risk to the participant is minimal. Before taking the questionnaire, participants were given an electronic version of the informed consent. The informed consent to participate in the questionnaire can be viewed in appendix B.

Risks associated to the participant are very low for this study. Risks involved in this study are both social and economic. Social risks may be involved if the participant chooses to share

results of their procedural knowledge questions and the results, they choose are not following the standard procedure or CLSI guidelines. There could be a negative connotation associated with incorrect responses. There could also be an economic impact to the participant if they choose to share their results with employees in a supervisory role and the decision was made to terminate the employee due to a perceived lack of knowledge demonstrated by the questionnaire. However, results will not be shared with the participant nor any other individual. Only the researcher will know the level of knowledge the participant exhibits by the questionnaire in the study. Risks associated with this research were reviewed by the internal review board (IRB) to ensure justification of the risk to the participant.

All results of the survey were kept in a locked box, in a locked desk only allowing the researcher access to survey information. Survey results will be kept through the entirety of this research and an additional ten years. After this time, survey results will be shredded and discarded.

Limitations

Decreased sample size was expected to be a potential limitation to this study. For this reason, additional clinical facilities in adjoining licensure and non-licensure states Virginia and West Virginia were included as a possible resource to obtain additional participants that meet target population qualifications. Additional facilities within surrounding states were added until participation goals were achieved. A list of clinical laboratories containing microbiology sections in these states Tennessee, Kentucky, Louisiana, Mississippi, Virginia, and West Virginia can be found in appendix E.

Other limitations exist among PT manufacturers whose results were requested. CMS lists six proficiency testing manufacturers that clinical microbiology laboratories can use for antibiotic susceptibility proficiency testing. Four PT manufacturers compile and publicly archive PT testing data for each PT event. This data is easily accessible through the PT manufacturer's website. Two PT manufacturers compile and archive PT testing data, but do not make data publicly available. This information is not required to move forward with the study but can add value to the study by increasing diversity.

Unethical behaviors are another limitation of this study. Laboratory professionals who perform unsuccessful proficiency testing due to unethical behaviors were recorded only as an unsuccessful PT event. Examples of unethical behaviors with proficiency testing may be willful neglect of proper environmental conditions, "cutting corners" during testing, or purposeful failures seen as "vindictive" behavior. Unethical behavior may also be conducted on questionnaires or during interviews when individuals may intentionally dishonest. Looking up correct answers to each question on the survey could also be attributed to unethical behavior by questionnaire participants. An assumption of using systems theory as the theoretical concept of this study, understands an assumption that most individuals will strive to do good work, but that individuals in the system are acted upon by diverse influences that are accounted for in functional systems (Anderson 2016). Using this theory, we must assume that most laboratory professionals will not act unethically, but performing this study using a stratified random sampling method will correct this possible bias.

As this study brings contributions to gaps in knowledge that exist in medical laboratory science, these gaps also provide limitations for this study. A lack of prior research conducted regarding error in clinical antibiotic susceptibility testing exists. A lack of literature also exists to

determine the relationship among variables licensure and certification with errors in the clinical laboratory. This lack of knowledge left limited resources to model this study after.

Limitations may exist that are causally related to the proficiency samples used for proficiency testing. One must assume the proficiency samples have been treated as required by the proficiency testing (PT) manufacturer during shipment and handled according to PT manufacturer instructions within the clinical laboratory.

Known confounding variables exist for this study. The amount of years actively practicing as a laboratory professional and education level are confounding variables. Known confounding variable may contribute to the outcome of the logistic regression analysis and could damage the internal validity of the experiment. Failing to control for the following variable could indicate a false correlation between procedural knowledge, licensure, and certification, leading to incorrectly rejecting the null hypothesis. The amount of years actively practicing as a laboratory professional could affect the outcome of this study. This is due to negative knowledge obtained on the job, as described in the theoretical concept. Laboratory professionals will make errors and corrections throughout their career. In this experience, laboratorians have contributed to their professional negative knowledge. Negative knowledge cannot be measured by this research, but experience in the clinical laboratory can be measured by the questionnaire. Question number eight on the questionnaire asks the experience level of the participant. Participants who have more than twenty years of experience were removed from the study to control for this confounding variable.

The second confounding variable is the level of education the laboratory professional has earned. As described by academic knowledge theory in the theoretical concept, a large body of knowledge results from collective academic inquiry. Data was collected in the questionnaire to

measure the level of education obtained by the laboratory professional, as it relates to laboratory medicine. This variable cannot be removed from the study; therefore, it was tested as a covariate in the multiple logistic regression. Random stratified sampling will aid in equal distribution for level of education among participants and for additional unknown confounding variables. Multiple logistics regression results were further analyzed for possible additional unknown confounding variables.

Delimitations

Many antibiotic susceptibility testing methods exist. Among those, many manufacturers also exist requiring use of their own product. Incorporating each method from each manufacturer would create a complicated questionnaire that would increase the probability of participant error in responding to questions or low response rates. For these reasons, this study focuses on the commonality among each testing method and each manufacturer. The dependent variable in this study is procedural knowledge regarding the preparation and use of an aerobic bacterial inoculum for antibiotic susceptibility testing. Questions testing procedural knowledge in the questionnaire focus specifically on this common testing method following Clinical Laboratory Standards Institute (CLSI) guidelines and standards.

Pre-analytical, analytical, and post analytical phases exist in testing the antibiotic susceptibility for aerobic bacterial. Each phase can consist of its own list of errors. This study focused on procedural errors that occur within the analytical phase of testing. Pre-analytical nor post analytical testing were analyzed for this study.

The questionnaire does not include questions pertaining to opinions, socioeconomic status of the clinical facilities laboratory, nor psychological factors of laboratorians, or other

variables that could possibly affect knowledge of laboratory professionals regarding antibiotic susceptibility testing.

The amount of years actively practicing as a laboratory professional could affect the outcome of this study. This is a known confounding variable that may contribute to the outcome of the logistic regression analysis and could damage the internal validity of the experiment. Failing to control for the following variable could indicate a false correlation between procedural knowledge, licensure, and certification, leading to incorrectly rejecting the null hypothesis. The amount of years actively practicing as a laboratory professional could affect the outcome of this study. This is due to negative knowledge obtained on the job, as described in the theoretical concept. Laboratory professionals will make errors and corrections throughout their career. In this experience, laboratorians have contributed to their professional negative knowledge. Negative knowledge cannot be measured by this research, but experience in the clinical laboratory can be measured by the questionnaire. Question number eight on the questionnaire asks the experience level of the participant. This can be seen on the questionnaire in appendix C. Participants who have more than twenty years of experience were removed from the study to control for this confounding variable.

Chapter IV: Data Analysis and Results

Data Screening Data Points

Five hundred and twenty-nine participant samples were determined to be qualified participants after a rigorous screening process. Guidelines for participant selection were set before participants were able to take the questionnaire, preventing a cherry-picking error. Participants must be employed within the United States of America as a laboratory professional and perform antibiotic susceptibility testing on aerobic bacterial colonies grown from patient samples.

Once participants had taken the questionnaire and data was compiled, a thorough evaluation of the original data was compared against duplicated data uploaded in SPSS statistical software. Missing data was examined for patterns and corrected by replacing the missing values with the group mean for the variable the missing value belongs to. This was chosen to reduce variability among results. Results were also screened for outliers to prevent any result from causing a high impact on the outcome of the statistical analysis. No outliers were detected in this series of data.

Normality

Normality was assessed by reviewing the skewness and kurtosis of the data sets. While skewness and kurtosis were used to access all of the variables, these apply more to continuous variables rather than those of categorical nature. Skewness results should be lower than 3.3 when reviewing data. The skewness results for state licensure held by a participant was 1.185. The skewness results for certification were 3.117. The skewness results for correct responses was -0.192. Kurtosis results should also be less than 3.3 for continuous variables. The kurtosis results for state licensure held by a participant are 0.023. Kurtosis for the certification data set was greater than 3.3, indicating more data is present in the tails of the bell curve. This can be seen in the histogram for this data, as indicated by Figure 2 seen below. However, this is a categorical nominal value and kurtosis measured in this manner applies more to continuous variables. The kurtosis for correct responses was -0.395. This data does demonstrate normality as demonstrated in Figure 2 seen on the following page.

Homoscedasticity

Homoscedasticity measures the variability in data among continuous variables to ensure the points are roughly the same distribution at all values. This can be done by viewing a scatterplot of the data. If variables are normally distributed, as indicated by the normality, continuous data points should also be homoscedastic. The homoscedasticity can be seen by viewing Figure 3 below. It is also important to note, none of the data points are above 3 on the Y-axis. This means that there are no residuals greater than 3. Residuals are deviations from the fitted line to the observed values. This can be valuable when determining the linearity for a set of data. This can be further viewed when data are plotted against a best fit line. This can be seen in Figure 4.

Figure 2. Histogram Demonstrating Normality

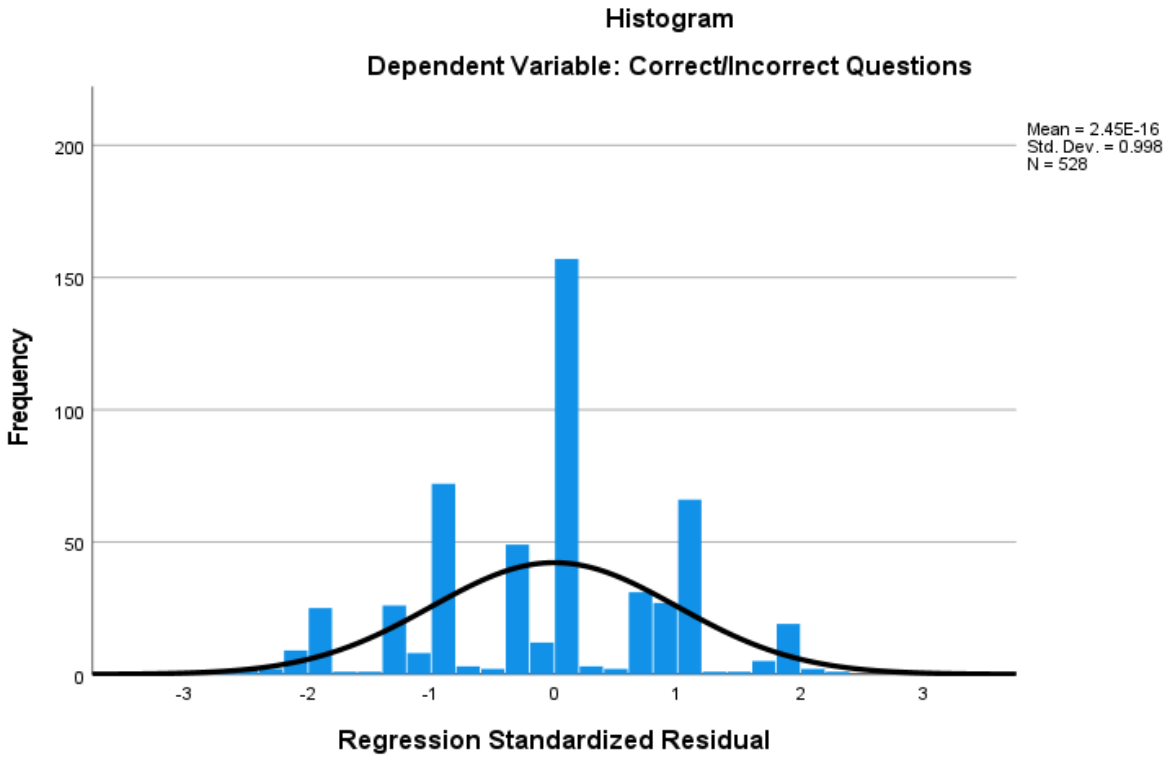


Figure 3. Scatterplot of Variables

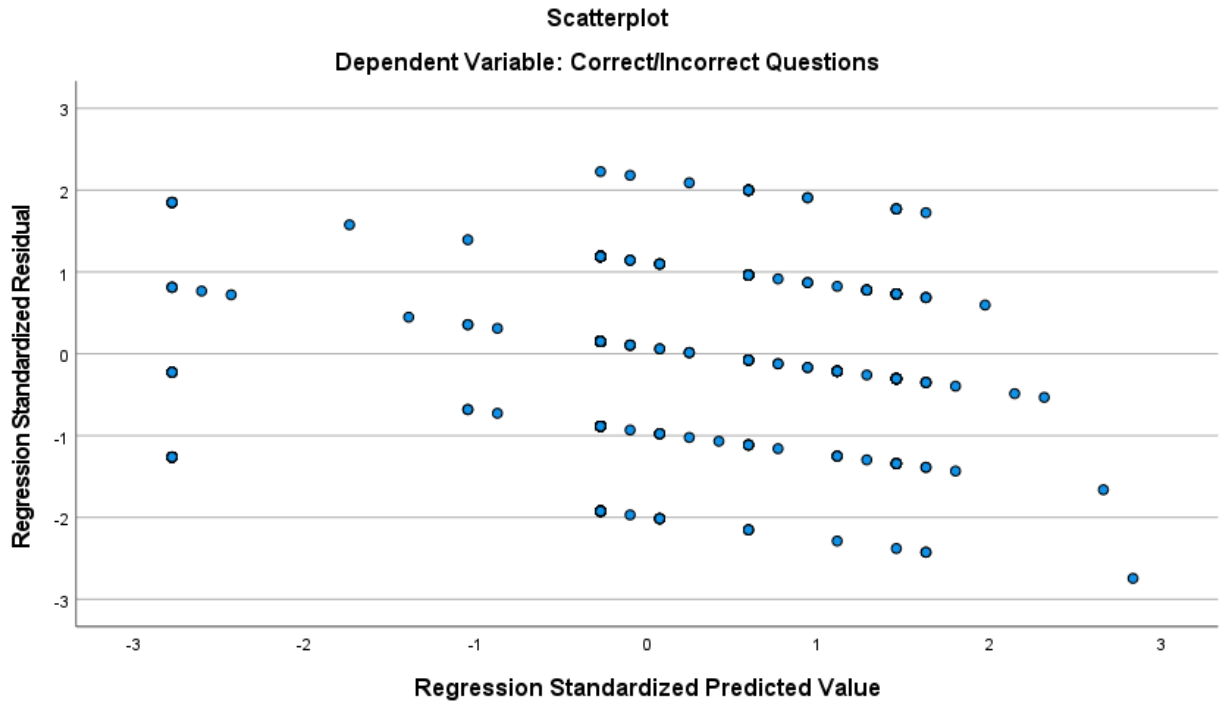
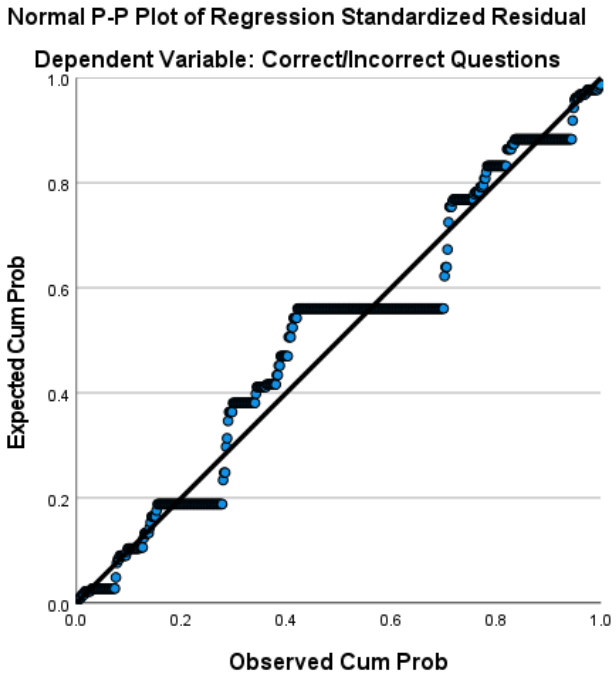


Figure 4. Data Points on a Best Fit Line

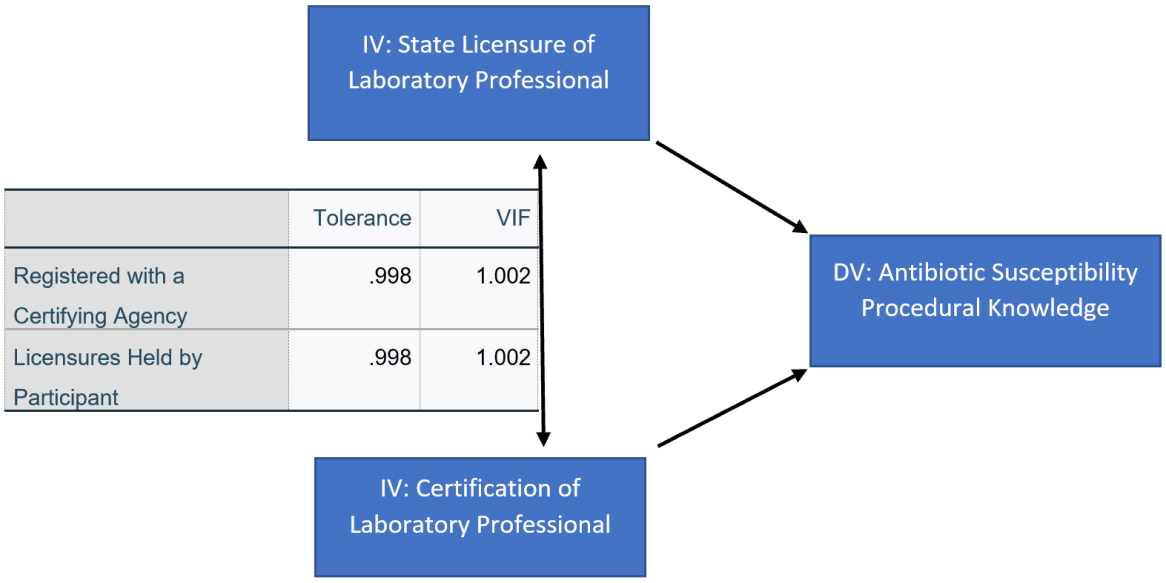


Collinearity

When checking for collinearity for a regression analysis, it is important the condition index is <15, the VIF is <4, and the tolerance is >0.2. The collinearity was checked for the dependent variable correct responses, and independent variables, certification and licensure. The condition index for the following variables was 1.000, 2.417, and 9.312 respectively. The VIF for these variables were 1.002 for each variable. The tolerance was 0.998 for each variable. This data demonstrates collinearity.

Multiple collinearity studies were performed against the independent variables to ensure one variable was not dependent on the other. The collinearity tolerance for certification and licensure was greater than the required >0.2. The collinearity VIF for the independent variables were less than the required numerical value of three. This statistical analysis proves there is no dependency between the independent variable's licensure and certification. This is illustrated in figure 5, below.

Figure 5. Collinearity among Independent Variables



Descriptive Statistics

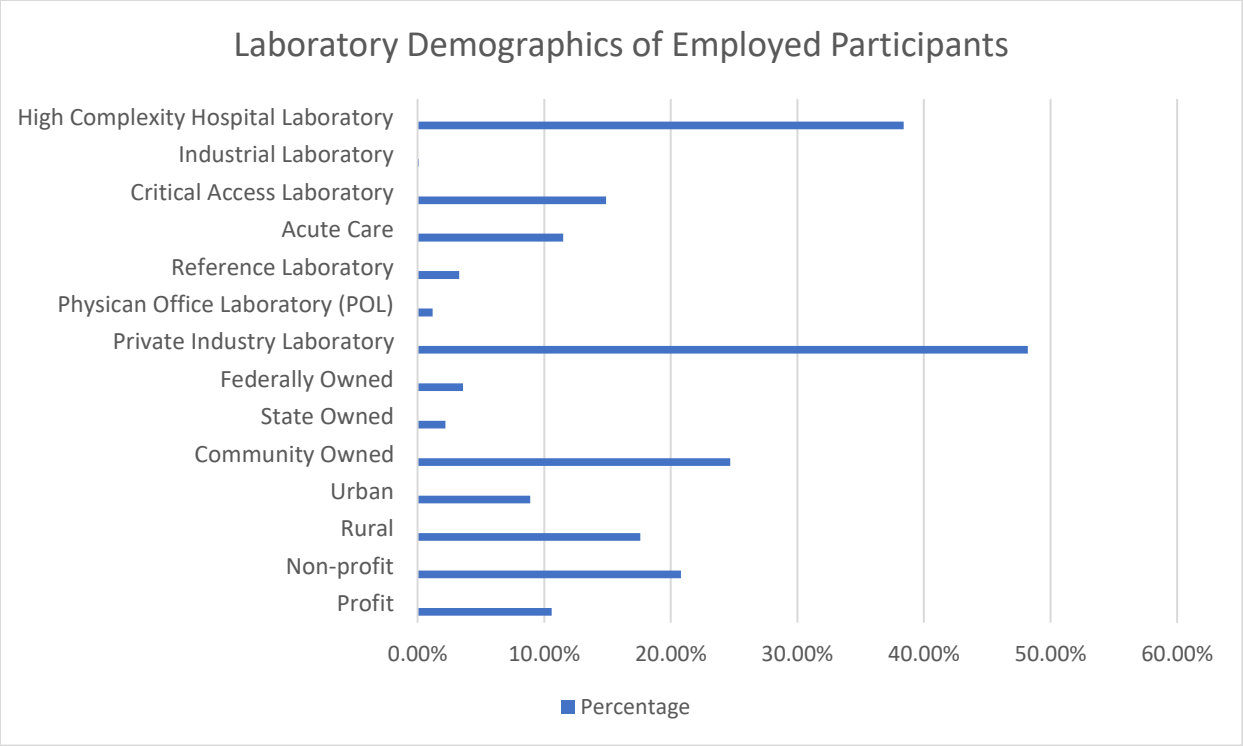
Proficiency testing results were observed for testing events one through three from years 2016 until 2018. This was performed for five proficiency manufacturers and distributors. Results were observed for accuracy with aerobic antibacterial susceptibility testing. Table 3 below demonstrates the error rate observed among five proficiency manufacturers for aerobic antibiotic susceptibility testing. When each profieincy manufacturer is viewed independently, the prevalence of error among proficiency testing ranges from roughly 1-12%. Each proficiency manufacturer's bacterial proficiency samples vary in which bacteria was chosen for the testing event. Proficiency manufacturers vary greatly in the quantity of respondents for each test. When each testing event is viewed independently, the quantity of error ranges from 1-34%. To see this detailed information regarding each testing event used to determine the prevalence of error, see Appendix N.

