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Approaches for Improving Antimicrobial Stewardship Programs

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Approaches for Improving Antimicrobial Stewardship Programs

A dissertation in partial satisfaction of the

requirements for the Doctor of Philosophy

in Environmental Health Sciences

by

Olivia Ellis

2020

ABSTRACT OF THE DISSERTATION

Approaches for Improving Antimicrobial Stewardship Programs

by

Olivia Ellis

Doctor of Philosophy in Environmental Health Sciences

University of California, Los Angeles, 2020

Professor Hilary Godwin, Co-Chair

Professor Richard Ambrose, Co-Chair

The overarching objective of the work described in this thesis is to explore approaches for improving Antimicrobial Stewardship Programs (ASPs) in clinical settings. Three discrete topics were explored: (1) using an Adenosine Triphosphate (ATP) assay to evaluate differences in bioburden and cleaning efficiency between surface types in operating rooms; (2) validating the use of a PCR-based assay to be used to tailor treatment recommendations for patients with *Neisseria gonorrhoeae*; and (3) investigation of antimicrobial susceptibility trends of

Stenotrophomonas maltophilia in a health system over time to provide insights into whether treatment guidelines for *S. maltophilia* need to be changed.

First, a commercial ATP detection assay was used to detect differences between the cleanliness of individual high touch surface types in operating rooms. This ATP testing assay revealed that irregularly shaped, uncovered surfaces (keyboards, overhead lights, and door handles) tend to harbor a larger bioburden than regularly shaped, covered surfaces (tables and mattresses). Additionally, surfaces were more likely to fail by ATP assay than by traditional Replicate Organism and Detection Plating (RODAC) methods. However, the majority of rooms (92%, 22/24) had least one surface that exceeded the 250 RLU threshold after turnaround cleaning via the ATP assay. Additionally, 42% (10/24) of the rooms had at least on surface that didn't pass as clean after turnaround cleaning via the RODAC surface sample test. This study suggests that further experimentation to determine whether single use covers for irregularly shaped surfaces in operating rooms could reduce bioburden.

In the second study reported in this thesis, a Real Time-Polymerase Chain Reaction (RT-PCR) assay for detecting ciprofloxacin-susceptible *Neisseria gonorrhoeae* was validated at three locations: Los Angeles (UCLA), San Francisco Public Health Lab (SFPHL), and Philadelphia Public Health Lab (PPHL). In this study, clinical specimens that had tested positive for *Neisseria gonorrhoeae* by Nucleic Acid Amplification Testing (NAAT) were further tested for susceptibility to ciprofloxacin using the validated RT-PCR assay that can detect a mutation in the gyrase A (*gyrA*) gene, which confers resistance to ciprofloxacin. At UCLA, 57% (n=319/557) of the specimens that could be genotyped were *gyrA* Wild Type (WT). At SFPHL, 72%

(n=134/185) of the genotyped specimens were WT and at PPHL, 64% (27/42) were WT. The goal of this study was to validate an Antimicrobial Susceptibility Test (AST) that would allow clinicians to more rapidly tailor treatment for patients with ciprofloxacin susceptible gonorrhea. We also assessed the utility of this assay for different samples types at different laboratories. For instance, the proportion of indeterminate *N. gonorrhoeae* varied significantly by testing laboratory and anatomical source ($p < 0.00001$, $\chi^2 = 28.7$). Therefore, post hoc pairwise Fisher's exact statistical testing was performed. There was a statistically significant number of indeterminate rectal and pharyngeal specimens between UCLA and SFPHL for ($p < 0.05$, $P = 0.0016$) as well as indeterminate urine and pharyngeal ($p < 0.05$, $P = 0.02$). The number of indeterminate pharyngeal and urine and indeterminate rectal and urine specimens also varied significantly between SFPHL and PPHL, respectively ($p < 0.05$, $P = 0.0013$ and $p < 0.05$, $P = 0.0221$).

The third study reported in this thesis explores antibiotic treatment options for *Stenotrophomonas maltophilia*, which is an intrinsically multi-drug-resistant organism that poses an increasing threat to the health of immunocompromised patients in hospital settings. Antimicrobial susceptibility trends of *Stenotrophomonas maltophilia* isolates from a single health system were analyzed to elucidate possible trends in antimicrobial resistance over time. While resistance to trimethoprim-sulfamethoxazole (Bactrim) has been reported globally, 98% of all specimens tested at the health system between 2009-2018 were susceptible to Bactrim. 99% of all isolates tested were also susceptible to minocycline monotherapy. These antibiotics also show the most susceptibility when analyzed in combination (one or the other antibiotics were susceptible 100% of the time). The results of this study suggest that using larger data sets — both through

combining data from multiple sites within the same network and by combining data from multiple years — may provide more robust treatment guidelines for clinicians who wish to choose the best empiric treatment for those with *S. maltophilia*.

Taken together, these studies provide support for improving the framework of ASPs through advanced environmental monitoring, reduction in AST turn-around time, and enhanced tracking of Antimicrobial Resistance (AMR) patterns. These studies provide important insights into how to improve the management and use of antimicrobials in clinical settings.

The dissertation of Olivia Ellis is approved.