Table 4. Overall Prevalence of Proficiency Testing Result Errors

Proficiency Manufacturer	Time Frame	Error Prevalence
American Proficiency Institute (API)	2016 Event 1-2018 Event 3	6.237%
American Academy of Family Physicians (AAFP)	2016 Event 1-2018 Event 3	0.796%
American Association of Bioanalysts (AAB)	2016 Event 1-2018 Event 3	0.913%
Medical Laboratory Evaluation (MLE)	2016 Event 1-2018 Event 3	5.845%
Wisconsin State Laboratory of Hygiene (WSLH)	2016 Event 1-2018 Event 3	11.4794%
Overall Error among all Proficiency Manufacturers Listed	2016 Event 1-2018 Event 3	5.938%

Five hundred and twenty-nine laboratory professionals participated in the questionnaire. Diverse randomized samples were obtained from all fifty states of the Unites States of America. Participants had varying degrees and subjects of education, varying certifications, levels of experience, and multiple testing platforms used by participants. When commonalities among demographics, such as the state practicing was performed, standard deviations were high. This confirmed great deviation from the mean, statistically confirming great deviations in responses. The diversity exhibited among participants by state of employment can be visualized in Appendix I. There is also great variegation among the types of laboratories worked in by participants. This is easily demonstrated in table 4, seen below. When each survey's demographics are viewed for each participant and common demographics, such as state practicing, hospital beds, state employed, and information volunteered by participants in the comment section are grouped; it is estimated over four-hundred and fifty varying clinical laboratories were used in this study.

Table 5. Laboratory Demographics of Employed Participants



There is also great diversity among education. The highest frequencies for the level of education was predominantly bachelor's degrees among participants (48.5%). Associates degrees were the second highest at twenty percent. Details regarding the education of each participant can be found in Appendix G. Participants predominantly were educated in the subject of laboratory science (49.6%). Biology was the next predominated field at roughly nine percent. Details regarding the subject background of each participant can be found in Appendix H.

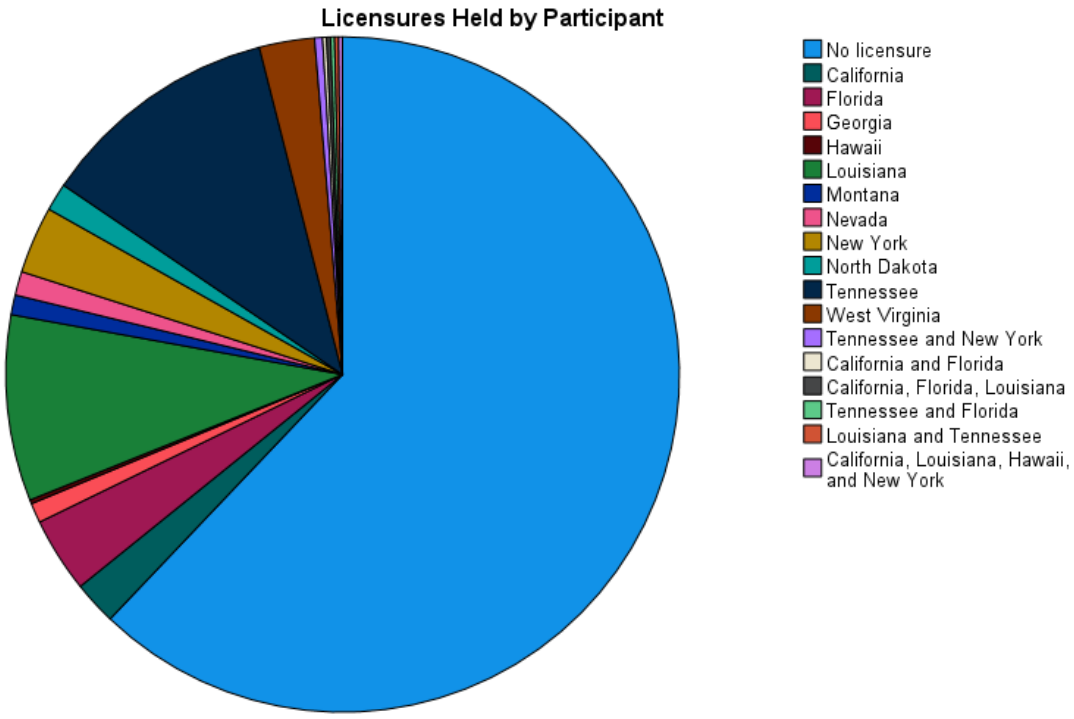
Participants were asked if they held a state licensure. 48.8% of participants did not hold a state licensure. The remaining participants held a licensure from at least one of the eleven states requiring licensure. The variations among participants is demonstrated in the pie graph seen below. Specific frequencies for each demographic can be found in Appendix J. 72.3% of participants are registered with a certifying agency. Of those participants registered, 74.4% are

registered with the American Society of Clinical Pathology (ASCP). The second most common certification held by participants was the American Medical Technologists (6.0%). Specific frequencies for each certification demographic can be found in Appendix K. The diversity among antibiotic sensitivity testing methods can be seen in Appendix L and demonstrates the most common instrument used for testing is the Vitek (39.1%), followed by Microscan (29.3%), and disc diffusion methods (3.9%).

Participants were asked questions about their performance when testing antibiotic susceptibility testing modeled from the Clinical Laboratory Standards Institute (CLSI). Each question models a step taken to perform antibiotic susceptibility testing to ultimately get a correct patient result. Literature exists translationally to indicate inaccuracies among antibiotic susceptibility testing when not conducted appropriately.

Participants were asked if they tested their technique by creating a bacterial inoculum using *Escherichia coli*, and then checked their concentration by counting colony growth. 7.4% of participants explained they performed this testing and obtained the recommended inoculum concentration due to obtaining a colony count of 5x10^5 CFU/mL. 8.9% of participants explain they perform bacterial colony counts using *E. coli* but obtain values other than the recommended 5x10^5 CFU/mL. 13.8% of participants were unaware of this recommendation by CLSI and were unaware of what this question was referring to. 21.9% of the participants explained this was not performed because their lab's Standard Operating Procedure (SOP) did not require them to perform this testing. 17.3% of participants explained they did not perform this testing simply because their lab did not perform it. 9.4% of participants explained they did not perform this testing because they were not trained by their facility to perform the testing.

Figure 6. Percentage of Participants with Licensures



Participants were asked how often they set up a purity plate for bacterial inoculums when performing antibiotic susceptibility testing. 58% of participants explained they set up a purity plate with each bacterial inoculum made. 5.7% of participants explained they only set up a purity plate when colonies are overgrowing or when they do not have good isolated colonies to pull from. 5.5% of participants were unaware of what a purity plate is. 3.9% of participants do not perform purity plates, because their lab does not require this type of set up when performing antibiotic susceptibility testing. 1.0% explained they did not perform purity plates because their Standard Operating Procedure (SOP) did not require they were set up. 4.6% of participants explained they did not set up purity plates because they were not trained to performed purity plates at their current laboratory.

Participants were asked what microbiology bacterial growing media was used to pull the colonies from, when performing antibiotic susceptibility testing. 31.3% of participants explained

they pulled colonies from non-inhibitory plates, such as 5% Trypticase Sheep Blood agar. 12.1% of participants explained when they are performing gram negative antibiotic susceptibility testing, they pull their organisms from plates selective for gram negative organisms, such as MacConkey agar. Gram positive organisms are selected from blood agar or plates selective for gram positive organisms, such as Columbia Naladixic Agar (CNA). 30.7% of participants explained they pull bacterial colonies from any media without limitation. 4.8% of participants were unaware of what this question was referring to.

Participants were asked what method was used to mix their bacterial inoculum once it was made. 54.9% of participants vigorously agitate or vortex the inoculum to ensure a homogenous mixture. 15.0% of participants gently rock the bacterial inoculum. 2.4% of participants explained they choose not to mix their bacterial inoculums. 2.1% of participants explain they do not mix their bacterial inoculums because their laboratory's Standard Operating Procedure (SOP) does not require their inoculums are mixed. 4.3% of participants explain they were not trained on the best methods to mix their bacterial inoculum to ensure a homogenous mixture.

Participants were asked how many antibiotic susceptibility tests they performed on a daily average and if those tested were batched (set up all at one time) or set up throughout the day as the colonies matured (set up one at a time). Participants were also asked if they were able to focus on antibiotic susceptibility tests or if they were required to perform multiple other tests at the same time. Diversity of responses were received regarding how many samples are set up on a daily basis. Generally, these values were about ten to fifty antibiotic susceptibility samples a day and all the samples received are performed at one time. The diversity in results can be seen in Appendix M. Only 35.9% of participants were able to focus on antibiotic susceptibility tests.

All of the other participants who answered this question explained they had responsibilities to other lab testing and performed multiple other tests while performing antibiotic susceptibility tests.

Participants were asked if they after they mixed the bacterial inoculum did they test the final turbidity to test the concentration and if so, what method is used. Only 53.1% of participants explained they tested the final turbidity of their bacterial inoculum for each sample. Of the 53% of participants who test the final turbidity, 22.8% manually use a McFarland Standard, 31.3% use an instrument to determine the final concentration, 0.6% use both a McFarland Standard and an instrument when applicable, 0.1% explain they use an instrument to determine bacterial concentrations of the inoculum when a prompt system is not used. Among those individuals who test the final turbidity of their bacterial inoculum, only 43.6% check the final turbidity with each sample. Other participants vary from daily to quarterly, and some only check the final turbidity with specific genius of bacteria, such a hemolytic *Streptococcus* species.

Bivariate

A bivariate correlation was conducted by performing a two-way ANOVA to determine the degree of relationship between dependent variable AST procedural knowledge (m=1.93, SD 0.993) and independent variable state licensure (m=3.74, SD 4.094). Spearman correlation model was used due to the data being ordinal in nature. The correlation coefficient indicated a correlation between the two variables (r= 0.209). The correlation between the variables is significant, $p< 0.001$.

A bivariate correlation was conducted by performing a two-way ANOVA to determine the relationship between between dependent variable AST procedural knowledge (m=1.93, SD 0.993) and certification (m=1.08, SD 0.271). The correlation coefficient between the two

variables was determined to be correlated (r= -0.166). The correlation between the variables is significant, p< 0.001. r(529)= 0.209, p< 0.001

In the parallel study conducted congruently, a bivariate correlation was conducted to determine the degree of relationship between incorrect results obtained in the questionnaire by a participant and state licensure. The correlation coefficient indicated a fair correlation between the two variables (r= 0.323). The correlation between the variables is significant (p<0.001). A Spearman bivariate correlation was conducted to determine the relationship between incorrect results obtained in the questionnaire by a participant and certification. The correlation coefficient between the two variables was determined to be a correlated (r= -0.166). The correlation between the variables is significant (p< 0.001). r(106)= -0.166, p<0.001

Multivariate Analysis

A multiple linear regression analysis was performed to determine if there was a correlation between the volume of incorrect results obtained in the questionnaire for a participant and if they had a certification and/or state licensure. The null hypothesis was rejected for both research questions two and three. The multiple linear regression determined a strong overall correlation between dependent and both independent variables ($F(2,525)= 18.376$, $p<0.001$, $R^2= 0.065$).

When the laboratory professional obtains state licensure (m=3.74, SD 4.094), AST procedural knowledge (m=1.93, SD 0.993) increases by 0.05 units when all other independent variables are held constant. The model predicted a 0.181 standard deviation increase in AST procedural knowledge with licensure. The null hypothesis was rejected.

Taken as a set, state licensure and certification account for 7% of the variance in laboratory professionals choosing the correct answers. This regression model was significant, $p< 0.001$. When looking at the coefficients, we can say a laboratory professional who holds a state licensure is a significant predictor for obtaining correct results, $p< 0.001$. Certification is also a significant predictor for obtaining correct results $p< 0.001$. The beta coefficients for certification was 0.044 and -0.637 for correct responses. For everyone standard deviation increases in licensure, there are 0.044 standard deviation increases in correct responses. For everyone standard deviation increases in certification, there is a -0.637 decrease in correct responses. ($y= 2.446 + 0.044 – 0.637$)

Each nationally recognized certification was also statistically correlated with AST procedural knowledge. When a laboratory professional obtains ASCP certification ($m=0.84$, $SD=0.391$), AST procedural knowledge ($m=1.93$, $SD= 0.993$) increases by 0.5 units when all other independent variables are held constant ($F(4, 522)=10.323$, $p<0.001$). The model predicted a 0.202 standard deviation increase in AST procedural knowledge when the participant help ASCP certification. When AMT certification was correlated against AST procedural knowledge, it was also statistically determined that laboratory professionals who hold AMT certification ($m=0.10$, $SD=0.346$), had a 0.4 unit increase in AST procedural knowledge ($m=1.93$, $SD=0.993$) when all other independent variables are held constant ($F(4, 522)=10.323$, $p<0.05$). This model predicted a 0.156 standard deviation increase in AST procedural knowledge with AMT certification. AAB certification was correlated with AST procedural knowledge. It was determined when a laboratory professional holds an AAB certification ($m=0.04$, $SD=0.254$), AST procedural knowledge ($m=1.93$, $SD=0.993$) decreases by 0.3 units when all other independent variables are held constant ($F(4, 522)=10.323$, $p>0.05$).

A parallel study was conducted congruently to ensure reproducibility. The parallel study focused on laboratory professionals in the licensure requiring state, Tennessee and laboratory professionals in the non-licensure requiring state, Kentucky. A multiple regression analysis was performed using these laboratory professionals only, to determine if there was a correlation between the volume of incorrect results obtained in the questionnaire for a participant and if they had a certification and/or state licensure. The multiple regression analysis determined a strong overall correlation between dependent and both independent variables ($F(2,103)= 8.698$, $p<0.05$, $R^2= 0.144$). Taken as a set, state licensure and certification account for 14% of the variance in laboratory professionals choosing the correct answers. This regression model was significant. When looking at the coefficients, we can say a laboratory professional who holds a state licensure in Tennessee is a significant predictor for obtaining correct results, $p= 0.032$. Certification is also a significant predictor for obtaining correct results, $p< 0.001$. The beta coefficients for certification was -0.619 and 0.565 for correct responses. For every standard deviation increase in certification, there are -0.619 standard deviation decrease in correct responses. For every one standard deviation increase in state licensure, there are 0.565 increase in correct responses ($y= 1.803 – 0.619 + 0.565$).

When correlation studies are conducted to determine variance among data, only a 7% variance existed, indicating results correlated 93%. This is much lower than expected, as the correlation between each study should have been <20% with at least 80% correlation. These results are consistent and hold true as both studies are comparable and produce similar results, indicating reproducible results.

A hierarchical multiple regression analysis was conducted to determine if suspected independent variables, years of experience with instrumentation/methodology and education

were covariates of the study. These variables were determined to have a covariate relationship with the independent variables in the study, both variables $p<0.05$. This statistical analysis was also valuable in ensuring no other unidentified covariate variables existed.

Logistical Regression Analysis

A logistical regression analysis was performed to look at each individual question and each state requiring licensure. A logistical regression analysis was performed because the dependent variable could no longer be classified as a continuous variable and was a dichotomous categorical level of measurement, as previously described. State licensure and certification again proved significant for individual questions found in the survey, $p<0.05$. Results for eah licensure state were erratic. No licensure state provided any more benefit for one question in the survey over another.

Evaluation of Findings

Research question one, asked what the prevalence for procedural errors among antibiotic susceptibility proficiency testing was for the clinical microbiology laboratory. After a performing a thorough retrospective review of pre-existing proficiency data among five different manufacturers, the overall error rate was calculated at 6%. When focus is brought to the individual proficiency events, the prevalence of error can be as high as 34%. Current literature does not provide descriptive statistical analysis to indicate the ubiquity of error with antibiotic susceptibility testing. This information brings to light the frequency of medical error that is occurring with antibiotic susceptibility testing in the clinical laboratory. The theory of error in medicine is best described by Leape (1994). This theory explains error is involved when humans

are involved, but high rates of error can be avoided with proper training, procedures, and standardization.

Research questions two and three asked if there was a relationship between laboratory personnel's lack of aerobic bacterial inoculum procedural knowledge, licensure, and certification. It was expected that all laboratory professionals would miss questions on the questionnaire to some degree, but it was hypothesized laboratory professionals who did not hold a nationally recognized certification and/or state licensure would miss enough questions to demonstrate a statistically significant difference. After data was collected, if was further analyzed by performing a multiple linear regression analysis to determine if there was a correlation between the amount of correct responses to the questionnaire and certification or licensure. When each of the three nationally recognized certifying agencies were statistically analyzed against AST procedural knowledge, ASCP who is the gold standard of the industry, was determined to be strongly significant ($p<0.001$). The AMT certification was also determined to be statistically significant when compared against AST procedural knowledge ($p<0.05$). When AAB certification was statistically compared to AST procedural knowledge, it was not determined to be significant ($p>0.05$). The results indicated state licensure and certification are both predictors for antibiotic susceptibility testing knowledge. It was also discovered when taken as a set, state licensure and certification account for 7% of the variance in laboratory professionals choosing the correct answers. The parallel study performed congruently indicated a 14% variance in laboratory professionals choosing the correct answers.

When individual questions in the survey were statistically analyzed against licensure requiring states, the significance of state licensure and certification was again proven. No licensure state showed value over another for individual questions. However, after careful

analysis, it was determined individuals who hold more than one state licensure had more correct responses when each question was viewed individually.

There currently is no literature available to describe the relationship between laboratory professional's antibiotic susceptibility testing knowledge, certification, and literature. However, the parallel study's findings being similar to those of the primary study not only indicates the study is reproducible, but also provides another avenue for result comparison due to the absence of existing literature.

Proficiency errors were calculated to range from 1-34% for individual proficiency events. Proficiency testing, designed to test if an individual is proficient, is taken quite seriously in the clinical laboratory. To have calculated values as high as 34% likely points to inaccuracies within the current system. Anderson (2016) explains in medicine, individuals strive to good work, but are acted upon by diverse influences. A functional system should be able to handle inaccuracies and human error. Systems theory focuses on areas such as the qualification of laboratory professionals to be employed in each state. It further focuses the systems involved with education, training, and certification. Systems theory focuses on the system and does to blame or hold the individual accountable for the inaccuracies.

Chapter V: Discussion and Conclusion

The system designed to prevent laboratory errors is faulty. Proficiency testing, put in place by the Clinical Laboratory and Standards Institute (CLSI), was implemented to ensure those performing testing are proficient. Analytical errors are occurring during patient testing leading to inaccuracies in antibiotic susceptibility testing and result interpretation for patient samples. The purpose of this research was to determine the prevalence of error occurring with antibiotic susceptibility proficiency testing; and to determine if there is a relationship between with antibiotic susceptibility testing procedural knowledge and certification and/or state licensure.

A retrospective review was conducted on antibiotic susceptibility proficiency testing results from five proficiency manufacturers for events one through three for years 2016 through

2018. When proficiency testing events were viewed individually, the prevalence of error ranged from 1-34%.

To determine what may be the contributing factors to these errors, an observation correlational quantitative study was performed. A stratified random sampling technique was used to obtain five hundred and twenty-seven laboratory professionals who participated in a survey to collect demographics regarding their education, background, if they had a certification, or held state licensure. Participants were asked a series of questions to determine their procedural knowledge regarding antibiotic susceptibility testing. Questionnaires were then graded for accuracy and scored correct for answers closely relating to standards required by the antibiotic susceptibility testing method they selected. A bivariate and multivariate analysis were conducted to determine the relationship between the scores, licensure, and certification. It was determined there is a relationship between antibiotic susceptibility testing knowledge and certification of laboratory professionals. It was also determined there is a relationship between antibiotic susceptibility testing knowledge and laboratory professionals who hold a state licensure.