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DEDICATION

With my deepest gratitude, I dedicate this work to my parents. I also extend my sincerest appreciation and acknowledgment to my friends, colleagues, educators, yoga and meditation instructors, and my mentors, past and present. I may not have conquered this achievement without your consistent devotion, patience, leadership, and support, which has been consistently displayed and exhibited to me throughout this journey. I am forever indebted to you for all the success my life brings.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
ASP	Antimicrobial Stewardship Program
AST	Antimicrobial Susceptibility Test
ATP	Adenosine Triphosphate
CFU	Colony Forming Units
CDC	Centers for Disease Control and Prevention
CER	Clinical Exposure Response
CLSI	Clinical Laboratory Standards Institute
DRO	Drug Resistant Organism
ECV	Epidemiologic Cutoff Value
FDA	Food and Drug Administration
gyrA	Gyrase A
HAI	Healthcare-associated Infection
HACCP	Hazard Analysis Critical Control Points
MALDI-TOF	Matrix Associated Laser Desorption-Time-of-Flight
MDRO	Multi Drug Resistant Organism
MIC	Minimum Inhibitory Concentration
MT	Mutant Type
NAAT	Nucleic Acid Amplification Test
PD	Pharmacodynamics
PK	Pharmacokinetics
RLU	Relative Light Units

LIST OF ABBREVIATIONS

RMMs	Rapid Microbiological Methods
RODAC	Replicate Organism Detection and Plating
RT-PCR	Real-Time Polymerase Chain Reaction
TAT	Turn-Around-Time
TVC	Total Viable Count
WHO	World Health Organization
WT	Wild Type

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CHAPTER 1

Introduction and Overview of the Organization of the Thesis

INTRODUCTION

Antimicrobial resistance (AMR) and healthcare-associated infections (HAIs) are two of the most pressing public health challenges of our time and are highly interrelated (Tacconelli, 2018; Medtech Europe, 2019; CDC, 2013). In fact, drug resistant organisms (DROs) are frequently the culprit of HAIs (ECDC, 2019). The World Health Organization (WHO) warns that these critical public health threats continue to increase in incidence and that AMR can result in longer hospital stays, higher medical expenses and increased mortality (WHO, 2018). Between 1997 to 2006, the number of HAIs in the US increased by 359% from 37,000 cases to nearly 170,000, respectively (Mainous *et al*., 2011). Each year in the United States, approximately 2 million people are infected with AMR organisms and nearly 23,000 individuals die from these infections (CDC, 2018). Overall, HAIs result in over 75,000 deaths and cost $28.4 to $33.8 billion per year in the United States (Scott II, R. D., 2009). The number of HAI deaths is projected to increase to a total of 10 million per year by 2050, more than cancer and diabetes combined (O'Neil, 2014).

These numbers are staggering and speak to the need for prevention and public health based approached to combatting AMR and HAIs. In this thesis, I explore processes and technologies that can be used to reduce morbidity and mortality related to AMR and HAIs that occur inside and outside of the hospital settings. In Chapter 2, I focus on the use of an Adenosine Triphosphate (ATP) monitoring technology to quantitatively determine which types of surfaces in an operating room are hardest to clean. In Chapter 3 and Chapter 4, respectively, I focus on two bacteria that have high levels of antibiotic resistance and pose threats to people worldwide:

Neisseria gonorrhoeae and *Stenotrophomonas maltophilia*.

Understanding Antimicrobial Resistance

To be able to understand how to combat antibiotic resistance, it is important to understand how bacteria become resistant to antibiotics in the first place. Bacterial resistance mechanisms can be either intrinsic or acquired. Bacteria develop intrinsic resistance via biochemical interactions in the environment produced by other microorganisms (Munita and Arias, 2016). Some bacteria have incredible genomic plasticity, which enables them to evade environmental and biochemical threats from other organisms in their vicinity that can threaten their existence (Munita 2016). For instance, chromosomal mutations can develop in the presence of antimicrobial medicines or the acquisition of external genomic resistance markers (horizontal gene transfer) (Munita and Arias, 2016). While bacteria have developed intrinsic resistance mechanisms via their commensal counterparts, the main concern in the clinical environment is related to acquired resistance mechanisms.

One of the trademarks of acquired antibiotic resistance is selective pressure. Selective pressure from antibiotic use and abuse has made bacteria both resistant and more virulent due to increasing levels of resistance (Zhang, 2015). Selective pressure can decrease the affinity of a drug, decrease drug uptake, activate an efflux pump to extrude the antibiotic, or cause global changes to the organism (Munita, 2016). Therefore, reducing antibiotic usage can subsequently reduce selective pressure (Zhang, 2015). Reducing selective pressure, by reducing the misuse of antibiotics, is one of the flagship practices of Antimicrobial Stewardship Programs (ASPs).

In clinical practice, determining antimicrobial resistance is complex and relies on the

establishment of criteria called susceptibility breakpoints (Munita and Arias, 2016). Susceptibility breakpoints are characterized as either susceptible, intermediate or resistant. These classifications rely on *in vitro* activity of antibiotics, that are tested against bacterial cultures, along with pharmacological parameters such as *in vivo* pharmacokinetic (PK) and pharmacodynamic (PD) studies (Munita and Arias, 2016). PK and PD are critical in the creation of breakpoints. Breakpoints are the criteria that are applied to the Minimum Inhibitory Concentrations (MICs) of culture isolates from patients within the clinical setting. Breakpoints are used to categorize the MIC of an isolate as susceptible, intermediate, susceptible-dose-dependent, non-susceptible, or resistant. A MIC is the concentration at which a bacterium is no longer viable when treated with an antibiotic. When the Clinical Laboratory Standards Institute (CLSI) sets breakpoints, they use several cut-offs (Simner and Miller, 2018):

- MICs of wild-type (WT) isolates (organisms that lack resistance markers to antibiotics) that provide an epidemiologic cutoff value (ECV).
- Animal or *in vitro* PK/PD models that provide a non-clinical PK/PD cutoff.
- PK/PD clinical exposure response (CER) data from patients in clinical trials that provide a CER cutoff.
- Success and failure data by MIC from clinical trial data that provide a clinical cutoff.

These cutoff values are key in developing a breakpoint that clinical microbiologists use in conjunction with MICs to provide susceptibility reports to clinicians (Simner and Miller, 2018). Breakpoints and MICs are then published in the CLSI M100, the document used as a reference for susceptibility reporting in the clinical laboratory (CLSI, 2015).

Antibiograms

The standard way to provide quantitative susceptibility data to clinicians for a facility or region is using a cumulative antibiogram. A cumulative antibiogram is a summary of annual Antimicrobial Susceptibility Testing (AST) data from a single healthcare facility. Antibiograms list the percentage of specimens, of a specific organism, that are susceptible to each drug or combination of drugs. Therefore, an antibiotic that shows 95% susceptibility for an organism is a better choice than a drug that has only 80% susceptibility. These percentages are obtained from Minimum Inhibitory Concentration (MIC) data for a variety of antibiotics for each pathogen (or class of pathogens) that are derived from *in vitro* AST studies conducted on samples from patients in a specific facility. This data can be used to assess trends in AST data from various organisms between years at a single institution even though the primary goal of an antibiogram is to assist clinicians in choosing the best empirical antimicrobial therapy, before an AST report is available.

While cumulative antibiograms are vital in supporting clinical efforts to reduce HAIs and AMR, an enhanced antibiogram or a non-traditional antibiogram may assist clinicians in making an even more informed decision regarding treatment over empiric therapy. One such example is a "combined antibiogram", which is used to assess the regional effectiveness of antibiotics. Combined antibiograms include a broader range of epidemiological data, subsequently improving the statistical quality of the results. These combined reports may not be needed on a regular basis, but they can answer more specific questions about susceptibility patterns within a health facility over time.

Antimicrobial Stewardship Programs: Focus on Control of HAIs and Control of Antibiotic Resistant Organisms

The threat of AMR and HAIs led to the creation of Antimicrobial Stewardship Programs (ASPs), which have been instituted at healthcare facilities across the United States to control the spread of antibiotics resistant organisms and infections. Antimicrobial stewardship is defined by the Association of Professionals in Infection Control and Epidemiology (APIC) as a coordinated program of interventions, which include: judicious use of antimicrobials, improving patient outcomes, reducing microbial resistance, and decreasing the spread of infections caused by drug resistant organisms (DROs) (APIC, 2019). ASPs have seven core elements that are designed and utilized to reduce the misuse of antibiotics. The seven core elements are: leadership commitment, accountability, drug expertise, action, tracking, reporting and education (APIC 2019).

Historically, infection prevention measures focused on handwashing, starting with the work of Dr. Ignaz Semmelweis in the mid-1800s, but they have evolved substantially since then. Although handwashing continues to be a paramount component of all ASPs, more sophisticated environmental infection control practices began in the 1960s and by the 1970's. Studies that began in the mid-20th century established that surfaces serve as environmental reservoirs within the hospital can propagate and transmit infectious agents, including DROs (CDC HAI, 2014; Dixon, 2011). By the 1990s, HAI control programs were increased substantially and operational in nearly every U.S. hospital (Dixon, 2011). As a result, infection prevention has expanded to include antiseptics and environmental cleaning. For instance, The California ASP Initiative is a part of the California Department of Public Health HAI Program, which signifies that ASPs should not solely focus on antibiotic stewardship but also on refining hygienic practices and improving environmental surveillance to optimize clinical outcomes (CDPH HAI, 2018). Even

though it is well known that surfaces and other fomites are a major route of disease transmission in many settings, operating rooms still rely heavily upon visual inspection or presence/absence bioburden testing as an assurance of cleanliness. Nevertheless, environmental cleaning is a critical step in the infection prevention process.

The evidence base for ASP best practices is still evolving (CDC, 2015). There are many unanswered, important questions about how different surface types in clinical environments contribute to disease transmission. Assessing the microbiome of the clinical setting is one important way that clinical laboratories can provide critical information to clinicians, which can improve patient safety and health outcomes. Therefore, enhanced environmental monitoring programs and antimicrobial surveillance may become an important aspect of ASPs of the future.

TWO ORGANISMS OF INTEREST: NEISSERIA GONORRHOEAE AND STENOTROPHOMONAS MALTOPHILIA

To combat the spread of antibiotic resistant infections, tools are also needed that would allow clinicians to better tailor treatment to the individual patient needs based on the bacteria that they are infected with and the latest information about drug susceptibility of those organisms. Two organisms for which these tools are particularly needed are *Neisseria gonorrhoeae* and *Stenotrophomonas maltophilia*.

Neisseria gonorrhoeae and *Stenotrophomonas maltophilia* are particularly challenging because standard culture-based approaches for determining antimicrobial susceptibility for these organisms require multiple days. Both *N. gonorrhoeae* and *S. maltophilia* rely on time-consuming culture-based methods that can take between 1 to 3 days to complete due to growth

constraints (Doern, 2018). The lack of rapid detection and reporting of antimicrobial resistance (AMR) for these organisms presents a real-world challenge that may be contributing to increasing incidence of infections caused by DROs. As a result, many labs no longer offer culture-based methods for diagnosis of *N. gonorrhoeae.*

While newer phenotypic, micro-fluidic, and nanotechnology-based tests offer promise for the future, none have been Food and Drug Administration (FDA) cleared for *N. gonorrhoeae* and *S. maltophilia* at the time that this thesis was written (August 2019). While reducing time to reporting remains the driver of these technologies, their improvement on health outcomes has yet to be proven (Doern, 2018).