Concerns of achieving enough participants was a limitation of this study. The geographical area of study was broadened until enough participant questionnaires were available to account for a reasonable representation of the population. Six proficiency manufacturers are currently approved by the Center for Medicare and Medicaid Services (CMS) for proficiency testing. A limitation to the study was the potential inability to obtain all six manufacturers proficiency data sets. Due to these limitations, a goal to obtain three manufacturer proficiency data sets was fixed. Five electronic proficiency data sets, provided by proficiency manufacturers, were used for this study.

This study will fill many literary and knowledge gaps that exist for antibiotic susceptibility testing in the clinical laboratory, as this type of research is the first of its kind. This is a limitation of the study. Repeated work has literature to reference and results that can be used as a comparison. As the first of its kind, limited literature exists, and no results are available for comparison. A parallel study was conducted congruently with this research. The parallel study proved valuable as the results were comparable with only 7% variation between studies. The parallel study proved the results were reproducible and also was used as a tool to compare results. Limitations for this study also involved confounding variables, experience and education.

Giving the many limitation of this original research, each was overcome successfully, and provided valuable information that can contribute to science, reduce medical error, and reduce potential harm to society. This chapter will discuss the implications of this research as well as provide recommendations on applying learned information and provide possibilities for future research.

Implications

It was an aim of this research to determine if there was a detectable presence of procedural error associated with proficiency testing samples. It was expected a small degree of error did exist among various proficiency testing events for antibiotic susceptibility testing. Results from the study indicated a prevalence of error exists among proficiency testing for antibiotic susceptibility. A retrospective review was conducted on antibiotic susceptibility proficiency testing results from five proficiency manufacturers to determine the prevalence of procedural errors among antibiotic susceptibility testing in the clinical microbiology laboratory. 83% of total available proficiency data regarding antibiotic susceptibility testing was obtained (5 of 6 manufacturers), 23% higher than expected. Results were analyzed for events one through

three for years 2016 through 2018. When proficiency testing events were viewed individually, the prevalence of error ranged from 1-34%. Prevalence of error and associated details describing each proficiency testing event for each manufacturer can be found in appendix N. Current literature does not exist for laboratory science describing analytical error that attributes to incorrect proficiency results.

Proficiency testing is performed by individuals who are regularly testing patient samples. This testing is taken quite seriously and is conducted to determine if the individual performing the testing is proficient. Only three proficiency events are conducted annually. Only one laboratory professional at the facility conducts the proficiency testing for each analyte per event. If you have a staff of ten laboratory professionals performing antibiotic susceptibility testing, it will take ten proficiency events over a course of four years to test each individual for antibiotic susceptibility proficiency. Current CLIA proficiency standards require a score of at least 80% to continue testing. If you were tested for proficiency every three years and are only able to maintain at least 80% on your proficiency, this allows for a considerable amount of "acceptable" testing error among patient samples, and ultimately error among patient diagnosis and treatment.

To determine what procedural error may be contributing to the error rates seen in proficiency testing, an observational correlational quantitative study was performed. A stratified random sampling technique was used to obtain five hundred and twenty-seven laboratory professionals. These participants were asked to participate in a survey that asked their demographics as it relates to their current employment in the clinical laboratory. Participants were also asked a series of questions to determine their procedural knowledge regarding antibiotic susceptibility testing. Questions focused on CLIA recommended steps to create a

correct aerobic bacterial inoculum used to obtain accurate antibiotic susceptibility testing results. Each question was graded for accuracy.

Each step in the creation of a bacterial inoculum has a potential for error. Using translational research, error in each step when creating a bacterial inoculum, has been attributed to inaccuracies in the result interpretation for antibiotic susceptibility testing. The conceptual framework for this research describes each individual step to create the bacterial inoculum and illustrates its connection to error in medicine, elucidating antibiotic resistance. When participant responses were analyzed, it became clear the hypothesized answer for research questions two and three were correct. Error does exist among all laboratory professionals, harnessing the second theory used in this research, to Error is Human.

After a patient's fluid sample has been inoculated onto microbiology bacterial growing media, and incubated for twenty-four hours, growth of pathogenic or opportunistic bacterial colonies are observed. If these types of bacteria are present, they must be eradicated from the host using antibiotics. To determine which antibiotics and what concentration would be most therapeutic in treating the host, an antibiotic susceptibility test must be performed. The first step is to remove isolated pathogenic or opportunistic bacterial colonies from the microbiology bacterial growing media. These colonies must be placed in a sterile liquid solution referred to as the inoculum broth and mixed to a homogenous mixture with a concentration of 5.0 x 10^5 CFU/mL.

Many times, organisms were set up on an array of microbiology bacterial growing media to aid in isolation of bacterial organisms. This media is selective and/or differential by nature aided by antibiotics, chemicals, and dyes. These components added to the media can affect the antibiotic sensitivity results. This can be prevented by using non-selective media, such as 5%

Sheep Blood Trypticase Soy Agar (SB-TSA) to remove isolated colonies to add to the inoculum broth. Participants were given multiple options to choose which microbiological media they primarily use to isolate bacterial organisms. 60% of participants explained they pull colonies for antibiotic susceptibility testing from any bacterial growing media or specifically explained they pulled all gram-negative bacteria from gram negative selective or differential plates and the same for gram positive organisms. Only 40% of participants explained they always take isolated bacteria from non-inhibitory plates, such as 5% Sheep Blood Trypticase Soy Agar. When analyzing these results, it is overwhelmingly apparent, more than half of laboratory professionals are likely causing inaccuracies in results simply by pulling colonies from bacterial growing media that can inhibit correct results or perpetuate incorrect results for antibiotic sensitivity testing.

The next important step in creating a bacterial inoculum is ensuring a completely homogenous mixture when adding the bacterial suspension to each minimum inhibitory concentration well or using to create a lawn of growth for disc diffusion or E-test methods. The purpose of this step is to standardize testing among each antibiotic. A homogenous bacterial inoculum ensures the concentration of bacteria is equally distributed among the testing platforms. CLSI explains the only method to ensuring a homogenous mixture is to vigorously agitate the bacterial inoculum by hand or by using a vortex. Participants were asked in a questionnaire what method they used to mix their bacterial inoculum broth. 69.8% of participants vigorously mix their samples by hand or use a vortex. 22.4% of participants either gently mix the inoculum broth or do not mix the inoculum at all. 5.4% of participants say they were never trained to mix their bacterial inoculums. 2.4% of participants further explained they did not mix the bacterial inoculum because their lab's Standard Operating Procedure (SOP) does not tell

them to mix it. 30% of bacterial inoculums made by participants likely contain heterogenous mixtures of bacteria because the bacterial inoculum is not mixed correctly. This means each minimum inhibitory concentration well is not standardized and contains varying degrees of bacterial concentration. Wells containing too little concentration of antibiotic will inaccurately report as 'bacteria susceptible to antibiotic.' Whereas wells that contain heavier concentrations of bacteria will inaccurately report as 'bacteria resistant to antibiotic.' This causes inaccuracies in antibiotic susceptibility testing results reported to a treating physician.

Participants were also asked if they were able to focus on antibiotic susceptibility testing or if they performed multiple other tests while performing antibiotic susceptibility testing. This is important, because literature demonstrates suspensions in solutions settle out due to gravity pulling the particulates out of solution in fifteen minutes. If this could happen, antibiotic susceptibility testing would be set up using non-homogenous mixtures, creating inaccurate results. For this reason, bacterial inoculums should be used for antibiotic susceptibility tests within fifteen minutes. 54% of participants explained they were unable to focus on antibiotic susceptibility testing and explained they performed multiple other tests while performing antibiotic susceptibility testing. This could also be another analytical error, as suspensions may be settling out while laboratory professionals are attempting to perform other tests.

It is also important to identify a bacterial inoculum is pure or contains only one bacterial organism. Bacterial plates containing human samples, will often times contain multiple organisms, as many locations on the human body are not sterile. It can be challenging to obtain at least three well isolated pathogenic or opportunistic bacterial colonies. Contamination of the bacterial inoculum can occur from accidently grabbing more than bacterial organism from the media or contamination can occur from an outside source. More than one organism growing on

an antibiotic susceptibility test can cause severely inaccurate results. Commensal organisms, those non-pathogenic bacteria or normal flora found on the host's body often times are quite resistant to antibiotics and can mask the presence of a more sensitive pathogen in the bacterial inoculum. These errors can be prevented by inoculating a purity plate made from the bacterial inoculum suspension. A purity plate should be set up each time a bacterial inoculum broth is created. Purity plates are incubated for twenty-four hours and monitored to ensure pure bacterial growth occurs. Participants were asked if they used a purity plate for antibiotic susceptibility testing and how often they used it. 73% of participants explained they set up a purity plate each time they created a bacterial inoculum. 14.8% explained they only used a purity plate if they felt it was challenging to get isolated bacterial colonies or they did not use purity plates at all. This is concerning because there are other methods of contamination. You can have two phenotypically similar bacterial species, that produce varying antibiotic susceptibilities. One could contaminate the bacterial inoculum from an external source. Without a purity plate you have no way of knowing if your antibiotic susceptibility test is pure. 6.4% of participants explained purity plates are not set up because the Standard Operating Procedure (SOP) does not tell them to. 5.8% of participants explain they were never trained at their facility to use a purity plate.

Once the bacterial inoculum has been set up, most of the time, laboratory professionals verify the final concentration. This can be done by comparing the bacterial inoculum to a 0.5 McFarland standard or the bacterial inoculum can be placed in a spectrophotometric instrument to measure the absorbance of light in the solution and perform a calculation to convert that information to bacterial concentration. The final concentration of the bacterial inoculum is critically important as a deviation in this step can cause gross inaccuracies in antibiotic testing. Only 67.5% of laboratory professionals test the final turbidity of their bacterial inoculum. Of this

amount, 55.7% of professionals check the final turbidity of each bacterial inoculum made. 5.5% check the final bacterial inoculum daily, 3.2% weekly, 2.9% monthly, 2.3% quarterly, 0.2% annually, 0.2% with *Staphylococcus* species only, and 0.2% hemolytic *Staphylococcus* species only.

With the large percentages of participants explaining behaviors in the questionnaire that are known to cause inaccurate antibiotic susceptibility testing, it is not surprising to see proficiency errors as high as 34% for some events. It is possible some bacterial organisms may be more sensitive to these errors than others and could explain large variations in proficiency error. Is there an already existing method available to reduce these analytical antibiotic susceptibility testing errors? Two variables were identified as having possible correlations with antibiotic susceptibility testing knowledge, certification, and state licensure.

Participants were asked if they were certified as medical laboratory technician, technologist, or scientist. They were then asked who their certification agency was to ensure the agency was nationally recognized. The three primary nationally recognized certification agencies for laboratory professionals in the United States are the American Society for Clinical Pathology (92%), American Medical Technologist (7.6%), and American Association of Bioanalysts (2.1%). A bivariate analysis was conducted to determine if there is a relationship between those laboratory professionals who are certified and antibiotic susceptibility testing knowledge. It was determined there is a strong relationship between certification and procedural knowledge regarding antibiotic susceptibility testing, $p < 0.001$. This is valuable to the field of medical laboratory science. While literature exists proving the value of certification for other areas of medicine, such as nursing and medical doctors, before this research, no literature existed proving

a correlation or relationship between certification and knowledge in testing areas of medical laboratory science.

Each certification agency was further analyzed independently to verify that a relationship exists between each certification agency and procedural knowledge of antibiotic susceptibility testing. This data determined the American Society of Clinical Pathology (ASCP) was the only nationally recognized certifying agency for laboratory professionals that exhibited a statistically strong relationship with procedural knowledge, $p < 0.05$. While preliminary data did identify a statistically significant relationship between certification and procedural knowledge, it is important to note ASCP accounts for 92% of certified laboratory professionals among this data. The most recent literature, provided by ASCP in 2000, explains their Board of Certification (BOC) is responsible for certifying 64% of laboratory professionals in the field. While the estimates have not been repeated in twenty years, it is estimated those values would be much higher in today's laboratory professional pool.

As the field is suffering immense staffing shortages for various reasons, facilities in non-licensure requiring states can employ individuals outside of the traditional arena for laboratory professionals. Not always, but usually these individuals are not certified as medical laboratory professionals. This research demonstrates a course of events, starting with certification, that leads to more accurate test results being reported to physicians for diagnosis and treatment of patients.

A bivariate analysis was also conducted to determine if there was a relationship between state licensure and antibiotic susceptibility testing procedural knowledge. There is currently no research that demonstrates the value of state licensure in the field of medical laboratory science. There are currently eleven known licensure requiring states, with only ten states requiring

licensure of individual laboratory professionals. These states are California, Florida, Hawaii, Louisiana, Montana, Nevada, New York, North Dakota, Tennessee, and West Virginia. Georgia is also considered a licensure requiring state, as it does require licensure. However, Georgia does not require the licensure of laboratory professionals, but does require the licensure of each clinical laboratory performing human sample testing. This is unique for clinical laboratories in most states in the United States. Each state has its own required qualifications for the licensed laboratory professional. The following list provides a series of requirements for those laboratory professionals seeking licensure in a licensure requiring state.

1. Academic Requirements
2. Background check
3. Finger printing
4. Certification
5. Annual or Biannual fee
6. Continuing Education Requirements
7. Training
8. Job Experience
9. Passport Photograph
10. Swearing of an Oath
11. Online Education Classes and Associated Quizzes
12. Infectious Disease Classes
13. State Licensure Exam
14. Recognizes Reciprocity of Other Licensures

No two states request the same requirements of their licensure seeking laboratory professionals. Educational requirements, for example, vary greatly from state to state. North Dakota simply requires a Bachelor of Science degree to meet the educational requirements for a medical laboratory scientist licensure. While New York, requires graduation from a clinical laboratory program or equivalent degree registered under the New York Inventory of Registered Programs. Should your program not reside in the state of New York, you are required to submit syllabi from each course requested. California and Tennessee licensure have specific educational credit requirements, where a specific number of biology and chemistries are requested. Continuing education also varies greatly between licensure states. States such as Hawaii, do not require continuing education. Other states such as North Dakota, who have lax academic requirements, require thirty continuing education credits every two calendar years. The table below demonstrates the diversity in the academic and continuing education requirements for each licensure requiring state. It is important to note, only licensure requiring states request academic and continuing education of medical laboratory professionals. The other thirty-nine non-licensure states, do not require academic nor continuing education requirements for laboratory personnel.

Table 6. Licensure States Academic and Continuing Education Requirements

State	Academic Requirements	Continuing Education
CA	MLS: 16 semester units of chemistry (must include clinical chemistry or analytical and biochemistry), 18 semester units of biology (must include hematology, immunology, and medical microbiology), 3 semester units of physics	12 CEU annually
FL	MLS Route 1: Must complete bachelor's degree (or higher) in clinical laboratory, chemical biological science, or with 24 hours of science (must include 6 hours of biological science and 6 of chemical science) MLS Route 2: Complete 90 semester hours of college credit (24 hours of science and must include 6 hours of biological science and 6 of chemical science) and complete a clinical laboratory training program	24 CEU biannually

GA	Not required in laboratory professional licensure	_
HI	Not required in laboratory professional licensure	_
LA	Not required in laboratory professional licensure	12 CEU calendar year
MO	MLS: Must have graduated from an accredited college or university with a bachelor's degree with at least 36 semester or 54 quarter hours in the physical and biological sciences.	14 CEU calendar year
NV	MLS Route 1: Obtain bachelor's degree in medical technology MLS Route 2: Obtain bachelor's degree in chemical, physical, or biological sciences and have one full year of documented full-time training Route 3: Take United States Department of Health and Human Services (USDHHS) or HEW exam	10 CEU biannually
NY	MLS Route 1: Receive bachelor's degree or higher in clinical laboratory technology from New York registered institution. MLS Route 2: Receive bachelor's degree or higher in biology, chemistry, or physical science with an advanced certificate proving licensure qualifying. Out-of-state programs must submit syllabi.	12 CEU annually
ND	MLS: bachelor's degree or higher in a science-related discipline.	30 CEU biannually
TN	MLS Route 1: bachelor's degree or higher in medical technology or in biological, chemical, or physical sciences and the completion of a medical laboratory technologist training program. MLS Route 2: bachelor's degree or higher, MLT certification, 3 years of fulltime clinical laboratory work experience, and science coursework equivalent to that required in a laboratory science education program. MLS Route 3: bachelor's degree or higher, five years of fulltime clinical laboratory work experience and completion of science coursework equivalent to that required in a laboratory science education program. Each qualifying bachelor's degree must include: 1. 16 semester hours of chemistry (including 1 year of general chemistry, organic chemistry or biochemistry with lecture and lab) 2. 16 semester hours of biological science (including microbiology with lecture and lab) 3. 3 semester hours of pre-science mathematics	24 CEU biannually
WV	MLS Route 1: Bachelor's degree or higher in medical laboratory technology/science AND has passed a national certifying examination MLS Route 2: Bachelor's degree or higher in a chemical, physical, or biological science AND 1 year of full-time experience or training	10 CEU annually and supervisor

	MLS Route 3: Passed a HEW exam between March 1, 1986 and December 31, 1987 MLS Route 4: Was qualified under CLIA guidelines (493.1489(b)(5)(1)) and was performing high complexity testing prior to April 25, 1995 Route 5: Was licensed as a CLP-MT immediately preceding the effective date of this rule (June 1, 2017) and has complied with the CLP application process.	signature required
	All non-licensure requiring states: Academic requirements nor continuing education requirements are requested from medical laboratory personnel from any of thirty-nine non-licensure requiring states	

Licensure states also vary in their requirements of background safety for their medical laboratory professionals. Not all states require background checks. Some states require additional precautions, such as passport photos and fingerprinting. It is important to note, that while diversity exists among those licensure states for background safety, non-licensure requiring states do not require background safety for medical laboratory professionals. The table below demonstrates this diversity in background safety among licensure requiring states.

Table 7. Background Safety in Licensure States

State	Background Check	Passport Photo	Fingerprinting
CA	Yes	No	No
FL	Yes	No	No
GA	No	No	No
HI	No	No	No
LA	Yes	Yes	No
MO	No	No	No
NV	No	No	No
NY	Yes	No	No
ND	No	No	No
TN	Yes	Yes	Yes
WV	No	No	No
Non-Licensure State	No	No	No

Licensure states that require background checks, screen the background of all laboratory professionals. This removes the responsibility of background screening from individual hiring laboratories. In licensure states that do not require background checks, it is up the hiring facility to access the rigor involving offenses found in the background of laboratory personnel.

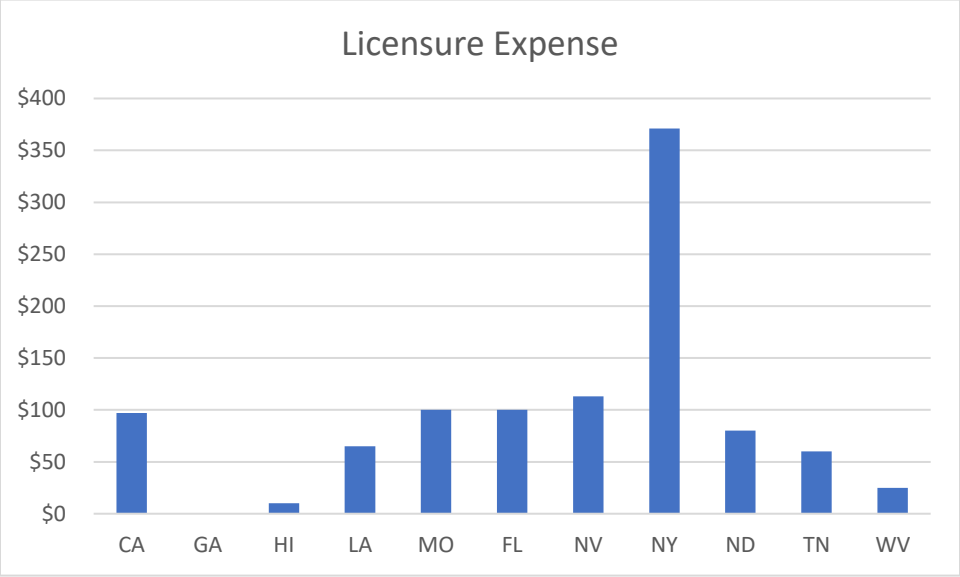
Certification requirements also vary greatly from one state to the next. California and Florida will accept certification from ASCP, ASCPi, AMT, or AAB. These states are the only states that will accept the international version of certification supplied by the American Society of Clinical Pathology. Licensure states Louisiana, Montana, Nevada, North Dakota, Tennessee, and West Virginia will accept ASCP, AMT, and AAB certification for licensure. Hawaii and New York will only accept ASCP certification when applying for medical laboratory licensure.

On the job training requirements also vary greatly from one state to the next. For most licensure states, the requirement of on-the-job training relies heavily on the route chosen to apply for licensure and is often based on the academic history of the licensee. California is the only states that maintains strict on-the-job training requirements post baccalaureate clinical training as a laboratory professional. On-the-job training in the state must be for a minimum of one year, be comprehensive and include all areas of the laboratory, must be clinical in nature, must be heavily documented including rotations and documentation of all testing methods performed. West Virginia does not require on-the-job training, but it the only state that comes close to the training requirements of California. Annually, state licensure is renewed in West Virginia. The renewal must include a comprehensive list of testing methodologies used by the licensee and includes detailed hours worked among each section of the laboratory.