As a result, most clinicians rely upon population level data to determine which antimicrobials to prescribe for their patients suffering from *Neisseria gonorrhoeae* and *Stenotrophomonas maltophilia*. In the case of *Neisseria gonorrhoeae,* physicians choose which antimicrobial to prescribe based upon treatment guidelines published by the CDC (CDC, 2015). As a result, azithromycin and ceftriaxone are commonly prescribed for *N. gonorrhoeae* and culture–based testing for antimicrobial susceptibility is not routinely performed.

In the case of *Stenotrophomonas maltophilia*, many clinicians rely on antibiogram reports for their facility or region when deciding what antimicrobial to prescribe for their patients. No FDA cleared susceptibility testing methods exist for *S. maltophilia*. The relatively common frequency of this infection along with lack of standardized susceptibility tests and interpretative criteria, poses a problem in the clinical laboratory, hindering the choice of a more suitable antibiotic treatment in some cases (Nicodemo, 2007). As a result, empiric use of Trimethoprim-Sulfamethoxazole (TMP-SMX) is frequently used to treat *Stenotrophomonas maltophilia*

infections. These prescription practices are not ideal because they contribute to selective pressure for the development of further antibiotic resistance in these organisms.

NEISSERIA GONORRHOEAE

Epidemiology of *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is the second most common STI in the United States and can lead to severe health problems if left untreated. Commonly, the bacterial infection is asymptomatic at various body sites including the genitals, throat and the rectum. The immuno-evasive characteristics of this bacteria make it more likely to be transmitted between sexual partners who are not aware of their disease state. For instance, women may acquire disseminated infections that lead to pelvic inflammatory disease that can ultimately cause sterility (Heymann, 2008). Newborns of infected mothers may develop infection of the eyes, sepsis, and in rare cases, gonococcal meningitis (Heymann, 2008). In symptomatic cases, gonorrhoeae can cause mucopurulent discharge and abnormal vaginal discharge in women as well as swelling of the epididymis and testes in men (Heymann, 2008). In extremely rare cases, *N. gonorrhoeae* can spread to the bones and joints causing arthritis, lead to septicemia and even endocarditis (an infection of the heart) (Heymann, 2008).

Gonorrhea cases are increasing at the local and global level. The World Health Organization (WHO) estimates that nearly 87 million new cases of gonorrhea occur each year (WHO, 2019). In 2017, a total of 555,608 cases of gonorrhea were reported in the United States, yielding a rate of 171.9 cases per 100,000 population (CDC, 2017). During 2016–2017, the rate of reported gonorrhea cases increased 18.6%, and increased 75.2% since the historic low in 2009 (CDC, 2017). As of 2017, California had the 13th highest rate of reported gonorrhea cases in the

United States (192 per 100,000) with most concentrated in San Francisco (602 per 100,000) and Los Angeles (257 per 100,000) counties (CDC, updated 2017). San Francisco also had the highest proportion of cases estimated to be in men who have sex with men (CDC, 2017).

Biology of *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is exclusively pathogenic to humans and is the causative agent of the sexually transmitted bacterial infection commonly referred to as gonorrhea. *Neisseria gonorrhoeae* is a fastidious, oxidase positive, Gram-negative diplococci. Although *N. gonorrhoeae* characteristically infects the urogenital epithelium, the *Neisseria* genus also inhabits the mucus membranes of the human body and several saprophytic species exist in the pharyngeal cavity (Heymann, 2008). Due to its fastidious nature *in vitro*, *N. gonorrhoeae* typically require nutrient enrichment for culture. While Nucleic Acid Amplification Tests (NAATs) can be used to rapidly identify the presence of *N. gonorrhoeae*, more lengthy culture methods allow for AST testing per Clinical Laboratory Standards Institute (CLSI) guidelines.

The Public Health Threat of Antibiotic-Resistant *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is considered by the Centers for Disease Control and Prevention (CDC) to be one of the three most urgent antimicrobial threats worldwide, in part because *N. gonorrhoeae* has so many different mechanisms for acquiring resistance (CDC, 2013). *N. gonorrhoeae* has evolved an arsenal of different drug resistance mechanisms because of genome plasticity and evolutionary selection pressure. For instance, Horizontal Gene Transfer (HGT), via conjugation, is responsible for the sequestration of a plasmid that encodes β-lactamase, a penicillin inhibitor (Umland, 1988). Analogously, the *tetA* gene for tetracycline resistance was

conferred by plasmids that encode antiporter and *tetR* ribosome-protection protein (Hu et al., 2005).

Drug resistance in *N. gonorrhoeae* is procured by various mechanisms other than conjugation. For instance, point mutations like the (V57M) in the *rspJ* gene encoding ribosomal protein S10 are caused by a single point mutation (Hu et al., 2005). By contrast, fluoroquinolone resistance is conferred by mutations on the chromosomal genes *gyrA* (S91F, D95N or D95G) and *parC* (S88P and E91G) (Seidner 2007, 2008). The combination of mutations in the *mtrR* efflux pump repressor protein and the 23s rRNA confer high-level resistance to azithromycin (Kirkcaldy 2015, Wu 2011). Additionally, mosaic-like structure of the penicillin binding protein (*penA* mosaic) in gonococci have been linked to a reduction in susceptibility to oral cephalosporins, the final class of antibiotics currently used to treat gonorrhea (Ochiai, 2008; Pandori, 2009; Whiley., 2007). The appearance of *N. gonorrhoeae* with reduced oral cephalosporin susceptibility has elevated concern for the decreasing effectiveness of antibiotics and the rise of untreatable gonorrhea (Bolan, 2012).

Over time, *N. gonorrhoeae* has developed resistance to many of the antibiotics that were once used to easily treat this widespread sexually transmitted infection (CDC, 2013). In the early 2000's, *N. gonorrhoeae* developed resistance to the empirical fluoroquinolone treatment that were previously used to control infections caused by the bacterium, including ciprofloxacin (CDC, 2013). Thus, the global public health achievements of the 20th century continue to be defied as antibiotics become less effective over time from overprescribing/misuse, environmental exposures, evolutionary pressures and genome plasticity.

The development of rapid AST algorithms and reporting mechanisms for *N. gonorrhoeae* can

help to enhance clinical practice by improving treatment options via distribution of the most informative AST data in a more targeted and individualized way. Luckily, a single point mutation at the serine 91 locus of the *gyrA* gene is enough to predict sensitivity and resistance to the fluoroquinolone ciprofloxacin (Klausner via Acquino). Utilizing a polymerase chain reaction method for detecting the wild-type or mutation of this gene via allows drug susceptibility for this organism to be determined more rapidly than using traditional methods. Therefore, the development of rapid AST algorithms and reporting mechanisms for *N. gonorrhoeae* can help to reduce selective pressure that leads to drug resistance by distributing AST information to clinicians in a more rapid, targeted and individualized way.

STENOTROPHOMONAS MALTOPHILIA

Epidemiology of *Stenotrophomonas maltophilia*

Between 1993 and 2004, *S. maltophilia* was among the 11 most frequently recovered organisms from intensive care units and *S. maltophilia* is increasingly the cause of opportunistic HAIs acquired from various environmental sources in hospitals including hospital water systems (Brooke, 2012). Patients with lung cancer, chronic obstructive pulmonary disease, cystic fibrosis or extended stays in the intensive care unit are most susceptible to *S. maltophilia* ventilator associated pneumonia and bloodstream infections. (Brooke, 2012). While this organism is not particularly virulent to healthy individuals, who tend to be colonized by the bacteria without infection, crude mortality rates in those who develop bloodstream infections can range from 14-69% (Brooke, 2012). Other infection locations include: soft tissue and skin, bone, urinary tract, meningitis and encephalitis (Brooke, 2012).

While most cases of *S. maltophilia* are susceptible to trimethoprim-sulfamethoxazole (TMP-SMX), the CDC has warned against the rising global threat of *S. maltophilia* resistant to TMP-SMX, via *sul* and *dfrA* genes, and the need for continued surveillance (Toleman, 2007). Resistance to this antibiotic has been observed globally and this fact poses a significant challenge to clinicians attempting to treat this disease. It has been suggested that since TMP-SMX is the mainstay empiric therapy for *S. maltophilia* infections, the mobilization of *sul* genes by means of class 1 integrons and IS*CR* elements is likely to increase with continued TMP-SMX use, leading to more resistance over time (Toleman, 2007). In 2016, researchers in China discovered that (116/300, 38.7%) of the *S. maltophilia* isolates from 25 hospitals in the country were resistant to TMP-SMX (Hu, 2016). Enhanced antibiograms may become a new normal of the future for TMP-SMX resistant *S. maltophilia* if this public health threat continues to emerge.

Biology of *Stenotrophomonas maltophilia*

S. maltophilia has been isolated from aquatic environments inside and outside of the hospital setting (Brooke, 2012). *S. maltophilia* is an aerobic Gram-negative bacterium that is ubiquitous in the environment (soil, plant roots, animals, washed salad, lakes, rivers and aquifers). *S. maltophilia* belongs to the family Xanthomonadaceae and is nonfermentative, catalase-positive, and oxidase-negative. *S. maltophilia* is closely related to the *Pseudomonas* genus and is similarly (intrinsically) resistant to all carbapenem antibiotics because its genome includes inducible chromosomal metallo-beta-lactamases (Brooke, 2012).

S. maltophilia is closely related to *Pseudomonas aeruginosa.* In fact, *S. maltophilia* has endured a rich history of genera nomenclature changes from *Pseudomonas* to *Xanthomonas,* related to its

complex genotypic and phenotypic traits (Lewis, n.d.). Although *S. maltophilia* displays broad ranging genotypic and phenotypic similarities to *Pseudomonas* and *Xanthomonas,* it was reclassified into its own genus in 1993 (Lewis, n.d.).

S. maltophilia has several resistance mechanisms. The multidrug efflux pump expressed by *S. maltophilia* is an important reason for its resistance to several commonly used classes of antimicrobials including beta-lactams, macrolides, and aminoglycosides (Brooke, 2012). *S. maltophilia* also has two chromosomally encoded β-lactamases, L1 and L2 and a chromosomally encoded carbapenemase, conferring resistance to nearly all the carbapenems (Brooke, 2012). Additional resistance mechanisms in *S. maltophilia* include: low outer membrane permeability, and drug resistant efflux systems (Adegoke, 2017). Other factors such as biofilm formation, quorum sensing, extracellular enzyme formation, flagella, and pili/fimbriae associated factors may contribute to organismal virulence, which may protect the organism from antibiotics or regulate host immune factors (Adegoke, 2017).