Florida and Louisiana require laboratory professionals take an oath upon licensure application and renewal. The oath speaks of maintaining good laboratory ethics, such as

reporting laboratory results honestly, and maintaining the integrity of patient reporting. Florida and Louisiana requires laboratory professionals to take a class before obtaining licensure, discussing infectious diseases in the laboratory, such as Human Immunodeficiency Virus (HIV). Both California and Louisiana require licensure seeking laboratory professionals take online education classes and associated quizzes, discussing the laws surrounding the laboratory and patient testing, as well as the implications when laws are not adhered to. North Dakota is the only state that recognizes reciprocity from other states also requiring licensure among laboratory professionals. California and New York require their own state licensure exams beyond national certification, as a requirement for medical laboratory licensure. The table below demonstrates the diversity among licensure expenses for the laboratory professional.

Figure 7. Licensure Expenses per State



While such diversity exists between states, a strong statistical relationship was exhibited between state licensure and procedural knowledge of antibiotic susceptibility testing, $p< 0.05$. By going through the application process for each licensure state, it is clear which element each state values of the laboratory professional's background, academic, criminal background, ethical integrity, or on-the-job experience. Each state varies greatly from one another and for this reason, no two states could be grouped together for additional testing purposes.

Many licensure states also require certification, another independent variable in this study. Multiple collinearity studies were performed against the independent variables to ensure one was not dependent on the other. The collinearity tolerance for certification and licensure was greater than the required >0.2. The collinearity VIF was less than the ideal numerical value of three. This statistical analysis proves there is no dependency between the independent variables licensure and certification. This proves there are valuable elements of licensure, besides certification, that correlate with antibiotic susceptibility procedural knowledge.

A parallel study was performed congruently with this research. The parallel study focused on laboratory professionals who are employed in the non-licensure state Kentucky who do not hold a state licensure and those laboratory professionals who hold a Tennessee state licensure. A multiple linear regression analysis was performed on the data and found the relationship between those who hold Tennessee state licensure and have procedural knowledge of antibiotic susceptibility testing to be strongly statistically significant, $p< 0.05$. It was also discovered those individuals who do not hold a state licensure and are employed in the non-licensure requiring state of Kentucky, have a strong statistically significant relationship with a lack of procedural knowledge of antibiotic susceptibility testing $p< 0.05$. This information supports the rejection of the null hypothesis.

A possible limitation that could have occurred when collecting data is dishonesty among questionnaire participants. It is possible, participants who do not hold certification with a national registry were dishonest and proclaimed to have certification when they did not. However, if this error occurred, procedural question responses would have remained the same and significance would not have been demonstrated in the study. This is because the significance of incorrect results would be correlated with certification. The correlation is not small enough to be affected by a few individuals who may have been dishonest and reported and incorrectly reported their values as such. This research focuses on systems theory, which explains most individuals will strive to do good work, but that individuals in the system are acted upon by diverse influences that are accounted for in functional systems (Anderson 2016). Using this theory, we must assume that most laboratory professionals will not act unethically, but performing this study using a stratified random sampling method will aid in dismantling this possible bias.

The last known limitation of the study involved the presence of confounding variables. The amount of years actively practicing as a laboratory professional and education level are confounding variables for this study. Known confounding variables may contribute to the outcome of the multivariate analysis and could damage the internal validity of the experiment. Failing to control for the variables could have indicated a false correlation between procedural knowledge, licensure, and certification, leading to incorrectly rejecting the null hypothesis. Experience in the clinical laboratory was measured by the questionnaire. The second confounding variable is the level of education the laboratory professional has earned. Data was collected in the questionnaire to measure the level of education obtained by the laboratory professional, as it relates to laboratory medicine. This variable cannot be removed from the

study; therefore, it was tested as a covariate in the multivariate regression. Random stratified sampling provided equal distribution for level of education among participants and for additional unknown confounding variables.

Unfortunately, in clinical or reference laboratories with greater volumes of laboratory professionals, it may take several years before you receive a proficiency sample for a particular type of testing, such as antibiotic susceptibility. Other mechanisms are put in place to catch error among staff and instrumentation, such as quality controls. However, for tests, such as antibiotic susceptibility testing, quality controls cannot be run daily and are often not ran by most individuals performing testing. If ten laboratory professionals perform antibiotic susceptibility testing, only one performs quality controls. This exemplifies the need for proficiency testing among all laboratory professionals in a timely manner.

Recommendations

Testing the correlation between certification and knowledge of antibiotic susceptibility testing was important because states that do not require licensure of laboratory personnel do not have state mandated qualifications, such as certification. This responsibility falls on the hiring management for the clinical laboratory at each facility instead of depending on statewide mandates. The required qualifications of laboratory staff often depend on what is available to fill the staffing gaps. Laboratory Managers explain in the ASCP (2005) survey, 60.3% of pathologists felt state licensure would cause decreased flexibility with hiring and vacancy, but would increase compensation in licensure states, the state regulates the type of individual to be hired in specific laboratory positions and the manager chooses the individual. Whereas in non-

licensure states, the hiring manager, or whoever is designated at the facility, is in charge of not only hiring the individual but also the type of individual (as CLIA allows) for the position. Laboratory professionals employed in non-licensure requiring states can have diverse backgrounds ranging from life science to independent study majors. Education requirements vary for job responsibilities and can also range from high school education or equivalent to a doctorate. These individuals may or may not have formal laboratory training or education and may not have graduated from a NACCLS accredited laboratory program. For non-licensure requiring states, certification is one of the only mechanisms that can be used to ensure a laboratory professional has the necessary fundamental knowledge to be successful at the job. While many laboratory professionals hold certification, this is not a national requirement. If not mandated on the national level by a regulating agency such as CLIA-88, this should be mandated on the state level. Because there is such strong statistical evidence in the value of certification, it should be required of all laboratory personnel who are performing antibiotic susceptibility testing. Further studies should be implemented to test the value of certification in other areas of the laboratory where no instrumentation supports the laboratory professional, and success of the method relies heavily on visual acuity and acute knowledge of the subject material at hand.

This research proves there is a correlation between antibiotic susceptibility knowledge and state licensure. There are currently eleven known licensure requiring states, with ten states requiring licensure of individual laboratory professionals. These states are California, Florida, Hawaii, Louisiana, Montana, Nevada, New York, North Dakota, Tennessee, and West Virginia. Georgia is often grouped with other licensure requiring states. However, Georgia only requires the licensure of laboratories and not their laboratory personnel. Each state is unique and has its own set of individual requirements. These requirements are listed below.

1. Academic Requirements
2. Background check
3. Finger printing
4. Certification
5. Annual or Biannual fee
6. Continuing Education Requirements
7. Training
8. Job Experience
9. Passport Photograph
10. Swearing of an Oath
11. Online Education Classes and Associated Quizzes
12. Infectious Disease Classes
13. State Licensure Exam
14. Recognizes Reciprocity of Other Licensures

From this research alone, we know certification, should be imperative for the laboratory professional no matter what state they are employed. Both bivariate and multivariate analysis demonstrate a strong statistical relationship between licensure and procedural knowledge of antibiotic susceptibility testing. Data did not demonstrate the value of each element of state licensure in testing accuracy. It is clear by this study, there is value to components of state licensure, other than certification. Future research should investigate the value of state mandated qualifications, other than certification, on procedural knowledge. States who require individuals to take an oath, require biannual laboratory law classes, and associated quizzes of laboratory professionals may have better ethics among their laboratory professionals and therefore may take

more pride in results accuracy and those professional prone to intentional error may avoid it. States who require stringent academic work, may see stronger values in academia and see value in the laboratory professional's strength of knowledge to prevent error. Those states who require rigorous on-the-job-training and request detailed work experience find value in this arena of the laboratory professional, to prevent error in laboratory results. Other states require high security background checks with inflexible boundaries, proving acceptable criminal clearance. Future research should focus on which element of the state licensure is valuable in preventing error in testing in the clinical laboratory.

Another recommendation comes over the concerns of over on-the-job training. Many participants in the questionnaire explained repeatedly they did not perform the specific step the question was referring to for antibiotic susceptibility testing because they were not trained to do so at their current facility. On-the-job training varies greatly from one institution to the next; and those requirements change drastically from non-licensure to many licensure requiring states. Some employers have rigorous training programs that prevent new hires from releasing patient results for tests such as gram stains, in less than twelve months. While other laboratory professionals are lucky to get two weeks of training, before being thrown on their own on a solo shift. CLIA does require a training schedule, checking off boxes as each item is taught, but at many facilities proctors or mentees are challenged to perform training duties as well as job responsibilities effectively. More research should investigate on-the-job training performed by laboratory professionals.

Future research should investigate why some proficiency events have higher error rates than others among the same proficiency manufacturer. It is possible some bacteria are more susceptible to procedural errors. If this is the case, error rates may be more significant and have

been masked by their tolerance to error. There is also a possibility higher error rates could be associated with a specific proficiency manufacturer. Some testing may prove more sensitive or more rigorous than other proficiency manufacturers. If this is the case, the Centers for Medicaid and Medicare Services will need to develop more rigorous standards for proficiency testing. It is also possible some manufacturers may have variations in their qualification for enrollment. This should all be investigated to further identify variations seen in proficiency testing event scores for antibiotic susceptibility.

Many participants explained they did not perform the specific step of antibiotic susceptibility testing due to that step not being included in their clinical laboratory's Standard Operating Procedures (SOP). Research should investigate if Standard Operating Procedures (SOP) for antibiotic susceptibility testing generally follow CLSI standards. If standards are not followed, what standardization is being used when implementing standard procedure?

Proficiency testing should be re-evaluated. CLIA should take a closer look at proficiency testing and remove the clinical laboratory management from overseeing and assigning proficiency. This would eliminate potential politics that may occur with assignment and result interpretation. Proficiency schedules and rotation should be handled by an offsite individual. Monitoring could be performed by an offsite individual who is not a laboratory professional. This would also free time with laboratory management and perhaps provide much needed hands on work in the short-staffed clinical laboratory. Laboratory professionals should enter and sign their own proficiency results into an electronic system without laboratory management access, eliminating proficiency results from touching managements hands.

Proficiency samples volume should be based on the capita of laboratory professionals employed in the clinical laboratory. Antibiotic susceptibility testing performed by twenty

individuals employed in the microbiology department, may only perform one proficiency test every 6-7 years, if left to an honest rotation. What value is proficiency testing if procedural errors may only be viewed once every few years? Thousands of patient sample are released over the course of a year, many may be with inaccuracies.

CLSI should recommend resuspension of laboratory professionals' bacterial inoculum by vigorous agitation or vortexing if has sat for fifteen minutes of longer. 54% of laboratory professionals are performing multiple other tests while attempting to perform antibiotic susceptibility testing. Literature demonstrates suspensions in solutions settle out due to gravity pulling the particulates out of solution in fifteen minutes. If this could happen, antibiotic susceptibility testing would be set up using non-homogenous mixtures, creating inaccurate results.

Conclusion

Research of this nature has not been performed for the clinical laboratory or medical laboratory professionals. This research has the possibility of opening the door to improve multiple avenues in medicine such as reducing medical error, reducing community antibiotic resistance, promoting certification, and increasing the accuracy of patient results reported to physicians.

This observational correlational quantitative research uncovered existing proficiency error that was shown to be distributed unevenly among testing events and proficiency manufacturers. Substantial frequencies in procedural errors were identified among enough participants to represent the nation's population of laboratory professionals. Both bivariate and

multivariate analysis were performed and identified a relationship between certification, state licensure, and procedural knowledge of antibiotic susceptibility testing.

It is clear from this research all laboratory professionals should be certified to perform antibiotic susceptibility testing in the clinical microbiology laboratory. Further research should be performed to determine if the same is true among other areas of the clinical laboratory where laboratory professionals do not rely on instrumentation. This research also clearly identified a strong statistical relationship between state licensure and procedural knowledge of antibiotic susceptibility testing. Future research should investigate the significance of each element of state licensure and results should be considered by each non-licensure state for their medical laboratory personnel.

References

Abraham, E.P., Chain, E., Fletcher, C.M., Gardner, A.D., Heatley N.G., Jennings, M.A., Florey, H.W. (1941). Further observations of penicillin. *Lancet*. 238. Pg.177-189. Doi: 10.1016/S0140-6736(00)72122-2

Aguda, E. (2017). *Hand off communication: A survey study of what anesthesia providers need to know*. Available from ProQuest Dissertations & Theses Global. (1979336274). Retrieved from https://search-proquest. com.ezproxy.trident.edu/docview/1979336274?accountid=28844

Akova, M. (2016). Epidemiology of antimicrobial resistance in bloodstream infections. *Virulence*, *7*(3), 252–266. http://doi.org/10.1080/21505594.2016.1159366

Aminov, R. (2010). A Brief History of the Antibiotic Era: Lessons Learned and Challenges for

the Future. *Frontiers in Microbiology*. 1. pp 134. http://doi.org/10.3389/fmicb.2010.00134

American Society for Microbiology (2017). The who, what, where of careers: Clinical Microbiology. American Society for Microbiology. Retrieved from https://www.asm.org/index.php/career-paths-clinical-microbiology.

Anderson B. R. (2016). Improving health care by embracing Systems Theory. *The Journal of thoracic and cardiovascular surgery*, *152*(2), 593-4.

Appold, K. (2013). Tackling Antibiotic Resistance. Journal of Continuing Education Topics and Issues. 15(3). pp. 92-94. Retrieved from https://search-proquest.com.ezproxy.trident.edu/docview/1443263845? accountid=28844

Badrick, T. and StJohn, A. (2014). Does Medical Science in the Workforce Deliver a Competent Profession. *Clinical Biochemistry*. 47 (2014). pp. 1-4. Retrieved from https://www.researchgate.net/profile/Tony_Badrick/publication/264462408_Does_registration_of_the_medical_science_workforce_deliver_a_competent_profession/links/55fcfa5c08aeafc8ac52fb12.pdf

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 45. Pg 493-496. Doi: 10.1093/ajcp/45.4_ts.493

Bayot, M. and Bragg, B. (2020). Antimicrobial Susceptibility Testing. In *StatPearls*. StatPearls Publishing.

Beckman Coulter (2015). *Dried Gram-Negative Procedural Manual.* Beckman Coulter.

Berendonk, T., Manaia, C., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., et al (2015). Tackling Antibiotic Resistance: The Environmental Framework. Nature. 13. pp 310-317. Doi: 10.1038/nrmicro3439

Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews.Microbiology*, *13*(1), 42-

51. doi: http://dx.doi.org.ezproxy.trident.edu:2048/10.1038/nrmicro3380

Brogan, D. M., Mossialos, E., (2016). A critical analysis of the review on antimicrobial resistance report and the infectious disease financing facility. *Global Health*. 12.8. doi: 10.1186/s12992-016-0147-y

Brook, I. (1989). Inoculum Effect. *Reviews of Infectious Diseases*. 11 (3). Pp. 361–368. https://doi.org/10.1093/clinids/11.3.361

Bureau of Labor and Statistics (2017). Occupational Employment Statistics. United Sates Department of Labor. Retrieved from https://www.bls.gov/oes/current/oes292011.htm#st

Bush, K., Courvalin, P., Dantas, G., Davies, J., Einstein, B., Huovinen, P., et al. (2011). Tackling Antibiotic Resistance. Microbiology. 9(2). pp 894-896. DOI: 10.1038/nrmicro2693.

Centers for Disease Control and Prevention (2014). Core elements of hospital antibiotic stewardship programs. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/getsmart/healthcare/ implementation/core-elements.html

Cohen, J. (1988), *Statistical Power Analysis for the Behavioral Sciences, 2nd Edition.* Hillsdale, N.J.: Lawrence Erlbaum.

Cox, G. and Wright, G. (2013). Intrinsic antibiotic Resistance: Mechanisms, origins, challenges, and solutions. *International Journal of Medical Microbiology.* 303. pp 287-292. http://dx.doi.org/10.1016/j.ijmm.2013.02.009

Delost, M., Miller, G., Chang, G., Korzun, W., and Nadder, T. (2009). Influence of Credentials of Clinical Laboratry Professionals on Proficiency Testing Performance. American Journal of Clinical Pathology. pp 550-554. 132. Doi: 10.1309/AJCPWCBSYISV1ASI.

Dintzis, S., Stetsenko, G., Sitlani, C., Gronowski, A., Astion, M., Gallagher, T. (2011) Communicating Pathology and Laboratory Errors: Anatomic Pathologists' and Laboratory Medical Directors' Attitudes and Experiences. *American Journal of Clinical Pathology*. 135(5). 760–765, https://doi.org/10.1309/AJCPJF1YUFG6GTFI

Edson, D. and Massey, L. (2007). Proficiency Testing Performance in Physican's Office, Clinic, and Small Hospital Laboratories, 1994-2004. LabMedicine. 38(4). Pp 237-239. Doi:

10.1309/6EV4QT8119DU7LXE.

Ferran, A., Dupouy, V., Toutain, P. L., & Bousquet-Mélou, A. (2007). Influence of inoculum size on the selection of resistant mutants of Escherichia coli in relation to mutant prevention concentrations of marbofloxacin. *Antimicrobial agents and chemotherapy*. 51(11). pp 4163-4166.

Fleming, A. (1929). On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. Influenza. *Journal of Exp. Pathology.* 10.226. doi: 10.1093/clinids/2.1.129

Forsman, L. (1992). Why is the laboratory an afterthought for managed care organizations. *Clinical Chemistry*. 42. pp 813–816. PMID: 8653920.

Forsyth, J. A. (2016). *The relationship between trust, commitment, and leader actions with intent to leave the united states army* (Order No. 10024300). Available from ProQuest Central; ProQuest Dissertations & Theses Global. (1770090193). Retrieved from https://search-proquest-com.ezproxy.trident.edu/docview/1770090193?accountid=28844

Foster, J.W., Woodruff, H.B. (1943). Microbiological aspects if penicillin: I. Methods of assay. *Journal of Bacteriology.* 46.187

Friedman, N., Temkin, E., and Carmeli, Y. (2016). The Negative Impact of Antibiotic Resistance. *European Society of Clinical Microbiology and Infectious Disease.* 22. pp 416-422. Doi: dx.doi.org/10.1016/j.cmi.2015.12.002

Frieri, M., Kumar, K., and Boutin, A. (2017). Antibiotic Resistance. *Journal of Infection and Public Health*. 10(4). Pp 369-378. Doi: doi.org/10.1016/j.jiph.2016.08.007

Gartmeier, M., Bauer, J., Gruber, H., and Heid, H. (2008). Negative Knowledge: Understanding

Professional Learning and Expertise. Vocations and Learning. 1:87-103. Doi: 10.1007/s12186-008-9006-1

Hallworth, M. (2011). The '70% claim': what is the evidence base? Annals of Clinical Biochemistry. 48. 487-488. Doi: 10.1258/acb.2011.011177

Hoyt, R.E., Levine, M.G., (1947). A method for determining sensitivity to penicillin and streptomycin. *Science*. 171. Doi: 10.1126/science.106.2747.171

Johnson, A. (2015). Surveillance of Antibiotic Resistance. Philosophical Transactions. 370. Doi: dx.doi.org/10.1098/rstb.2014.0080

Khan, Z., Siddiqui, M., and Park, S. (2019). Current and Emerging Methods of Antibiotic Susceptibility Testing. *Diagnostics*. 9(2).49. doi:10.3390/diagnostics9020049

Kohn, L. T., Corrigan, J., & Donaldson, M. S. (2000). To err is human: Building a safer health system. Washington, D.C: National Academy Press.

Leape, L. (1994). Error in Medicine. JAMA. 272(23). 1851-1857. doi:10.1001/jama.1994.03520230061039

Li, J., Xie, S., Ahmed, S., Wang, F., Gu., Y., Zhang, C., *et al* (2017). Antibacterial Activity and Resistance: Influencing Factors. Frontier Pharmacologic. Doi.org/10.3389/fphar.2017.00364

Morency-Potvin, P., Schwartz, D., Weinstein, R. (2017). Antimicrobial Stewardship: How the microbiology laboratory can right the ship. Clinical Microbiology. Rev 30:1. 381–407. doi.org/10.1128/CMR.00066-16.

Murrell, D. and Harrington, A. (2016). Impact of Limited Incubation of Bacterial Growth-Positive Cultures and Antibiotic Sensitivity Testing. *Open Forum Infectious Diseases*. 3. pp 165. https://doi.org/10.1093/ofid/ofw172.32

Park, J., Leung, S., and Wang, J. (2016). Licensure in the Era of Genomic Medicine. *Archives of Pathology and Laboratory Medicine*. 140(7). 623-624. doi: http://dx.doi.org.ezproxy.trident.edu:2048/10.5858/arpa.2016-0103-ED

Perkins, I. (2016). Error Disclosure in Pathology and Laboraotry Medicine: A Review of the

Literature. *Journal of Ethics.*18(8):809-816. doi:10.1001/journalofethics.2016.18.8.nlit1-1608.