The Public Health Threat of *S. maltophilia*

The number of nosocomial *S. maltophilia* cases continues to rise (Sanchez, 2015). This may be due in part to the co-infective nature of the organism or its commensal relationship with organisms in its natural habitat, soil, a known reservoir for several antibiotic producing fungi. Additionally, *S. maltophilia* treatment can be problematic because it is intrinsically resistant to several antibiotics and cleaners that are used in hospital settings (Klausner, 1999). This can be further complicated by the fact that, *S. maltophilia* can acquire new resistance via horizontal gene transfer and genomic mutations (Lamani 2011, Brooke 2012, Sanchez 2015).

The standardized Minimum Inhibitory Concentration (MIC) breakpoint data for *S. maltophilia* are limited at most institutions due to its high levels of intrinsic resistance and reliability of empiric treatment with Trimethoprim-Sulfamethoxazole TMP-SMX (Bactrim). Although *S. maltophilia* is frequently treated successfully with TMP-SMX, resistance to this drug has been known since 2007 with the discovery of *sul1 and sul2* genes (Toleman, 2007).

Overall, there are few studies published that assess the susceptibility patterns of *S. maltophilia*, and these are typically data for *in vitro* studies, only involve a small number of isolates, and/or have not been completed for contemporary isolates (Gajdacs, 2019; Adegoke, 2019). For example, few studies have evaluated the efficacy of moxifloxacin on *S. maltophilia* (Chung, 2012). Moxifloxacin has been shown to be more active than ciprofloxacin and levofloxacin *in vitro* studies and results from previous studies suggest that moxifloxacin may be used in place of levofloxacin (Nicodemo, 2007; Chung 2012). In fact, some moxifloxacin has demonstrated the most efficacy for reducing the adherence and biofilm formation of *S. maltophilia* at suboptimal MICs (Brooke, 2012). However, hydrophobicity of the cell surface appears to be a strain-dependent phenomenon and individual strains would need to be evaluated to determine the efficacy of moxifloxacin activity against biofilm formation by this opportunistic pathogen (Brooke, 2012).

FOCUS AND ORGANIZATION OF THIS THESIS

Although antibiotic stewardship has existed for many years, there is room for improvement because the evaluation of stewardship interventions to date have chiefly focused on the successes of metrics such as the optimization of antibiotic use and cost savings (McGowan, 2012). While it is true that ASPs were introduced to improve antibiotic therapy and prevent adverse medical

outcomes like Healthcare-Associated Infections (HAIs), the results produced from studies that evaluate health outcomes are limited by the ecologic nature of their study design and uncontrolled confounding (Lesprit, 2008). Existing efforts have minimized the unintended consequences of antibiotic misuse, but these current programmatic practices continue to leave room for improvement and standardization. To improve current practices in ASPs, alternative methods must be explored, which include environmental surveillance, new diagnostics, improved treatment recommendations along with updated local cumulative antibiograms. To successfully meet these goals, environmental monitoring (a key contributing factor of the epidemiological triad) should be included in a comprehensive plan for all ASPs.

Focus of the first study in this thesis

To lower the prevalence of HAIs, supplementary evidence-based approaches are needed to improve cleaning in clinical settings. Adenosine Triphosphate (ATP) bioluminescence assays have been proposed as a rapid, inexpensive, and semi-quantitative way to monitor for microbial bioburden but have not been validated for different types of surfaces or different hospital wards.

Few studies have examined if specific surfaces carry a higher bioburden based on their surface type or shape. Chapter 2 explores the use of Adenosine Triphosphate (ATP) detection as a convenient and efficient methodology for detecting the bioburden differences between five high touch surfaces (door handle, overhead light, computer keyboard, side table and patient mattress) in operating rooms, which may contribute to the development of HAIs. This would provide, evidence for more strategic, science-based infection control/ASP practices for reducing bioburden and HAIs in clinical settings.

In Chapter 2, my collaborators and I compare the use of Adenosine Triphosphate (ATP) and microbiological based environmental monitoring in the operating room on different high touch surfaces to identify bioburden differences on a per surface basis in the clinical setting. This study is useful in ascertaining whether improvements can be made to the current practices and approaches to turn-around cleaning in the operating room or other clinical surfaces. The study addresses current challenges associated with programmatic differences and inconsistencies between individual ASPs, potential risks associated with the hospital environment, and recommended strategies for improving environmental cleaning and monitoring.

Focus of the second study in this thesis: Control of Antibiotic-Resistant Neisseria gonorrhoeae

AST algorithms for *Neisseria gonorrhoeae* are culture based and have a lengthy turn-around-time, which prevents clinicians from empirically treating patients with the appropriate antibiotics. Consequently, the recommended empirical treatment of azithromycin and ceftriaxone is used in place of a more individualized therapeutic antibiotic regimen. This empiric therapy runs the risk of increasing resistance to the macrolide and cephalosporin antibiotics. Therefore, a great need exists for the development and use of rapid AST methods for *Neisseria gonorrhoeae*, which has become multi-drug resistant in recent years.

Chapter 3 describes the validation of a Real Time-Polymerase Chain Reaction (RT-PCR) assay for detecting ciprofloxacin-susceptible *Neisseria gonorrhoeae* at three locations. This Chapter includes research data that that allows us to ascertain whether improvements can be made to the *N. gonorrhoeae* screening and treatment recommendations. The chapter also discusses the practical implications of Nucleic Acid Amplification Testing (NAAT) versus traditional culture

methods for AST by reducing Turn-Around-Time (TAT) for individual patients by using rapid molecular testing techniques for AST. Overall, the study can be utilized to assist clinical and public health professionals to understand interventional strategies that can be used to curtail the public health dilemma that may result from increasing incidence of drug-resistant *N. gonorrhoeae*.