Prous, M. and Ponto, M. (2016). Testing Knowledge of Eye Donation: a survey of intensive care nurses. *Progress in Health Sciences.* 6(1). 86-94. doi:10.5604/01.3001.0009.5131

Qualtrics (2018). Sample Size Calculator. Calculating Sample Size. Retrieved from https://www.qualtrics.com/blog/calculating-sample-size/

Rodriguez-Rojas, A., Rodriguez- Beltran, J., Couce, A., and Blazquez, J. (2013). Antibiotics and antibiotic resistance: A bitter fight against evolution. *International Journal of Medical Microbiology.* 303. pp 293-297. http://dx.doi.org/10.1016/j.ijmm.2013.02.004

Steward, C. and Schulze, M. (2005). ASCP Survey on Laboratory Personnel State Licensure. *Laboratory Medicine*. 36(9). pp 524–527. Doi: doi.org/10.1309/6GRMPW6W3XQD1GBJ

Vincent, J.G., Vincent, H.W., Morton, J. (1944). Filter paper disc medication of the Oxford Cup penicillin determination. *Professional Society Exp Biology Medicine.* 55. Pg 162-164. Doi: 10.3181/00379727-55-14502

World Health Organization (2014). *Antimicrobial resistance: global report on surveillance*. World Health Organization. Retrieved from http://www.who.int/drugresistance/documents/surveillancereport/en/

Zapata, A., & Ramirez-arcos, S. (2015). A comparative study of McFarland turbidity standards and the densimat photometer to determine bacterial cell density. *Current Microbiology*. 70(6). 907-909. doi: http://dx.doi.org.ezproxy.trident.edu:2048/10.1007/s00284-015

Appendices

Appendix A: Antibiotic List

Antibiotics	
Amikacin	Tobramycin
Amoxicillin	Trimethoprim
Clavulanic	Vancomycin
Ampicillin	
Ampicillin/Sulbactam	
Azithromycin	
Aztreonam	
Cefazolin	
Cefdinir	
Cefepime	
Cefixime	
Cefotaxime	
Cefotetan	
Cefoxitin	
Cefpodoxime	
Ceftazdime	
Ceftriaxone	
Cefuroxine	
Chloramphenicol	

Ciprofloxacin
Colistin
Doripenem
Ertapenem
Erythromycin
Gentamycin
Imipenem
Levofloxacin
Meropenem
Moxifloxacin
Nalidixic acid
Nitrofurantoin
Norfloxacin
Penicillin
Piperacillin/Tazobactam
Sulfa/Trimethoprim
Tetracycline
Ticarcillin/Clavulanate

Appendix B: Questionnaire Informed Consent

Dear questionnaire participant,

You are being invited to participate in a research study to determine the clinical laboratory professional's impact on antibiotic resistance. This research is being conducted for partial fulfillment of a doctoral degree at Trident University for Heather Phillips. Data collected from this survey was used to identify possible contributors of antibiotic resistance. You were chosen to participate in this study, because you are a laboratory professional who performs and interprets bacterial antibiotic susceptibility testing on human specimens.

In this survey, you are being asked to honestly answer the demographic questions as they relate to your current job as a laboratory professional; and then answer antibiotic susceptibility procedural questions. This questionnaire should not take you longer than 5 minutes to complete. You are completing this survey as an individual and not as a part of any institution, such as a hospital.

I hope you agree that both knowledge and learning are critical to change and agree to complete this survey. Your participation in this survey is completely voluntary; I offer no incentive for participation. At any point, you can voluntarily choose to stop taking the survey without penalty. By submitting the survey incomplete or in its entirety, implies consent to participate in this study. You are agreeing that you are at least eighteen years of age, have read,

and understood this consent form. I ask that you please do not use additional aids to complete this questionnaire.

Strict anonymity for each participant was exhibited with this study. No personal identifiers are associated with this survey. The researcher does not have access to identifying information for study participants and survey results cannot be connected to survey participants. Regardless, survey data was confidential and kept in lock-and-key circumstances by the researcher alone. There is a minimal social or economic risk if you choose to participate in this study. This risk may occur if you discuss your survey with those at your institution. Your decision to participate will not affect your position with your employer. I am the only person with access to surveys, statistical data, as well as responses to survey questions. Survey links can be forwarded and completed through personal email.

If you have questions regarding this research feel free to contact my dissertation chair, Dr. John Forsyth at, John.Forsyth@trident.edu, or the Institutional Review Board at Trident University International, 5757 Plaza Drive, Suite 100, Cypress, California 90630; Telephone: (714) 226-9840. I look forward to receiving your responses and sincerely appreciate your participation in this study.

Respectfully,
Heather L. Phillips
Heather.Phillips@my.trident.edu

Appendix C: Questionnaire

Directions: Please select the answer that best describes your demographics as a laboratory professional and your performance when creating bacterial inoculums for antibiotic susceptibility testing for human patient aerobic bacterial pathogens. It is important, answers focus on your work only as a professional laboratorian and no other laboratorian's work who may perform testing in your department.

There is not a correct answer for the following questions. Any applicable answer selected is useful in providing valuable information for research purposes. Please, complete this questionnaire in its entirety.

1. The first few questions are designed to obtain demographic information to ensure you are a target participant for this study. Are you currently employed as a laboratory professional in the United States?
    1. Yes
    2. No
2. If you answered yes to question 1, indicate which state you are currently employed as a laboratory professional within the United States of America.

1. Alabama
2. Alaska
3. Arizona
4. Arkansas
5. California
6. Colorado
7. Connecticut
8. Delaware
9. Florida
10. Georgia
11. Hawaii
12. Idaho
13. Illinois
14. Indiana
15. Iowa
16. Kansas
17. Kentucky
18. Louisiana
19. Maine
20. Maryland
21. Massachusetts
22. Michigan
23. Minnesota
24. Mississippi
25. Missouri
26. Montana
27. Nebraska
28. Nevada
29. New Hampshire
30. New Jersey
31. New Mexico
32. New York
33. North Carolina
34. North Dakota
35. Ohio
36. Oklahoma
37. Oregon
38. Pennsylvania
39. Rhode Island
40. South Carolina
41. South Dakota
42. Tennessee
43. Texas
44. Utah
45. Vermont
46. Virginia

47. Washington
48. West Virginia
49. Wisconsin
50. Wyoming

3. Do you hold an active state licensure as a medical laboratory professional? If so, what state do you have a state license for? If not, move to the next question.

   Open ended question:

4. Do you work, as a clinical/medical laboratory professional, in the clinical microbiology department? This includes full-time, part-time, and PRN or "as needed" positions.

   1. Yes
   2. No

5. Do you set up, perform, and interpret results for antibiotic susceptibility testing in the clinical laboratory for which you are currently employed as a laboratory professional?

   1. Yes
   2. No

6. How many beds does your hospital have?

   Open ended question

7. Select each item below which best describes the clinical facility that houses your clinical laboratory.
   1. Reference lab
   2. Rural hospital
   3. Urban hospital
   4. Critical access
   5. For profit
   6. Non-profit
   7. Acute care or general hospital
   8. Rehabilitation
   9. Nursing home or long-term care
   10. Physician's office laboratory
   11. Specialty Care Facility
   12. Community hospital (not federally owned)
   13. Federal hospital (Veterans Affairs (VA))
   14. State owned hospital

8. What is your highest completed level of education?

1. High school diploma or equivalent
2. Associated degree (aka A.S.)
3. Bachelor's degree (aka B.S., baccalaureate, or baccalaureates)
4. Master's degree
5. Ph.D. or doctorate
6. Medical Doctor (MD)
7. Other: open ended answer

9. What is the title of your degree reported I question 8 (ie chemistry, health science, medical laboratory science, biology, …)?

   Open ended question

10. Have you graduated from an accredited laboratory program?
    1. Yes
    2. No

11. Are you currently registered with a national board of certification as a clinical/medical laboratory professional, such as American Society of Clinical Pathology (ASCP), American Medical Technologist (AMT), or American Association of Bioanalysts (AAB)?
    1. Yes
    2. No

12. If you answered yes to question 11, indicate which certifying board you are currently registered. Do not answer this question, if you answered no for question 11.
    1. American Society of Clinical Pathology (ASCP)
    2. American Medical Technologist (AMT)
    3. American Association of Bioanalysts (AAB)
    4. Other: open ended answer

13. How many years of experience do you have as a laboratory professional in the microbiology department performing antibiotic susceptibility testing?

    Open ended question

14. Which of the following testing methods are primarily used to perform antibiotic susceptibility testing for pathogens identified in human specimen?

    1. Disk Diffusion (DD) with antibiotic disks
    2. E-test (gradient method)
    3. Microscan (Minimum Inhibitory Concentration [MIC] broth dilution)
    4. Vitek (Minimum Inhibitory Concentration [MIC] broth dilution)
    5. Sinsititre (Minimum Inhibitory Concentration [MIC] broth dilution)
    6. Other: open ended answer

15. The rest of the questions are going to ask you about antibiotic susceptibility testing performed over the last 12 months. When performing bacterial antibiotic susceptibility testing over the last 12 months, have you tested your bacterial inoculum concentration by performing colony counts using the organism *E. coli* ATCC 25922? Please, indicate the answer below that best describes your inoculum colony counts.

    1. I am unaware of what this question is referring to.

    2. My inoculum contains <1 x 10^5 CFU/mL

    3. My inoculum contains 1 x 10^5 CFU/mL to 4.9 x 10^5 CFU/mL

    4. My inoculum contains 5 x 10^5 CFU/mL

    5. My inoculum contains >5 x 10^5 CFU/mL to 9.9 x 10^5 CFU/mL

    6. My inoculum contains >10 x 10^5 CFU/mL

    7. Our lab's procedure does not require I perform periodic colony counts for my inoculum.

    8. The lab I am currently employed, does not perform bacterial inoculum colony counts to check concentration.

9. I was not trained or advised to perform bacterial inoculum colony counts by my supervisor when I was hired.

16. Which of the following applies to using purity plates for your bacterial inoculum?

1. I do not know what a purity plate is.

2. A purity plate is performed for each bacterial inoculum made.

3. A purity plate is only used for bacterial inoculums that may contain more than one bacterial organism.

4. Our lab does not perform purity plates on bacterial inoculums.

5. Our lab does not have a procedure for performing purity plates on bacterial inoculums.

6. I was not trained or advised to perform purity plates for my bacterial inoculums when bacterial antibiotic susceptibility testing is performed.

17. Over the last twelve months, when performing bacterial antibiotic susceptibility testing, bacterial colonies are taken from which of the following microbiology media?

1. I am unaware of what this question refers to.

2. Bacterial colonies are always taken from non-inhibitory plates, such as a blood agar plate (BAP or SBA) not impregnated with antibiotics.

3. Gram negative bacterial colonies are always taken from gram negative selective media and gram-positive bacterial colonies are always taken from blood agar plates or gram positive selective media.

4. Bacterial colonies are taken from any plate which contains the most morphologically similar colonies and exhibits the best colony isolation.

5. Other: open ended answer

18. When performing antibiotic susceptibility testing, which of the following best describes the quantity of colonies used to create the bacterial inoculum?
    1. I am unaware of what this question refers to.
    2. The quantity of colonies taken is irrelevant and consistently varies between patient samples.

3. I consistently use between one and three bacterial colonies to set up my bacterial inoculums.
4. Three bacterial colonies are always obtained regardless of colony size
5. Three bacterial colonies are usually obtained, but less than three bacterial colonies may be utilized if poor isolation occurs.
6. 4-5 large colonies and 5-10 small bacterial colonies
7. I often use greater than ten bacterial colonies each time I make a bacterial inoculum for antibiotic susceptibility testing.
8. I was not trained to use a specific number of bacterial colonies when performing a bacterial inoculum. The number of colonies used to perform bacterial inoculums varies based on consistency of the colony.
9. Laboratory procedure does not dictate how many bacterial colonies are used to make each bacterial inoculum. While the number of bacterial colonies used to make bacterial inoculums is not consistent, it often ranges between three to ten colonies.

19. On an average day, how many antibiotic susceptibility tests are set up at one time? To further elaborate, do you focus on one patient at a time? Do you line up al incubated samples and set up antibiotic susceptibility test at once?

    Open ended question

20. Which of the following best describes the method used to mix the bacterial inoculum?
    1. I do not mix the bacterial inoculum after adding the bacteria
    2. I gently rock the bacterial inoculum by hand
    3. I vigorously agitate the bacterial inoculum by hand
    4. I use a vortex to mix the bacterial inoculum
    5. I was not trained on how to mix the bacterial inoculum
    6. My laboratory procedure does not require the mixing of the bacterial inoculum. For this reason, the bacterial inoculum is not mixed.

21. At the time you perform antibiotic susceptibility testing, is this the only testing performed or do you do multiple testing in other areas of the lab at the same time?
    1. I can focus on only antibiotic susceptibility testing
    2. I perform multiple other tests while performing antibiotic susceptibility testing

22. Do you test the final turbidity or concentration of the bacterial inoculum used for antibiotic susceptibility testing? If you answered yes to this question answer questions 23 and 24. If you answered no, you have completed this survey.
    1. No

2. Yes

23. Continue only if you answered yes to question 22. How do you test the turbidity or concentration of the bacterial inoculum used for antibiotic susceptiblity testing?
    1. A McFarland standard
    2. An instrument that measures the turbidity or concentration
    3. We do not test the bacterial inoculum turbidity ir concentration
    4. Other: open ended answer

24. Continue if you answered yes to question 22. Do you check the final turbidity or concentration of the bacterial inoculum for each sample or on a regular schedule?
    1. The bacterial inoculum concentration is checked with each sample
    2. Checked daily
    3. Checked weekly
    4. Checked monthly
    5. Checked quarterly
    6. Other: open ended answer

25. Anything else you would like to add? Comment below.
    Open ended answer

Thank you for your participation in this this survey. Your responses will greatly contribute to science and the medical/clinical laboratory field.

Appendix D: Bacterial Inoculum Procedure for Microdilution, Disk Diffusion, and Gradient Method Antibiotic Susceptibility Testing for Aerobic Gram Negative and Gram Positive Human Bacterial Pathogens

1. Prepare inoculum
    1. using a sterile applicator stick, loop, or swab touch the surface of 4-5 large or 5-10 small bacterial colonies
        1. each touched colony should be morphologically similar
        2. each colony should be well isolated
        3. colonies should be pulled from a non-inhibitory plate
        4. colonies should be 18-24 hours in age
    2. emulsify colonies in inoculum water for 2-3 seconds
        1. autoclaved deionized water (DI water)
        2. emulsification of colonies may occur by vigorously agitating or vertexing suspension
    3. Final turbidity should be equivalent to 0.5 McFarland Turbidity Standard
        1. turbidity can be measured by compared inoculum or bacterial suspension to McFarland turbidimetric scale or by using spectrophotometric methods such as a turbidity meter

2. correct turbidity is considered obtained when using spectrophotometric methods achieve a range of 0.08 ± 0.02 at a wavelength of 625 nm

4. Bacterial inoculum should be streaked on a purity plate using sterile instrumentation and incubated under appropriate conditions
    1. only morphologically similar colonies should exist on purity plates
    2. purity plates exhibiting >1 bacterial colony types indicate antibiotic susceptibility panels contain more than 1 organism
        1. purity plates and antibiotic susceptibility panels should be discarded and set up using sterile equipment and well isolated morphologically similar colonies
5. CLSI recommends periodically checking bacterial inoculum densities by using colony count recommendations from document M07-A9.
    1. Using *Escherichia coli* ATCC 25922, colony counts should be 5 x 10^5 CFU/mL

Appendix E: Hospitals in Kentucky, Tennessee, Louisiana, Mississippi, West Virginia, and Virginia

Kentucky Hospitals* (Total 127)	**Internal Microbiology Department**
1. Baptist Hospital East	No
2. Baptist Hospital Northeast	No
3. Baptist Regional Center	No
4. Blanchfield Army Community Center	Yes
5. Bluegrass Community Hospital	Yes
6. Bourbon Community Hospital	Yes
7. Breckinridge Memorial Hospital	Yes
8. Caldwell County Hospital	Yes
9. Carroll County Memorial Hospital	Yes
10. Cassey County Hospital	Yes
11. Caverna Memorial Hospital	Yes
12. Central Baptist Hospital	Yes
13. Central State Hospital	Yes
14. Clark Regional Medical Center	Yes

15. Clinton County Hospital	Yes
16. Crittenden County Hospital	Yes
17. Cumberland County Hospital	Yes
18. Eastern State Hospital	
19. Ephraim McDowell Reg Medical Hospital	Yes
20. Federal Medical Center	Yes
21. Flaget Memorial Hospital	Yes
22. Fleming County Hospital	Yes
23. Fort Logan Hospital	Yes
24. Frankfort Regional Medical Center	Yes
25. Gateway Regional Health	Yes
26. Georgetown Community Hospital	Yes
27. Greenview Regional Hospital	Yes
28. Hardin Memorial Hospital	Yes
29. Harlan ARH Hospital	Yes
30. Harrison Memorial Hospital	Yes
31. Hazard ARH Regional Medical Center	Yes
32. Highlands Regional Medical Center	Yes
33. Ireland Army Community Hospital	Yes
34. Jackson Purchase Medical Center	Yes
35. James B Haggin Memorial Hospital	Yes
36. Jane Todd Crawford Hospital	Yes
37. Jenkins Community Hospital	Yes
38. Jennie Stewart Medical Center	Yes
39. Jewish Hospital	Yes
40. Jewish Hospital- Shelbyville	Yes
41. Kentucky River Medical Center	Yes
42. Kindred Hospital- Louisville	Yes
43. King's Daughter Medical Center	Yes
44. Knox County Hospital	Yes
45. Lake Cumberland Regional	Yes
46. Lincoln Trail Behavioral System	Yes
47. Livingston Hospital and Healthcare	Yes
48. Logan Memorial Hospital	Yes
49. Lourdes Hospital	Yes
50. Manchester Memorial Hospital	Yes
51. Marcum and Wallace Memorial Hospital	Yes
52. Marshall County Hospital	Yes
53. Mary Breckinridge Hospital	Yes
54. Marymount Medical Center	Yes
55. McDowell ARH Hospital	Yes
56. Meadowview Regional Medical	Yes
57. Medical Center at Franklin	Yes
58. Medical Center at Scottsville	Yes
59. Medical Center – Bowling green	Yes

60. Methodist Hospital	Yes
61. Methodist Hospital Union County	Yes
62. Middlesboro ARH Hospital	Yes
63. Monroe County Medical Center	Yes
64. Morgan ARH Hospital	Yes
65. Muhlenberg Community Hospital	Yes
66. Murray-Calloway County Hospital	Yes
67. New Horizon Health System	Yes
68. Nicholas County Hospital	Yes
69. Norton Audobon Hospital	Yes
70. Norton Suburban Hospital	Yes
71. Oak Tree Hospital	Yes
72. Ohio County Hospital	Yes
73. Our Lady of Bellefonte Hospital	Yes
74. Our Lady of the Way Hospital	Yes
75. Owensboro Medical Health System	Yes
76. Parkway Regional Hospital	Yes
77. Pattie A Clay Regional Medical Center	Yes
78. Paul B Hall Regional Medical Center	Yes
79. Pikeville Medical Center	Yes
80. Regional Medical Center of Hopkins County	Yes
81. Rockcastle Hospital	Yes
82. Russell County Hospital	Yes
83. Saint Joseph Hospital	Yes
84. Saint Joseph Hospital East	Yes
85. Samaritan Hospital	Yes
86. Shriners Hospital for Children	Yes
87. Spring View Hospital	Yes
88. St Claire Regional Medical Center	Yes
89. Saint Elizabeth Medical Center South	Yes
90. Saint Elizabeth Medical Center	Yes
91. St Luke Hospital East	Yes
92. St Luke Hospital West	Yes
93. St Mary and Elizabeth Hospital	Yes
94. TJ Samson Community Hospital	Yes
95. Taylor Regional Hospital	Yes
96. Ten Broeck Hospital	Yes
97. Three Rivers Medical Center	Yes
98. Trigg County Hospital	Yes
99. Twin Lakes Regional Medical Center	Yes
100. University of Louisville Hospital	Yes
101. University of Kentucky Hospital	Yes
102. VA Medical Center- Louisville	Yes
103. Veterans AFF Medical Center – Lexington	Yes
104. Wayne County Hospital	Yes

105. Western Baptist Hospital	Yes
106. Western State Hospital	Yes
107. Westlake Regional Hospital	Yes
108. Whitesburg ARH Hospital	Yes
109. Williamson ARH Hospital	Yes

* Kentucky hospital chart information obtained American Hospital Directory (2018). Individual Hospital Statistics for Kentucky. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_KY.html

The following hospitals located in United States state Kentucky and were removed from the survey list due to the lack of an internal clinical microbiology department.

Baptist East, Northeast, Ten Broeck Dupont, Southern Kentucky Rehab Hospital, Select Specialty Hospital, Saint Joseph Berea, Rivervalley Behavioral Hospital, Rivendell Behavior Health, Ridge Behavioral Health System, Pineville Community Hospital Association, Our Lady of Peace, Northkey Community Care, Healthsouth Rehab Hospital, Gateway Rehabilitation Hospital, Commonwealth Reg Specialty Hospital, Frazier Rehab Institute, Cumberland Hall Hospital, Cardinal Hill Specialty Hospital, Continuing Care Hospital, Cardinal Hill Rehab Hospital,

Tennessee* Hospitals (Total 153)	**Internal Microbiology Department**
1. Athens Regional Medical Center	Yes
2. Baptist Hospital of Cooke County	Yes
3. Baptist Hospital for Women	No
4. Baptist Hospital West	
5. Baptist Memorial Hospital for Women	Yes
6. Baptist Memorial Hospital Lauderdale	
7. Baptist Memorial Hospital Union City	Yes
8. Baptist Memorial Care Hospital	Yes
9. Baptist Memorial Hospital Tipton	
10. Baptist Memorial Hospital Memphis	Yes
11. Baptist Memorial Hospital Collierville	Yes
12. Baptist Rehab Germantown	
13. Bedford County Medical Center	Yes
14. Blount Memorial Hospital	Yes
15. Bolivar General Hospital	Yes
16. Camden General Hospital	Yes
17. Centennial Medical Center	Yes
18. Claiborne County Hospital	Yes
19. Cookeville Regional Medical Center	Yes

20. Copper Basin Medical Center	Yes
21. Crockett Hospital	Yes
22. Cumberland Hall Psych Hospital	No
23. Cumberland Medical Center	Yes
24. Cumberland River Hospital	Yes
25. Decatur County General Hospital	Yes
26. Dekalb Community Hospital	Yes
27. Delta Medical Center	Yes
28. Dyersburg Regional Medical Center	Yes
29. East Tennessee Children's Hospital	Yes
30. Erlanger Bledsoe Hospital	Yes
31. Erlanger Medical Center	Yes
32. Fort Loudoun Medical Center	Yes
33. Fort Sanders Regional Medical Center	Yes
34. Fort Sanders Sevier Medical Center	Yes
35. Gateway Health System	Yes
36. Gibson General Hospital	Yes
37. Grandview Medical Center	Yes
38. Grandview Medical Center	Yes
39. Hardin Medical Center	Yes
40. Harton Regional Medical Center	Yes
41. Haywood Park Community Hospital	Yes
42. Healthsouth Cane Creek Hosptial	Yes
43. Healthsouth Chattanooga Hospital	Yes
44. Healthsouth Rehab Hospital	Yes
45. Healthsouth Rehab Hospital Center	Yes
46. Healthsouth Rehab Hospital North	Yes
47. Henderson County Community Hospital	Yes
48. Hendersonville Medical Center	Yes
49. Henry County Medical Center	Yes
50. Hickman Community Hospital	Yes
51. Hillsdale Hospital	Yes
52. Horizon Medical Center	Yes
53. Humboldt General Hospital	Yes
54. Indian Path Medical Center	Yes
55. Jackson-Madison County General Hospital	Yes
56. James H Quillen VA Medical Center	Yes
57. Jamestown Regional Medical Center	Yes
58. Jellico Community Hospital	Yes
59. Johnson City Medical Center	Yes
60. Johnson City Specialty Hospital	
61. Johnson County Community Hospital	Yes
62. Kindred Hopsital Nashville	
63. Kindred Hospital Chattanooga	
64. Lakeshore Mental Health Institution	No

65. Lakeside Behavioral Health System	No
66. Lakeway Regional Hospital	Yes
67. Laughlin Memorial Hospital	Yes
68. Lincoln County Health System	Yes
69. Livingston Regional Hospital	Yes
70. Macon County General Hospital	Yes
71. Marshall Medical Center	Yes
72. Maury Regional Hospital	Yes
73. McKenzie Regional Hospital	Yes
74. McNairy Regional Hospital	Yes
75. Medical Center of Manchester	Yes
76. Memorial Healthcare System	Yes
77. Memphis Mental Health Institute	No
78. Methodist Healthcare	Yes
79. Methodist Healthcare Somerville	No
80. Methodist Healthcare University Hospital	Yes
81. Methodist Medical Center of Oakridge	Yes
82. Middle Tennessee Mental Health Institution	No
83. Middle TN Medical Center	Yes
84. Milan General Hospital	Yes
85. Moccasin Bend Mental Health Institution	No
86. Nashville General Hospital	Yes
87. Nashville Rehabilitation Hospital	No
88. North Side Hospital	Yes
89. Northcrest Medical Center	Yes
90. Parkridge Medical Center	Yes
91. Parkwest Medical Center	Yes
92. Pathways of TN	No
93. Peninsula Hospital	Yes
94. Perry Community Hospital	Yes
95. Plateau Mental Health Center	No
96. Quillen Rehabilitation hospital	No
97. Regional Hospital of Jackson	Yes
98. Regional Medical Center at Memphis	Yes
99. Rhea Medical Center	Yes
100. Ridgeview Psych Hospital and Center	No
101. Riverpark Hospital	Yes
102. Riverview Regional Medical Center North	Yes
103. Riverview Regional Medical Center South	Yes
104. Roane Medical Center	Yes
105. Saint Francis Hospital Bartlett	Yes
106. Saint Francis Hospital Memphis	Yes
107. Saint Thomas Hospital Nashville	Yes
108. Scott County Hospital	Yes
109. Select Specialty Hospital Memphis	No

110.	Select Specialty Hospital Knoxville	No
111.	Select Specialty Hospital Nashville	No
112.	Select Specialty Hospital Bristol	No
113.	Select Specialty Hospital East Oak Hill Knoxville	No
114.	Siskin Hospital for Physical Rehab	No
115.	Skyline Madison Campus	No
116.	Skyline Medical Center	Yes
117.	Skyridge Medical Center	Yes
118.	Southern Hills Medical Center	Yes
119.	Southern TN Medical Center	Yes
120.	St Jude Children's Res Hospital	Yes
121.	St Mary Jefferson Memorial Hospital	Yes
122.	St Mary Medical Center Follette	Yes
123.	St Mary Medical Center Knoxville	Yes
124.	Stonecrest Medical Center	Yes
125.	Stones River Hospital	Yes
126.	Summit Medical Center	Yes
127.	Sumner Regional Medical Center	Yes
128.	Sweetwater Hospital	Yes
129.	Sycamore Shoals Hospital	Yes
130.	Takoma Regional Hospital	Yes
131.	The Center for Spinal Surgery	No
132.	Three Rivers Hospital	Yes
133.	Trinity Hospital	Yes
134.	Trousdale Medical Center	Yes
135.	Unicoi County Memorial Hospital	Yes
136.	United Regional Medical Center	Yes
137.	University of TN Medical Center	Yes
138.	University Medical Center Lebanon	Yes
139.	VA TN Valley Healthcare System	No
140.	Vanderbilt Stallworth Rehab	No
141.	Vanderbilt University Medical Center	Yes
142.	Veterans Affairs Medical Center	Yes
143.	Volunteer Community Hospital	Yes
144.	Wayne Medical Center	Yes
145.	Wellmount Bristol Reg Medical Center	Yes
146.	Wellmount Hancock County Hospital	Yes
147.	Wellmount Hawkins County Memorial Hospital	Yes
148.	Wellmount Holston Valley Medical Center	Yes
149.	Western Mental Health Institute	Yes
150.	White County Community	Yes
151.	Williamson Medical Center	Yes
152.	Woodridge Hospital	Yes
153.	Woods Memorial Hospital District	No

* Tennessee hospital chart information obtained American Hospital Directory (2018). Individual Hospital Statistics for Tennessee. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_TN.html

The following hospitals located in United States state Tennessee and were removed from the survey list due to the lack of an internal clinical microbiology department.

Baptist Hospital for Women, Woods Memorial Hospital District, Vanderbilt Stallworth Rehab, VA TN Valley Healthcare System, The Center for Spinal Surgery, Skyline Madison Campus, Siskin Hospital for Physical Rehab, Select Specialty Hospital East Oak Hill Knoxville, Select Specialty Hospital East Oak Hill Knoxville, Select Specialty Hospital Bristol, Select Specialty Hospital Nashville, Select Specialty Hospital Memphis, Ridgeview Psych Hospital and Center, Quillen Rehabilitation hospital, Plateau Mental Health Center, Pathways of TN, Nashville Rehabilitation Hospital, Moccasin Bend Mental Health Institution, Middle Tennessee Mental Health Institution, Memphis Mental Health Institute, Lakeside Behavioral Health System, Lakeshore Mental Health Institution, Cumberland Hall Psych Hospital

Louisiana Hospitals* (Total 211)	**Internal Microbiology Department**
1. Abbeville General Hospital	Yes
2. Abrom Kaplan Memorial Hospital	Yes
3. Acadia Rehabilitation Hospital	
4. Acadia Vermilion Hospital	Yes
5. Acadia-St Landry Hospital	Yes
6. Acadian Medical Center	Yes
7. Allen Parish Hospital	Yes
8. American Legion Hospital	Yes
9. Assumption Community Hospital	Yes
10. Avoyelles Hospital	Yes
11. Bastrop Rehabilitation Hosp	
12. Baton Rouge Gen Med Center	Yes
13. Bayne-Jones Army Comm Hospital	Yes
14. Beauregard Memorial Hospital	Yes
15. Behavioral Hosp of Baton Rouge	No
16. Behavioral Hosp of Shreveport	No
17. Behavioral Hospital - Lutcher	No
18. Benton Rehabilitation Hospital	
19. Bienville Medical Center	Yes

20. Bogalusa Medical Center	Yes
21. Bossier Specialty Hospital	
22. Brentwood Hospital	Yes
23. Bunkie General Hospital	Yes
24. Byrd Regional Hospital	Yes
25. Caldwell Memorial Hospital	Yes
26. Central Louisiana State Hosp	Yes
27. Children's Hospital	Yes
28. CHRISTUS Coushatta Health Care	
29. CHRISTUS Schumpert Health System	
30. CHRISTUS St Frances Hospital	Yes
31. CHRISTUS St Patrick Hospital	Yes
32. Citizens Medical Center	Yes
33. Community Care Hospital	Yes
34. Community Rehabilitation Hosp	
35. Community Specialty Hospital	Yes
36. Community Specialty Hospital	
37. Cornerstone Hosp SW Louisiana	Yes
38. Cornerstone Hosp-West Monroe	
39. Cornerstone Hospital	Yes
40. Crossroads Regional Hospital	Yes
41. Crowley Rehabilitation Hosp	Yes
42. Cypress Psychiatric Hospital	No
43. Dauterive Hospital	Yes
44. De Soto Regional Health System	
45. DeQuincy Memorial Hospital	Yes
46. Doctor's Hospital of Opelousas	Yes
47. Doctors Hospital of Slidell	Yes
48. Doctors' Hosp of Shreveport	Yes
49. Dubuis Hosp of Lake Charles	Yes
50. Dubuis Hospital of Alexandria	Yes
51. Dubuis Hospital of Shreveport	Yes
52. E A Conway Medical Center	Yes
53. Earl K Long Medical Center	Yes
54. East Carroll Parish Hospital	Yes
55. East Jefferson Gen Hospital	Yes
56. Eastern Louisiana Health System	
57. Edgewood Hospital	Yes
58. Eunice Extended Care Hospital	Yes
59. Evangeline Extended Care Hospital	No
60. Extended Care of SW Louisiana	No
61. Fairway Medical Center	Yes
62. Franklin Foundation Hospital	Yes
63. Glenwood Regional Med Center	Yes
64. Golden Age Senior Care Hospital	Yes

65. Green Clinic Surgical Hospital	Yes
66. Greenbiar Hospital	Yes
67. Gulf States LTAC of Covington	
68. Gulf States LTAC of Feliciana	
69. Gulf States LTAC of Hammond	
70. Gulf States LTAC of Morgan	
71. Gulf States LTAC of Washington	
72. Gulf States of Denham Springs	
73. Hardtner Medical Center	Yes
74. Healthsouth Rehab Hospital	
75. Healthsouth Riverside Hospital	Yes
76. Healthsouth Specialty Hospital	
77. Healthwest Rehab Hospital	
78. Heart Hospital of Lafayette	Yes
79. Homer Memorial Hospital	Yes
80. Hood Memorial Hospital	Yes
81. Huey P Long Medical Center	Yes
82. Iberia Extended Care Hospital	No
83. Iberia Medical Center	Yes
84. Jackson Parish Hospital	Yes
85. Jennings American Legion Hosp	Yes
86. Kindred Hospital - New Orleans	Yes
87. La Place Rehabilitation Hosp	
88. Lady of the Sea General Hospital	Yes
89. Lafayette General Med Ctr	Yes
90. Lafayette General Surg Hosp	Yes
91. Lafayette Surgical Specialty Hospital	No
92. Lake Charles Memorial Hospital	Yes
93. Lakeview Regional Medical Center	Yes
94. Lallie Kemp Medical Center	Yes
95. Lane Regional Medical Center	Yes
96. Lasalle Medical Hospital	Yes
97. Leesville Rehabilitation Hosp	
98. Leonard J Chabert Medical Ctr	Yes
99. Lifecare Hospitals	Yes
100. Lillian Louise Behavioral Hosp	No
101. Lincoln General Hospital	Yes
102. Louisiana Extended Care Hosp	No
103. Louisiana Medical Center & Heart Hospital	Yes
104. LSU Medical Center-Univ Hosp	Yes
105. LTAC of Acadiana	
106. Luling Rehabilitation Hospital	
107. Madison Parrish Hospital	Yes
108. Meadowbrook Specialty Hospital	
109. Medical Center of LA	Yes

110.	Minden Medical Center	Yes
111.	Monroe Surgical Hospital	Yes
112.	Morehouse General Hospital	Yes
113.	Natchitoches Regional Med Ctr	Yes
114.	Neuro Medical Center Hospital	Yes
115.	New Orleans Adolescent Hosp	Yes
116.	North Caddo Medical Center	Yes
117.	North Oaks Medical Center	Yes
118.	North Oaks Rehab Hospital	
119.	NorthShore Regional Med Center	Yes
120.	Oakdale Community Hospital	Yes
121.	Oakdale Behavioral Hospital	No
122.	Ochsner Baptist Medical Center	Yes
123.	Ochsner Clinic Foundation	No
124.	Ochsner Med Ctr-Baton Rouge	Yes
125.	Ochsner Medical Center-Kenner	Yes
126.	Ochsner Medical Ctr-West Bank	Yes
127.	Ochsner St Anne General Hosp	Yes
128.	Omega Hospital	Yes
129.	Opelousas General Health Syst	
130.	Ouachita Surgical Hospital	
131.	Our Lady of Lake Reg Med Ctr	Yes
132.	Our Lady of Lourdes Reg Center	Yes
133.	Overton Brooks VA Med Center	Yes
134.	P & S Surgical Hospital	
135.	Park Place Surgical Hospital	
136.	Physicians Surg Spec Hospital	No
137.	Pointe Coupee General Hospital	Yes
138.	Premier Rehab Hospital	
139.	Prevost Memorial Hospital	Yes
140.	Promise Hospital of Ascension	Yes
141.	Promise Spec Hosp of Miss Lou	Yes
142.	Promise Specialty Hospital	Yes
143.	Promise Specialty Hospital-Medical Ctr Dr	Yes
144.	Rapides Regional Medical Ctr	Yes
145.	Red River Behavioral Center	No
146.	Regency Hospital of Covington	Yes
147.	Rehab Hospital of DeQuincy	
148.	Richardson Medical Center	Yes
149.	Richland Parish Hospital	Yes
150.	River Oaks Child & Adolescent	
151.	River Oaks Hospital	Yes
152.	River Parishes Hospital	Yes
153.	River West Medical Center	
154.	Riverland Medical Center	Yes

155.	Riverside Medical Center	Yes
156.	Sabine Medical Center	Yes
157.	Sage Rehabilitation Institute	
158.	Savoy Medical Center	Yes
159.	Select Specialty Hospital	
160.	Shriners Hosps for Children	Yes
161.	Slidell Memorial Hospital	Yes
162.	South Baton Rouge Rehab Hosp	Yes
163.	SE LA Hospital	
164.	Southeast Regional Med Center	Yes
165.	Southern Surgical Hospital	
166.	Southwest Medical Center	Yes
167.	Springhill Medical Center	Yes
168.	St Anne Rehabilitation Hosp	
169.	St Charles Parish Hospital	Yes
170.	St Elizabeth Hospital	Yes
171.	St Francis Medical Center	Yes
172.	St Francis North Hospital	Yes
173.	St Francis Specialty Hospital	
174.	St Helena Parish Hospital	Yes
175.	St James Parish Hospital	Yes
176.	St John's Specialty Hospital	
177.	St Landry Extended Care Hosp	No
178.	St Luke's Rehabilitation Hosp	
179.	St Luke’s Specialty Hospital	
180.	St Martin Hospital	Yes
181.	St Patrick's Psychiatric Hospital	No
182.	St Tammany Parish Hospital	Yes
183.	Sterlington Rehab Hospital	
184.	Surgical Specialty Centre	
185.	Teche Regional Medical Center	Yes
186.	Terrebonne General Medical Ctr	Yes
187.	Thibodaux Regional Medical Ctr	Yes
188.	Touro Infirmary	
189.	Touro Rehabilitation Center	
190.	Tri Parish Rehabilitation Hos	Yes
191.	Tri-Ward General Hospital	Yes
192.	Tulane Univ Hospital & Clinic	Yes
193.	Tulane-Lakeside Hospital	Yes
194.	Union General Hospital	Yes
195.	University Med Ctr-Psych Unit	No
196.	University Medical Center	Yes
197.	Veterans Affairs Medical Ctr	Yes
198.	Villa Feliciana Med Complex	
199.	Ville Platte Medical Center	Yes

200. Vista Surgical Hospital	
201. Vital Source Specialty Hosp	
202. W O Moss Reg Medical Ctr	Yes
203. West Calcasieu Cameron Hosp	Yes
204. West Carroll Memorial Hospital	Yes
205. West Feliciana Parish Hospital	Yes
206. West Jefferson Medical Center	Yes
207. WestEnd Hospital	Yes
208. Willis-Knighton Bossier Center	
209. Willis-Knighton Medical Center	
210. Winn Parish Medical Center	
211. Woman's Hospital	Yes

* Louisville hospital chart information obtained American Hospital Directory (2018). Individual Hospital Statistics for Louisville. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_LA.html

The following hospitals located in United States state Louisiana and were removed from the survey list due to the lack of an internal clinical microbiology department.