Antibiotics, alongside vaccinations, improved hygiene and sanitation, have substantially reduced mortality from infectious disease (MMRW, 1999). Unfortunately, antibiotic use and misuse has also increased emergence of antibiotic resistant organisms, which are increasingly difficult to treat and control. Therefore, judicious use of antibiotics is an essential component of preserving their benefits.

Chapter 4 provides evidence to support the importance of enhancing antibiogram surveillance as a part of ongoing antibiotic resistance monitoring for isolates of *S. maltophilia* in the clinical setting. Cumulative antibiograms and active surveillance of trends may assist us in providing targeted treatment for individuals infected with *S. maltophilia*.

Chapter 5 provides a general summary of insights gained from the studies presented in Chapters 2-4 and recommendations for future studies. Molecular methods and combined antibiograms, discussed in Chapters 4 and 5, can improve ASPs by reducing turn-around-time on AST results and improving the accuracy of AST data available to clinicians over larger periods of time. Overall, there is a need for more research and development into approaches for comprehensive and rapid AST. The development of new testing strategies and their subsequent evaluation can provide a more targeted approach to treating individual patients and potentially improve public health outcomes of the future by slowing the tide of antimicrobial resistance.

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CHAPTER 2

How to better monitor and clean irregular surfaces in operating rooms: Insights gained by using both ATP luminescence and RODAC assays

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ABSTRACT

Background: A major limitation to developing evidence-based approaches to infection prevention is the paucity of quantitative, real-time methods for monitoring cleanliness of environmental surfaces in clinical settings. One solution that has been proposed is Adenosine Triphosphate (ATP) bioluminescence monitoring, but additional studies are needed that demonstrate the effectiveness of this approach for assessing the cleanliness of different types of surfaces that are frequently found in clinical settings.

Materials/Methods: An ATP bioluminescence assay to assess cleaning of five different types of high-touch surfaces (overhead lights, door handles, anesthesia keyboards, mattresses, and side tables) in 24 operating rooms (ORs). ATP results obtained after cleaning were compared to results obtained after cleaning using Replicate Organism Detection and Counting (RODAC) plates.

Results: Cleaning was found to be more effective for flat, covered surfaces (mattresses and side tables) than for irregularly-shaped surfaces (overhead lights, door handles, and anesthesia keyboards). Irregularly shaped surfaces were more likely to pass by RODAC testing than ATP bioluminescence after cleaning.

Conclusion: Systematic use of ATP bioluminescence monitoring for assessing cleaning efficacy of high-touch surfaces in an OR suggests that irregularly shaped surfaces may require enhanced covering, cleaning and monitoring protocols.

BACKGROUND

Cleaning and disinfection of environmental surfaces and patient care equipment are essential components of infection prevention in the healthcare setting (Rutala, 2008). The environment serves as a reservoir for healthcare-associated infections (HAIs) (CDPH, 2016). Furthermore, well-documented evidence links the transmission of pathogens to contaminated hospital surfaces (Rutala, 2013; Allen, 2014). Therefore, frequent and effective disinfection of "high-touch surfaces" is a critical step for protecting patient safety (Hughes, 2008).

Evidence-based recommendations for cleaning, disinfection and sterilization in hospitals are published by the Healthcare Infection Control Practices Advisory Committee (HICPAC) at the Centers for Disease Control and Prevention (CDC) (Rutala, 2008). Disinfection, as defined by HICPAC, is the process by which many or all pathogenic microorganisms, other than spores, are eliminated from inanimate objects (Rutala, 2008). This process begins with appropriate environmental cleaning and removal of organic or inorganic debris from surfaces with the use of an Environmental Protection Agency (EPA)-registered hospital grade disinfectant (Rutala, 2008). Hospital operating rooms (ORs) have policies in place that require environmental services staff and perioperative staff to follow cleaning guidelines established by HICPAC and the Association of peri-Operative Registered Nurses (AORN) in an effort to reduce HAIs (Rutala, 2008; Allen, 2014), ORs must be disinfected between each procedure, and every OR should be terminally disinfected at the end of each day (Allen 2014).

In spite of these disinfection practices, approximately one out of every twenty-five hospitalized patients develop a HAI (CDC HAI, 2016). Among these, surgical site infections (SSIs) account for 31%, of all HAIs, and are associated with the greatest additional healthcare cost, estimated at $2.5-$10 billion annually in the U.S. (CDPH, 2016; CDC HAI, 2016; Magill et al., 2012). ORs contain a variety of high touch surfaces that can serve as fomite reservoirs of microorganisms and pathogens. Inadequate cleaning of these environmental surfaces likely contributes to SSIs (CDPH, 2016; Allen, 2014; Spagnolo, 2013). Therefore, methodologies are needed that would allow for routine environmental monitoring for microbial contamination and potential pathogens present on a broad range of different surfaces types in an OR setting.