University Med Ctr-Psych Unit, St Patrick's Psychiatric Hosp, St Landry Extended Care Hosp, Red River Behavioral Center, Physicians Surg Spec Hospital, Ochsner Clinic Foundation, Oakdale Behavioral Hospital, Louisiana Extended Care Hosp, Lillian Louise Behavioral Hosp, Lafayette Surgical Specialty Hospital, Iberia Extended Care Hospital, Extended Care of SW Louisiana, Evangeline Extended Care Hospital, Cypress Psychiatric Hospital, Behavioral Hosp of Baton Rouge, Behavioral Hosp of Shreveport, Behavioral Hospital – Lutcher

Mississippi Hospitals* (Total 113)	**Internal Microbiology Department**
1. Alliance Health Center	Yes
2. Alliance HealthCare System	
3. Baptist Mem Hosp-Booneville	
4. Baptist Mem Hosp-North MS	
5. Baptist Mem Hosp-Union County	
6. Baptist Mem Hospital-Desoto	
7. Baptist Memorial Hosp-Golden	
8. Batesville Specialty Hospital	
9. Beacham Memorial Hospital	Yes
10. Biloxi Regional Medical Center	Yes
11. Bolivar Medical Center	Yes
12. Boswell Regional Center	Yes
13. Brentwood Behavioral Health	Yes
14. Calhoun Health Services	Yes

15. Central Mississippi Med Ctr	Yes
16. Choctaw County Medical Center	Yes
17. Choctaw Health Center	Yes
18. Claiborne County Hospital	Yes
19. Covington County Hospital	Yes
20. Delta Regional Medical Center	Yes
21. Diamond Grove Center	Yes
22. East Mississippi State Hosp	Yes
23. Field Memorial Comm Hospital	Yes
24. Forrest General Hospital	Yes
25. Franklin County Mem Hospital	Yes
26. G V Montgomery VA Med Center	Yes
27. Garden Park Medical Center	Yes
28. George County Hospital	Yes
29. Gilmore Mem Reg Med Ctr	Yes
30. Greene County Hospital	Yes
31. Greenwood Leflore Hospital	Yes
32. Greenwood Specialty Hospital	Yes
33. Grenada Lake Medical Center	Yes
34. Gulf Coast Medical Center	Yes
35. H C Watkins Memorial Hospital	Yes
36. Hancock Medical Center	Yes
37. Hardy Wilson Memorial Hospital	Yes
38. Highland Community Hospital	Yes
39. Humphreys County Mem Hospital	Yes
40. Jasper Community Hospital	Yes
41. Jeff Anderson Reg Medical Ctr	Yes
42. Jefferson Davis Community Hospital	Yes
43. Kilmichael Hospital	Yes
44. King's Daughters Hospital	Yes
45. King’s Daughter Medical Center	Yes
46. Laird Hospital	Yes
47. Lawrence County Hospital	Yes
48. Leake Memorial Hospital	Yes
49. Madison County Medical Center	Yes
50. Magee General Hospital	Yes
51. Magnolia Regional Health Ctr	Yes
52. Marion General Hospital	Yes
53. Memorial Hospital at Gulfport	
54. Methodist Rehabilitation Center	
55. Mississippi Baptist Med Center	Yes
56. Mississippi State Hospital	Yes
57. Montfort Jones Mem Hospital	Yes
58. MS Hosp for Restorative Care	No
59. Natchez Community Hospital	Yes

60. Natchez Regional Medical Ctr	Yes
61. Neshoba County Gen Hospital	Yes
62. Newton Regional Hospital	Yes
63. North Mississippi Med Center	
64. North Mississippi Medical Ctr	Yes
65. North Mississippi Medical Ctr	
66. North Mississippi State Hosp	Yes
67. North MS Medical Center-Eupora	
68. North MS Medical Center-Iuka	
69. North Oak Regional Medical Ctr	Yes
70. North Sunflower Medical Center	Yes
71. Noxubee General Hospital	Yes
72. NW Miss Regional Med Center	Yes
73. Oktibbeha County Hospital	Yes
74. Parkwood Hlth System	
75. Pearl River County Hospital	Yes
76. Perry County General Hospital	Yes
77. Pioneer Community Hospital	Yes
78. Promise Specialty Hospital	
79. Quitman County Hospital	Yes
80. Rankin Medical Center	Yes
81. Regency Hosp of Hattiesburg	Yes
82. Regency Hospital of Jackson	Yes
83. Regency Hospital of Meridian	Yes
84. Riley Hospital	Yes
85. River Oaks Hospital	Yes
86. River Region Medical Center	Yes
87. Rush Foundation Hospital	Yes
88. S E Lackey Memorial Hospital	Yes
89. Scott Regional Hospital	Yes
90. Select Specialty Hospital	
91. Sharkey-Issaquena Comm Hosp	
92. Simpson General Hospital	Yes
93. Singing River Hospital System	Yes
94. South Central Regional Medical Center	Yes
95. South Mississippi State Hosp	Yes
96. South Sunflower Cnty Hospital	Yes
97. Southwest Mississippi Med Cntr	Yes
98. Specialty Hospital of Meridian	
99. St Dominic-Jackson Mem Hosp	Yes
100. Stone County Hospital	Yes
101. Tallahatchie General Hospital	Yes
102. Tippah County Hospital	Yes
103. Trace Regional Hospital	Yes
104. Tri-Lakes Medical Center	Yes

105. Tyler Holmes Memorial Hospital	Yes
106. University Hospital and Clinic	Yes
107. USAF Medical Center Keesler	
108. VA Gulf Coast Veterans Health	Yes
109. Walthall County Gen Hospital	Yes
110. Wayne General Hospital	Yes
111. Westley Medical Center	Yes
112. Winston Medical Center	Yes
113. Woman's Hospital at River Oaks	

* Mississippi hospital chart information obtained American Hospital Directory. (2018). Individual Hospital Statistics for Mississippi. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_MS.html

The following hospitals located in United States state Mississippi and were removed from the survey list due to the lack of an internal clinical microbiology department.

MS Hosp for Restorative Care

Virginia Hospital* (Total 102)	**Internal Microbiology Department**	**Beds**
1. Augusta Health		207
2. Bath Community Hospital (critical access)	Yes	25
3. Bon Secours DePaul Medical Center		238
4. Bon Secours Mary Immaculate Hospital		123
5. Bon Secours Maryview Medical Center		342
6. Bon Secours Memorial Regional Medical Center		225
7. Bon Secours Richmond Community Hospital		101
8. Bon Secours St. Francis Medical Center		130
9. Bon Secours St. Mary's Hospital		391
10. Buchanan General Hospital	Yes	49
11. Carilion Clinic St. Albans Hospital (Psych)	No	36
12. Carilion Franklin Memorial Hospital	Yes	18
13. Carilion Giles Memorial Hospital (critical access)	Yes	25
14. Carilion New River Valley Medical Center	Yes	146
15. Carilion Roanoke Community Hospital	Yes	
16. Carilion Roanoke Memorial Hospital	Yes	855
17. Carilion Stonewall Jackson Hospital (critical access)	Yes	25
18. Carilion Tazewell Community Hospital	Yes	56
19. Centra Bedford Memorial Hospital		50
20. Centra Lynchburg General Hospital	Yes	385

21. Centra Southside Community Hospital		40
22. Centra Virginia Baptist Hospital	Yes	317
23. Chesapeake Regional Medical Center	Yes	310
24. Children's Hospital of Richmond at VCU	Yes	87
25. Children's Hospital of The King's Daughters	Yes	212
26. CJW Medical Center(Chippenham & Johnston-Willis)		667
27. Clinch Valley Medical Center	Yes	200
28. Culpeper Regional Hospital (UVA owned)	Yes	70
29. Cumberland Hospital	Yes	84
30. Danville Regional Medical Center	Yes	250
31. Dickenson Community Hospital (critical access)	Yes	
32. Dominion Hospital (psych)	No	100
33. Fauquier Health System		97
34. Fort Belvoir Community Hospital	Yes	120
35. Graydon Manor (drug/alcohol rehab)	No	
36. Haymarket Medical Center	Yes	60
37. Henrico Doctors' Hospital—Henrico Campus		767
38. Henrico Doctors' Hospital—Parham Campus		
39. Inova Alexandria Hospital	Yes	318
40. Inova Children's Hospital	Yes	452
41. Inova Fair Oaks Hospital		182
42. Inova Fairfax Hospital		833
43. Inova Loudoun Hospital		183
44. Inova Mount Vernon Hospital		237
45. Inova Women's Hospital		
46. John Randolph Medical Center	Yes	112
47. Johnston Memorial Hospital	Yes	116
48. Lake Taylor Transitional Care Hospital		296
49. LewisGale Hospital Alleghany		110
50. LewisGale Hospital Montgomery		146
51. LewisGale Hospital Pulaski		42
52. LewisGale Medical Center		521
53. Martinsville Memorial Hospital	Yes	237
54. Mary Washington Hospital	Yes	437
55. Mountain View Regional Medical Center	Yes	98
56. Naval Medical Center Portsmouth	Yes	
57. Norton Community Hospital	Yes	129
58. Page Memorial Hospital (critical access)	Yes	15
59. Pioneer Community Hospital of Patrick (critical access)	Yes	
60. Poplar Springs Hospital	Yes	
61. Prince William Health System		170
62. Rappahannock General Hospital	Yes	
63. Reston Hospital Center	Yes	187
64. Retreat Doctors' Hospital		116

65. Riverside Behavioral Health Center	No	
66. Riverside Doctors' Hospital Williamsburg		13
67. Riverside Regional Medical Center	Yes	215
68. Riverside Rehabilitation Institute		
69. Riverside Shore Memorial Hospital		130
70. Riverside Tappahannock Hospital		67
71. Riverside Walter Reed Hospital	Yes	67
72. Russell County Medical Center	Yes	50
73. Sentara Bayside Hospital	Yes	
74. Sentara CarePlex Hospital		144
75. Sentara Halifax Regional Hospital		80
76. Sentara Leigh Hospital		238
77. Sentara Martha Jefferson Hospital		176
78. Sentara Norfolk General Hospital		563
79. Sentara Northern Virginia Medical Center		183
80. Sentara Obici Hospital		168
81. Sentara Princess Anne Hospital		160
82. Sentara RMH Medical Center		238
83. Sentara Virginia Beach General Hospital		276
84. Sentara Williamsburg Regional Medical Center		145
85. Shenandoah Memorial Hospital (critical access)	Yes	20
86. Smyth County Community Hospital	Yes	44
87. Southern Virginia Regional Medical Center	Yes	80
88. Southampton Memorial Hospital	Yes	90
89. Southside Regional Medical Center	Yes	300
90. Spotsylvania Regional Medical Center	Yes	133
91. Stafford Hospital	Yes	83
92. StoneSprings Hospital Center	Yes	124
93. Twin County Regional Healthcare	Yes	78
94. University of Virginia Children's Hospital	Yes	111
95. University of Virginia Health System	Yes	645
96. VCU Health Community Memorial Hospital		260
97. VCU Medical Center	Yes	865
98. Virginia Hospital Center	Yes	342
99. Warren Memorial Hospital	Yes	46
100. Wellmont Lonesome Pine Mt. View Hospital	Yes	21
101. Winchester Medical Center	Yes	429
102. Wythe County Community Hospital	Yes	20

* Virginia hospital chart information obtained American Hospital Directory (2018). Individual Hospital Statistics for Virginia. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_VA.html

The following hospitals located in United States state Virginia and were removed from the survey list due to the lack of an internal clinical microbiology department.

Carilion Clinic St. Albans Hospital (Psych), Dominion Hospital (psych), Graydon Manor (drug/alcohol rehab), Riverside Behavioral Health Center

West Virginia Hospitals* (Total 36)	Internal Microbiology Department	Beds
1. Beckley ARH Hospital		160
2. Beckley VA Medical Center	Yes	0
3. Berkeley Medical Center	Yes	177
4. Bluefield Regional Medical Center		92
5. Cabell Huntington Hospital	Yes	327
6. CAMC Women and Children's Hospital	Yes	0
7. Camden-Clark Medical Center - Memorial Campus		245
8. Charleston Area Medical Center General Hospital		877
9. Charleston Area Medical Center Memorial Hospital		0
10. Charleston Area Medical Center Teays Valley Hospital		70
11. Charleston Surgical Hospital		35
12. Davis Medical Center	Yes	90
13. Fairmont Regional Medical Center	Yes	207
14. Greenbrier Valley Medical Center	Yes	98
15. Huntington VA Medical Center		0
16. Logan Regional Medical Center		140
17. Louis A. Johnson VA Medical Center		0
18. Martinsburg VA Medical Center	Yes	0
19. Mon Health Medical Center	Yes	189
20. Ohio Valley Medical Center	Yes	203
21. Pleasant Valley Hospital	Yes	194
22. Princeton Community Hospital	Yes	226
23. Raleigh General Hospital	Yes	300
24. Reynolds Memorial Hospital	Yes	90
25. Ruby Memorial Hospital	Yes	535
26. Saint Francis Hospital	Yes	142
27. Saint Mary's Medical Center		379
28. Stonewall Jackson Memorial Hospital	Yes	70
29. Summersville Regional Medical Center	Yes	101
30. Thomas Memorial Hospital	Yes	206
31. United Hospital Center	Yes	264
32. Weirton Medical Center	Yes	167
33. Welch Community Hospital	Yes	108
34. Wetzel County Hospital	Yes	48
35. Wheeling Hospital		343
36. Williamson Memorial Hospital	Yes	76

* West Virginia hospital chart information obtained American Hospital Directory (2018). Individual Hospital Statistics for West Virginia. American Hospital Directory. Retrieved from https://www.ahd.com/states/hospital_WV.html

There were no hospitals with obvious indications or who claimed to lack of an internal clinical microbiology department who were located in United States state West Virginia.

Appendix F: Dummy Codes Used for Variables

Q1: (Excel column A) The first few questions are designed to obtain demographic information to ensure you are a target participant for this study. Are you currently employed as a laboratory professional in the United States of America?

Response	Code
Yes	2
No	1

Q2: (Excel column B) If you answered yes to question 1, indicate which state you are currently employed as a laboratory professional within the United States of America.

State	Accepted Responses	Code
Alabama	Alabama, alabama, AL, Al, al	1
Alaska	Alaska, alaska, AK, Ak, ak	2
Arizona	Arizona, arizona AZ, Az, az	3
Arkansas	Arkansas, arkansas AR, Ar, ar	4
California	California, california CA, Ca, ca	5
Colorado	Colorado, colorado CO, Co, co	6
Connecticut	Connecticut, connecticut CT, Ct, ct	7
Delaware	Delaware, Delaware, DE, De, de	8
Florida	Florida, florida, FL, Fl, fl	9
Georgia	Georgia, Georgia, GA, Ga, ga	10
Hawaii	Hawaii, hawaii, HI, Hi, hi	11
Idaho	Idaho, Idaho, ID, Id, id	12
Illinois	Illinois, Illinois, IL, Il, il	13
Indiana	Indiana, Indiana, IN, In, in	14
Iowa	Iowa, iowa, IA, Ia, ia	15
Kansas	Kansas, kansas, KS, Ks, ks	16
Kentucky	Kentucky, kentucky, KY, Ky, ky	17
Louisiana	Louisiana, louisiana, LA, La, la	18
Maine	Maine, maine, ME, Me. me	19
Maryland	Maryland, maryland, MD, Md. md	20
Massachusetts	Massachusetts, massachusetts, MA, Ma, ma	21
Michigan	Michigan, michigan, MI, Mi, mi	22
Minnesota	Minnesota, minnesota, MN, Mn, mn	23

Mississippi	Mississippi, mississippi, MS, Ms, ms	24
Missouri	Missouri, missouri, MO, Mo, mo	25
Montana	Montana, montana, MT, Mt, mt	26
Nebraska	Nebraska, nebraska, NE, Ne, ne	27
Nevada	Nevada, nevada, NV. Nv, nv	28
New Hampshire	New Hampshire, new hampshire, NH, Nh, nh	29
New Jersey	New Jersey, new jersey, NJ, Nj, nj	30
New Mexico	New Mexico, new mexico, NM, Nm, nm	31
New York	New York, new york, NY, Ny, ny	32
North Carolina	North Carolina, north carolina, NC, Nc, nc	33
North Dakota	North Dakota, north dakota, ND, Nd, nd	34
Ohio	Ohio, ohio, OH, Oh, oh	35
Oklahoma	Oklahoma, oklahoma, OK, Ok, ok	36
Oregon	Oregon, oregon, OR, Or, or	37
Pennsylvania	Pennsylvania, Pennsylvania, PA, Pa, pa	38
Rhode Island	Rhode Island, rhode island, RI, Ri, ri	39
South Carolina	South Carolina, south carolina, SC, Sc, sc	40
South Dakota	South Dakota, south dakota, SD, Sd, sd	41
Tennessee	Tennessee, tennessee, TN, Tn, tn	42
Texas	Texas, texas, TX, Tx, tx	43
Utah	Utah, utah, UT, Ut, ut	44
Vermont	Vermont, vermont, VT, Vt, vt	45
Virginia	Virginia, virgina, VA, Va, va	46
Washington	Washington, washington, WA, Wa, wa	47
West Virginia	West Virginia, west virginia, WV, Wv, wv	48
Wisconsin	Wisconsin, wisconsin, WI, Wi, wi	49
Wyoming	Wyoming, wyoming, WY, Wy, wy	50
District of Columbia	District of Columbia, district of Columbia, Dist Colum, DC, dc, D.C., d.c.	51
Out of Country	Out of States, Out of USA, not in states, not in country, not in USA	52

Q3: (Excel column C) Do you hold an active state licensure as a medical laboratory professional? If so, what state do you have a state license for? If not, move to the next question.

Response	Code	Response	Code
No	1	Tennessee and New York	13
California	2	California and Florida	14
Florida	3	Louisiana, Florida, and California	15
Georgia	4	Tennessee and Florida	16
Hawaii	5	Louisiana and Tennessee	17
Louisiana	6	Tennessee and New York	18
Montana	7	California, Louisiana, Hawaii, and New York	19
Nevada	8		
New York	9		
North Dakota	10		
Tennessee	11		
West Virginia	12		

Q4: (Excel column D) Do you work, as a clinical/medical laboratory professional, in the clinical microbiology department? This includes full-time, part-time, and PRN or "as needed" positions.

Response	Code
Yes	1
No	2

Q5: (Excel column E) Do you set up, perform, and interpret results for antibiotic susceptibility testing in the clinical laboratory for which you are currently employed as a laboratory professional?

Response	Code
Yes	1
No	2

Q6: (Excel column F) How many beds does your hospital have?

Response	Code
0 - 20,000	0 - 20,000

Q7: (Excel column G) Select each item below which best describes the clinical facility that houses your clinical laboratory.

Response	Code
Acute Care	1
Community Hospital (not federally owned)	2
Critical Access	3
Federal Hospital (ie, Veteran's Affairs)	4
General Hospital	5
Long Term Care	6
Non-profit	7
Nursing Home	8
Physician's Office Laboratory	9
Reference Lab	10
Rehabilitation	11
Rural Hospital	12
Specialty Care Facility	13
State Owned Hospital	14
Urban Hospital	15
Unknown	16
For Profit	17
Industry Lab	18

Q8: (Excel column H) What is your highest completed level of education?

Response	Code
High School Diploma or Equivalent	1
Associates Degree (ie A.S., A.A.)	2
Bachelor's Degree (ie B.S., B.A.)	3
Master's Degree (ie M.S., M.B.A., M.A)	4
Doctorate (ie Ph.D.)	5
Medical Doctor (ie M.D.)	6
No Response	7

Q9: (Excel column I) What is the title of your degree reported in question 8?

Response	Code
Medical Laboratory Science, Medical Lab Science, M.L.S., Medical Technology, M.T., Medical Technologist, Clinical Laboratory Science, Clinical Lab Science, Clinical Laboratory Scientist, C.L.S., Science Medical Laboratory, MT (ASCP), BSMT, Medical Lab, Laboratory Scientist, Medical laboratory Technician, M.L.T, Medical Laboratory, Applied Medical Science, ASCP	1
Administration	2
Applied Technology	3
Associate of Applied Science	4
Biology, B.S. Biology	6
Biochemistry	7
Education	8
Forensics	9
Health Science, Health, Clinical	10
Health Services Administration	11
Master Business of Arts, M.B.A.	13
Master of Health	14
Master of Science, M.S.,	15
Microbiology	16
Molecular Biology	17
Natural Science	18
Science	19

Technology	20
Medical Assistant	21
Healthcare Informatics	22
History	23
Immunohematology	24
Cellular Biology	25
Physiology and Genetics	26
Bacteriology	27
Public Health	28
Biomed	29
Zoology	30
Psychology	31
Combined Science	32
Management in Allied Health	33
Lab Operations	34
Healthcare Management	35
Math and Science	36
Management of HIV and Related Infections	37
Allied Health Leadership	38
Accounting	39
Workforce Training and Development	40
Biotechnology	41
Art	42
General Studies	43

Q10. (Excel column J) Have you graduated from a NACCLS accredited laboratory program?

Response	**Code**
Yes	1
No	2

Q11. (Excel column K) Are you currently registered with a national board of certification as a clinical/medical laboratory professional, such as American Society of Clinical Pathology (ASCP), American Medical Technologist (AMT), or American Association of Bioanalysts (AAB)?

Response	**Code**
Yes	1
No	2

Q12. (Excel column L) If you answered yes to question 11, indicate which certifying board you are currently registered. Do not answer this question, if you answered no for question 11.

Response	Code
American Society of Clinical Pathology (ASCP)	1
American Medical Technologist (AMT)	2
American Association of Bioanalysts (AAB)	3
American Society of Clinical Pathology (ASCP) and American Medical Technologist (AMT)	4
(HPCSA)	5
(HEW)	6
Canadian Society of Medical Laboratory Scientist (CSMLS)	7
College of Medical Laboratory Technologists of Ontario (CMLTO)	8
None	9
Health and Care Professionals Council (HCPC)	11
Canadian Society of Medical Laboratory Scientist (CSMLS) and College of Medical Laboratory Technologists of Ontario (CMLTO)	12
American Society of Clinical Pathology (ASCP) and American Medical Technologist (AMT) and American Association of Bioanalysts (AAB)	13

Q13. (Excel column M) How many years of experience do you have as a laboratory professional in the microbiology department performing antibiotic susceptibility testing?

Response	Code
0-100	0-100

Q14. (Excel column N) Which of the following testing methods are primarily used to perform antibiotic susceptibility testing for pathogens identified in human specimens?

Response	Code
No response	0
Disk Diffusion (DD) with antibiotic disks	1
E-test (gradient method)	2
Microscan (Minimum Inhibitory Concentration [MIC] broth dilution)	3
Vitek (Minimum Inhibitory Concentration [MIC] broth dilution)	4
Sinsititre (Minimum Inhibitory Concentration [MIC] broth dilution)	5
Pheonix	6
Hotel	7
Disk Diffusion and E-test	8
Vitek and E-test	9
Microscan and E-test	10
Vitek and Disk Diffusion	11
Microscan and Disk Diffusion	12
Use multiple instruments	13
Hotel, Disc Diffusion (DD), and E-test	14

Vitek, Microscan, and E-test	15
Pheonix, Disc Diffusion (DD), and E-test	16
Pheonix and Microscan	17
Pheonix and E-test	18
Vitek, Disc Diffusion (DD), and E-test	19

Q15. (Excel column O) The rest of the questions are going to ask you about antibiotic susceptibility testing performed over the last 12 months. When performing bacterial antibiotic susceptibility testing, have you tested your bacterial inoculum concentration by performing colony counts using the organism E. coli ATCC 25922? Please, indicate the answer below that best describes your inoculum colony counts.

Response	**Code**
I am unaware of what this question is referring to	1
My inoculum contains <1 x 10^5 CFU/mL	2
My inoculum contains 1 x 10^5 CFU/mL to 4.9 x 10^5 CFU/mL	3
My inoculum contains 5 x 10^5 CFU/mL	4
My inoculum contains >5 x 10^5 CFU/mL to 9.9 x 10^5 CFU/mL	5
My inoculum contains >10 x 10^5 CFU/mL	6
Our lab's procedure does not require I perform periodic colony counts for my inoculum.	7
The lab I am currently employed, does not perform bacterial inoculum colony counts to check concentration.	8
I was not trained or advised to perform bacterial inoculum colony counts by my supervisor when I was hired.	9

Q16. (Excel column P) Which of the following applies to your use of a purity plate for your bacterial inoculum?

Response	**Code**
I do not know what a purity plate is.	1
A purity plate is performed for each bacterial inoculum made.	2

A purity plate is performed only used for bacterial inoculums that may contain more than one bacterial organism.	3
Our lab does not perform purity plates on bacterial inoculums.	4
Our lab does not have a procedure for performing purity plates on bacterial inoculums.	5
I was not trained or advised to perform purity plates for my bacterial inoculums when bacterial antibiotic susceptibility testing is performed.	6

Q17. (Excel column Q) When performing bacterial antibiotic susceptibility testing, bacterial colonies are taken from which of the following microbiology media?

Response	**Code**
I am unaware of what this question refers to.	1
Bacterial colonies are always taken from non-inhibitory plates, such as a blood agar plate (BAP or SBA) not impregnated with antibiotics	2
Gram negative bacterial colonies are always taken from gram negative selective media and gram-positive bacterial colonies are always taken from gram positive selective media.	3
Bacterial colonies are taken from any plate (or microbiology media) which contains the most morphologically similar colonies and exhibits the best colony isolation.	4

Q18. (Excel column R) When performing antibiotic susceptibility testing, which of the following best describes the quantity of colonies used to create the bacterial inoculum?

Response	**Code**
I am unaware of what this question refers to	1
The quantity of colonies taken is irrelevant and colony numbers vary between patient samples or the type of bacteria growing.	2
I consistently use between one and three bacterial colonies to set up my bacterial inoculums.	3
Three bacterial colonies are always obtained regardless of colony size	4

Three bacterial colonies are usually obtained, but less than three bacterial colonies may be utilized if poor isolation occurs.	5
4-5 large bacterial colonies and 5-10 small bacterial colonies are used	6
I often use greater than ten bacterial colonies each time I make a bacterial inoculum for antibiotic susceptibility testing.	7
I was not trained to use a specific number of bacterial colonies when performing a bacterial inoculum. The number of colonies used to perform bacterial inoculums varies based on consistency of the colony.	8
Laboratory procedure does not dictate how many bacterial colonies are used to make each bacterial inoculum. For this reason, there is a consistent number of bacterial colonies used.	9
I consistently use five colonies when making my inoculum.	10

Q19. (Excel column S) On an average day, how many antibiotic susceptibility tests are set up at one time? To further elaborate, do you focus on one patient at a time? Do you line up all incubated samples and set up antibiotic susceptibility tests at once?

Response	**Code**
0-10 set up individually throughout day	1
0-10 set up as a batch	2
0-10	3
11-20 set up individually throughout day	4
11-20 set up as a batch	5
11-20	6
21-30 set up individually throughout day	7
21-30 set up as a batch	8
21-30	9
31-40 set up individually throughout day	10
31-40 set up as a batch	11
31-40	12
41-50 set up individually throughout day	13
41-50 set up as a batch	14
41-50	15
51-100	18
Not answered by participant	19
>300 set up as a batch	20
51-100 set up individually throughout day	21

51-100 set up as a batch	22
101-200 set up individually throughout day	23
101-200 set up as a batch	24
101-200	25

Q20. (Excel column T) Which of the following best describes the method used to mix the bacterial inoculum?

Response	**Code**
I do not mix the bacterial inoculum after adding the bacteria.	1
I gently rock the bacterial inoculum by hand.	2
I vigorously agitate the bacterial inoculum by hand.	3
I use a vortex to mix the bacterial inoculum.	4
I was not trained on how to mix the bacterial inoculum.	5
My laboratory procedure does not require the mixing of the bacterial inoculum. For this reason, the bacterial inoculum is not mixed.	6

Q21. (Excel column U) At the time you perform antibiotic susceptibility testing, is this the only testing performed or do you do multiple testing in other areas of the lab at the same time?

Response	**Code**
I am able to focus on only antibiotic susceptibility testing	1
I perform multiple other tests while performing antibiotic susceptibility testing	2

Q22. (Excel column V) Do you test the final turbidity or concentration of the bacterial inoculum used for antibiotic susceptibility testing? If you answered yes to this question answer question 23 and 24. If you answered, no. You have completed this survey.

Response	**Code**
No	1
Yes	2

* If answer is No, it may be because they are using a prompt system

Q23. (Excel column W) Continue only if you answered yes to question 22. How do you test the turbidity or concentration of the bacterial inoculum used for antibiotic susceptibility testing?

Response	**Code**
a McFarland standard	1
an instrument that measures the turbidity or concentration	2
We do not test the bacterial inoculum turbidity or concentration	3

A turbidity meter is not used for instrument testing. It is used only when disk diffusion and E-testing are performed.	4
McFarland is used only for quality control and calibration but not patient samples.	5
Inoculum is made by instrument	6
McFarland standard and instrument are used	8
Instrument is used for manual dilutions when prompt systems are not used.	9
Serial Dilution	10

Q24. (Excel column X) Continue if you answered yes to question 22. Do you check the turbidity or concentration of the bacterial inoculum for each sample or on a regular interval?

Response	**Code**
The bacterial inoculum concentration is checked with each sample.	1
Checked Daily	2
Checked Weekly	3
Checked Monthly	4
Checked Annually	5
Concentration is not checked	6
Checked Quarterly	7
Only checked with Staphylococcus isolates	8
Only checked with hemolytic Staphylococcus	9

Q25. (Excel column Y) Comments

Derivative of Q6. Profitable Institution (Excel column Z)

Profitable Institution	**Code**
Unidentified	0
For Profit	1
Not for Profit	2

Derivative of Q6. Rural or Urban Hospital (Excel column AA)

Rural or Urban Hospital	Code
Unidentified	0
Rural Hospital	1
Urban Hospital	2

Derivative of Q6. Stakeholders (Excel column AB)

Stakeholder	Code
Unidentified	0
Community Owned Hospital	1
State Owned Hospital	2
Federal Hospital	3
Other (private industry)	4

Derivative of Q6. Type of Laboratory Facility (Excel column AC)

Type of Laboratory Facility	Code
Unidentified	0
Physician's Office Laboratory	1
Reference Lab	2
Acute Care Only Laboratory	3
Critical Access Hospital Lab	4
Industrial Lab	5
Hospital Laboratory (acute emergency care, inpatient labs, outpatient labs, long term care, med/surg, rehabilitation, specialty care, etc.)	6

Comparing Question 14, 15, 22 Responses (Excel column AD)

Q14 Response	Q15 Response	Q22 Response	Code
Microscan	Checking inoculum concentrations with colony counts-correct results	Yes No	1 2
Microscan	Checking inoculum concentrations with colony counts - incorrect results	Yes No	3 4
Microscan	Not checking inoculum concentrations with colony counts for various reasons (participant doesn't know what this is, not in SOP, lab does not do it, or not training staff to perform)	Yes No	5 6

Vitek	Checking inoculum concentrations with colony counts-correct results	Yes No	7 8
Vitek	Checking inoculum concentrations with colony counts - incorrect results	Yes No	9 10
Vitek	Not checking inoculum concentrations with colony counts for various reasons (participant doesn't know what this is, not in SOP, lab does not do it, or not training staff to perform)	Yes No	11 12
Pheonix, Sensititre, or Hotel	Checking inoculum concentrations with colony counts-correct results	Yes No	13 14
Pheonix, Sensititre, or Hotel	Checking inoculum concentrations with colony counts - incorrect results	Yes No	15 16
Pheonix, Sensititre, or Hotel	Not checking inoculum concentrations with colony counts for various reasons (participant doesn't know what this is, not in SOP, lab does not do it, or not training staff to perform)	Yes No	17 18
Manual Method	Checking inoculum concentrations with colony counts-correct results	Yes No	19 20
Manual Method	Checking inoculum concentrations with colony counts - incorrect results	Yes No	21 22
Manual Method	Not checking inoculum concentrations with colony counts for various reasons (participant doesn't know what this is, not in SOP, lab does not do it, or not training staff to perform)	Yes No	23 24

Comparing Questions 15, 16, 17, and 20 Responses (Excel column AE)

Questions 15, 16, 17, and 20	**Code**
0 questions correct	0
1 question correct	1
2 questions correct	2
3 questions correct	3
4 questions correct	4

Q15 Responses (Excel column AF)

Q15 Response	**Code**
Incorrect answer	1
Correct answer	2
Internal lab issue	3
Training issue	4

Q16 Responses (Excel column AG)

Q16 Response	**Code**
Incorrect answer	1
Correct answer	2
Internal lab issue	3
Training issue	4

Q17 Responses (Excel column AH)

Q17 Response	Code
Incorrect answer	1
Correct answer	2

Q20 Responses (Excel column AI)

Q15 Response	**Code**
Incorrect answer	1
Correct answer	2
Internal lab issue	3
Training issue	4

Appendix G. Highest Level of Education Obtained by Participants

Highest Level of Education Obtained by Participant

	N	%
Highschool Diploma or Equivalent	3	0.4%
Associates Degree	139	20.7%
Bachelor's Degree	326	48.5%
Master's Degree	55	8.2%
Doctorate	6	0.9%

Appendix H. Title of Degree and Frequencies

Title of Highest Degree

	N	%
Laboratory Science	333	49.6%
Administration	1	0.1%
Applied Technology	11	1.6%
Applied Science	19	2.8%
Biology	57	8.5%
Biochemistry	4	0.6%
Education	4	0.6%
Forensics	6	0.9%
Health Science	14	2.1%
Health Services Administration	7	1.0%
MBA	8	1.2%
Master of Health	23	3.4%
Molecular Biology	5	0.7%
Natural Sceince	7	1.0%
Medical Assistant	1	0.1%
Healthcare Informatics	1	0.1%
History	1	0.1%

Immunohematology	1	0.1%
Cellular Biology	1	0.1%
Physiology and Genetics	1	0.1%
Bacteriology	2	0.3%
Public Health	2	0.3%
Biomed	1	0.1%
Zoology	2	0.3%
Psychology	1	0.1%
Combined Science	1	0.1%
Management in Allied Health	1	0.1%
Lab Operations	1	0.1%
Healthcare Management	2	0.3%
Math and Science	1	0.1%
Allied Health Leadership	1	0.1%
Accounting	1	0.1%
Workforce Training and Development	1	0.1%
Biotechnology	3	0.4%
Art	1	0.1%
General Studies	1	0.1%

Appendix I. State Employed by Participant and Frequencies

State Employed by Participant

	N	%
Alabama	2	0.3%
Alaska	1	0.1%
Arizona	5	0.7%
Arkansas	14	2.1%
California	4	0.6%
Colorado	7	1.0%
Connecticut	2	0.3%
Delaware	2	0.3%
Florida	14	2.1%
Georgia	6	0.9%
Idaho	3	0.4%
Illinois	18	2.7%
Indiana	7	1.0%
Iowa	8	1.2%
Kansas	4	0.6%
Kentucky	44	6.5%
Louisiana	47	7.0%

Maine	3	0.4%
Maryland	4	0.6%
Massachusetts	6	0.9%
Michigan	15	2.2%
Minnesota	8	1.2%
Mississippi	39	5.8%
Missouri	7	1.0%
Montana	5	0.7%
Nebraska	5	0.7%
Nevada	6	0.9%
New Hampshire	3	0.4%
New Jersey	5	0.7%
New Mexico	3	0.4%
New York	17	2.5%
North Carolina	7	1.0%
North Dakota	4	0.6%
Ohio	20	3.0%
Oklahoma	5	0.7%
Oregon	4	0.6%
Pennsylvania	14	2.1%
Rhode Island	5	0.7%
South Carolina	10	1.5%
South Dakota	5	0.7%
Tennessee	63	9.4%
Texas	22	3.3%
Utah	5	0.7%
Vermont	3	0.4%
Virginia	9	1.3%
Washington	7	1.0%
West Virginia	13	1.9%
Wisconsin	12	1.8%
Wyoming	6	0.9%
District of Columbia	1	0.1%

Appendix J. Licensure Held by Participant

Licensures Held by Participant

	N	%
No licensure	328	48.8%
California	11	1.6%
Florida	19	2.8%
Georgia	5	0.7%
Hawaii	1	0.1%
Louisiana	47	7.0%
Montana	5	0.7%
Nevada	6	0.9%
New York	17	2.5%
North Dakota	7	1.0%
Tennessee	61	9.1%
West Virginia	14	2.1%
Tennessee and New York	2	0.3%
California and Florida	1	0.1%
California, Florida, Louisiana	1	0.1%
Tennessee and Florida	1	0.1%
Louisiana and Tennessee	1	0.1%
California, Louisiana, Hawaii, and New York	1	0.1%

Appendix K. Percentages Graduated from Accredited Lab and Registered with Certification and Agency

Graduated from Accredited Lab Program

	N	%
Yes	485	72.2%
No	44	6.5%

Registered with a Certifying Agency

	N	%
Yes	487	72.5%
No	42	6.3%

Certifying Board Registered

	N	%
American Society of Clinical Pathology (ASCP)	433	64.4%
American Medical Technologist (AMT)	40	6.0%
American Association of Bioanalysts (AAB)	11	1.6%
ASCP and AMT	2	0.3%
HEW	2	0.3%

ASCP, AMT, and AAB	1	0.1%

Appendix L. Antibiotic Susceptibility Testing Methods Used by Participants

AS Testing Method Used by Participant

	N	%
No response	1	0.1%
DD	26	3.9%
E-Test	1	0.1%
Microscan	197	29.3%
Vitek	263	39.1%
Sinsititre	1	0.1%
Pheonix	12	1.8%
Hotel	1	0.1%
DD and E-Test	5	0.7%
Vitek and E-test	3	0.4%
Microscan and E-test	1	0.1%
Microscan and DD	1	0.1%
Multiple Instruments	1	0.1%
Hotel, DD, and E-test	1	0.1%
Vitek, Microscan, and E-tets	1	0.1%
Pheonix, DD, and E-test	1	0.1%
Pheonix and Microscan	1	0.1%
Pheonix and E-test	1	0.1%

Vitek, DD, and E-test	1	0.1%

Appendix M. Antibiotic Susceptibility Testing Quantities

Number of AST set up at one time

	N	%
0-10 a day, set up one at a time	20	3.0%
0-10 batched	94	14.0%
0-10	52	7.7%
11-20 a day, set up one at a time	7	1.0%
11-20 batched	54	8.0%
11-20	27	4.0%
21-30 a day, set up one at a time	7	1.0%
21-30 batched	31	4.6%
21-30	9	1.3%
31-40 a day, set up one at a time	4	0.6%
31-40 batched	8	1.2%
31-40	3	0.4%
41-50 a day, set up one at a time	4	0.6%
41-50 batched	15	2.2%
41-50	5	0.7%
51-100	5	0.7%
Not answered by participant	148	22.0%
>300 batched	1	0.1%

51-100 a day, set up one at a time	9	1.3%
51-100 batched	14	2.1%
101-200 a day, one at a time	1	0.1%
101-200 batched	5	0.7%
101-200	1	0.1%
50	1	0.1%

Ability of Lab Professional to Focus on AST

	N	%
Focus on AST only	241	35.9%
Perform multiple other tests while performing AST	278	41.4%

Appendix N. Prevalence of Proficiency Testing Result by Event

Prevalence of Proficiency Testing Result by Event

Proficiency Manufacturer	Time Frame	Error Prevalence	Organism Tested	Total Responses
American Proficiency Institute (API)	2018 Event 1	3.256%	*Enterococcus faecium*	8439
American Proficiency Institute (API)	2018 Event 2	6.424%	*Escherichia coli (ESBL strain)*	16,358
American Proficiency Institute (API)	2018 Event 3	0.914%	*Klebsiella pneumoniae*	15,896
American Proficiency Institute (API)	2017 Event 1	0.7898%	*Escherichia coli*	16,586
American Proficiency Institute (API)	2017 Event 2	34.899%	*Pseudomonas aeruginosa*	8,771
American Proficiency Institute (API)	2017 Event 3	4.275%	*Enterococcus faecium*	7,650

American Proficiency Institute (API)	2016 Event 1	5.294%	*Staphylococcus aureus*	12,183
American Proficiency Institute (API)	2016 Event 2	0.7458%	*Escherichia coli*	16,358
American Proficiency Institute (API)	2016 Event 3	9.9865%	*Proteus vulgaris*	15,531
American Academy of Family Physicians (AAFP)	2018 Event A	0.000%	*Escherichia coli*	70
American Academy of Family Physicians (AAFP)	2018 Event B	1.3889%	*Klebsiella pneumonia*	72
American Academy of Family Physicians (AAFP)	2018 Event C	1.639%	*Proteus spp*	61
American Academy of Family Physicians (AAFP)	2017 Event A	1.1763%	*Enterobacter aerogenes*	85
American Academy of Family Physicians (AAFP)	2017 Event B	0%	*Escherichia coli*	20
American Academy of Family Physicians (AAFP)	2017 Event C	0.000%	*Pseudomonas spp*	20
American Academy of Family Physicians (AAFP)	2016 Event A	Not listed by manufacturer	Not listed by manufacturer	Not listed by manufacturer
American Academy of Family Physicians (AAFP)	2016 Event B	1.980%	Not listed by manufacturer	101
American Academy of Family Physicians (AAFP)	2016 Event C	0.000%	Not listed by manufacturer	109
American Association of Bioanalysts (AAB)	2018 Quarter 1	0.463%	*Klebsiella pneumoniae*	1296
American Association of Bioanalysts (AAB)	2018 Quarter 2	0.405%	*Escherichia coli*	1234
American Association of Bioanalysts (AAB)	2018 Quarter 3	1.403%	*Enterococcus faecalis*	713

American Association of Bioanalysts (AAB)	2017 Quarter 1	0.650%	*Enterobacter aerogenes*	1077
American Association of Bioanalysts (AAB)	2017 Quarter 2	2.342%	*Enterococcus faecalis*	726
American Association of Bioanalysts (AAB)	2017 Quarter 3	0.3671%	*Escherichia coli*	1234
American Association of Bioanalysts (AAB)	2016 Quarter 1	0.7950%	*Enterococcus faecalis*	713
American Association of Bioanalysts (AAB)	2016 Quarter 2	1.3295%	*Enterobacter aerogenes*	1077
American Association of Bioanalysts (AAB)	2016 Quarter 3	1.1802%	*Enterococcus faecalis*	726
Medical Laboratory Evaluation (MLE)	2018 M1	15.385%	*Pseudomonas aeruginosa*	208
Medical Laboratory Evaluation (MLE)	2018 M2	2.2843%	*Klebsiella pneumonia*	394
Medical Laboratory Evaluation (MLE)	2018 M3	0.000%	*Staphylococcus aureus*	236
Medical Laboratory Evaluation (MLE)	2017 M1	10.000%	*Steptococcus agalactiae*	200
Medical Laboratory Evaluation (MLE)	2017 M2	34.9398%	*Providencia stuartii*	83
Medical Laboratory Evaluation (MLE)	2017 M3	6.2189%	*Enterobacter aerogenes*	402
Medical Laboratory Evaluation (MLE)	2016 M1	2.828%	*Escherichia coli*	389
Medical Laboratory Evaluation (MLE)	2016 M2	1.2821%	*Klebsiella pneumonia*	312
Medical Laboratory Evaluation (MLE)	2016 M3	Not listed by manufacturer	-	-
Wisconsin State Laboratory of Hygiene (WSLH)	2018 Event 1	Not listed by manufacturer	-	-
Wisconsin State Laboratory of Hygiene (WSLH)	2018 Event 2	Not listed by manufacturer	-	-
Wisconsin State Laboratory of Hygiene (WSLH)	2018 Event 3	Not listed by manufacturer	-	-

Wisconsin State Laboratory of Hygiene (WSLH)	2017 Event 1	2.505%	*Pseudomonas aeruginosa*	479
Wisconsin State Laboratory of Hygiene (WSLH)	2017 Event 2	11.7211%	*Staphylococcus aureus*	674
Wisconsin State Laboratory of Hygiene (WSLH)	2017 Event 3	4.0888%	*Acinetobacter baumannii*	856
Wisconsin State Laboratory of Hygiene (WSLH)	2016 Event 1	3.506%	*Staphylococcus aureus*	713
Wisconsin State Laboratory of Hygiene (WSLH)	2016 Event 2	2.734%	*Enterococcus faecium*	512
Wisconsin State Laboratory of Hygiene (WSLH)	2016 Event 3	3.704%	*Stenotrophomonas maltophilia*	783