Previous studies have shown that Adenosine Triphosphate (ATP) bioluminescence monitoring has the potential to provide rapid quantitative measures of effective cleaning in hospitals (Lewis 2008, Sciortino and Giles, 2012). Environmental ATP monitoring offers an advantage over microbiologic methods, which use an array of swabs and sponges accompanied by various broths and agars, which are time and labor-intensive (Powitz, 2016). Although ATP is an excellent biomarker, the ATP detection can result from viable organisms or non-viable cellular material and does not differentiate between pathogenic and non-pathogenic organisms (Powitz, 2016). As a result, the magnitude of the signal obtained from this technique is not a direct measure of pathogenicity or patient risk (Powitz, 2016). Still, ATP monitoring is regularly performed in a number of industries including aerospace, food and beverage, ecology, cosmetics and clinical (Powitz, 2016). The food safety industry and the planetary protection division of the National Aeronautics and Space Administration (NASA) have reported that the ATP assay is the best real-time option for monitoring cleanliness that is currently available (Powitz, 2016; NAS, 2006).

However, there are still several challenges associated with use of ATP monitoring for cleanliness in clinical settings such as operating rooms. For instance, individual surfaces in operating rooms have different likelihoods of becoming contaminated. Additionally, different amounts or types of contamination on individual surfaces may pose various risks to patients, which is dependent on the surgical procedure, organismal viability, contamination level and the patient's overall health status (Kramer, 2006). Furthermore, the patient risks are dependent upon exposure to particular surfaces and likely not random as contamination is rarely distributed evenly over individual surfaces (Whiteley, 2016). Due to the non-random distribution of contamination, there is a low level of precision between ATP measurements and there is a large amount of variation in detection limits between sampling devices (Whiteley, 2016). Furthermore, a universally accepted threshold has not been established for ATP bioluminescence monitoring and there are a number of suggested maximum Relative Light Units (RLU) measurements amongst the different ATP sampling devices on the market (Whiteley, 2016; 3M, 2010; Dancer, 2004; Boyce, 2001; Mulvey, 2011; Price, 2017) Thus, continued development of procedures that are inexpensive, real-time, and evidence-based quantitative measures of cleanliness in ORs is still needed. We wanted to know if we could apply ATP bioluminescence testing as a proxy for monitoring effective cleaning of different surface types in the OR.

MATERIALS AND METHODS

We compared the results obtained from real-time ATP monitoring of 5 different types of high-touch surfaces in an operating room after cleaning to those before cleaning. The surfaces were also assessed after cleaning using a traditional quantitative microbiologic method (Replicate Organism Detection and Counting or "RODAC" plates) in addition to the ATP assay. Matrix-

Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry was used to identify organisms isolated from the RODAC plates. Air samples were collected on RODAC plates (inside an impactor) in parallel with RODAC surface samples. We compared results for regularly shaped (flat), covered surfaces (mattresses and side tables) and for irregularly shaped, uncovered surfaces (overhead lights, door handles, and anesthesia keyboards) in order to determine how surface types affected the utility of ATP bioluminescence testing.

Sampling Strategy

Samples were collected over 5 consecutive weeks from 24 ORs in a 520-bed teaching hospital. ATP and bacterial load (RODAC) sampling were performed on five different types of high-touch OR surfaces (door handles, overhead lights, anesthesia keyboards, side tables, and patient mattresses) in three different types of ORs (cardiac surgery, neurosurgery, general OR). The surface types were specifically selected to represent both flat, covered surfaces (mattresses and side tables) and uncovered, irregularly-shaped surfaces (overhead lights, door handles, and anesthesia keyboards). Hospital environmental services staff performed regular turnaround cleaning alongside peri-operative anesthesia staff according to existing hospital protocols. Turnaround cleaning was always performed between patients, utilizing either quaternary ammonia with microfiber cloths and/or disposable bleach disinfectant wipes. The disinfectant was left to dry on each surface before sampling. Surfaces were swabbed for ATP before and after cleaning. We split each test surface into its respective quadrants and all samples were collected from the same quadrant on each surface before and after cleaning. RODAC air and surface samples were also collected after turnaround cleaning.

Surface Sampling

ORs were sampled for viable bacterial contamination within 20 minutes of the completion of cleaning procedures. RODAC plates were used to culture bacteria from surfaces and contained five neutralizers, (sodium bisulfate, sodium thioglycollate, sodium thiosulfate, lecithin, and polysorbate 80) which inactivate disinfectant agents. To sample, the lid of a RODAC plate was removed and the plate was gently pressed the agar surface to the test surface for ten seconds. In cases where the surface was curved, the plate was gently rolled such that the entire agar surface met the test surface over a 10-second period. The lids of the RODAC plates were snapped back into place, inverted and incubated at 35°C ± 2°C for 48 ± 4 hours. Visible colonies were enumerated, and the number of Colony Forming Units (CFU)/plate was recorded. Cleaning compliance was assessed using previously established cutoff benchmarks of 64 CFU/plate: pass ($\leq$2.5 CFU/cm^2) and fail (> 2.5 CFU/cm^2) (Dancer, 2004; Boyce, 2001; Griffith, 2000; Mulvey, 2011). Three colonies from each plate were subcultured to individual Blood Agar Plates (BAPs) following in-house standard operating procedures and identified via Biomerieux VITEK-2 MALDI-TOF mass spectrometry.

Air Sampling

Air samples were collected on the floor near the door opening to the outside hallway of each OR after cleaning using a MicroBio MB2 portable impactor (flow rate = 100 L/min) loaded with a RODAC plate. Air samples were collected for a total of 5 minutes or 0.5 L^3 volume. Three colonies from each air plate were subcultured to BAP and identified, per in-house standard operating procedures, via Biomerieux VITEK-2 MALDI-TOF mass spectrometry.

ATP sampling

OR surfaces were swabbed for ATP both before and after turnaround cleaning with the 3M™ CleanTrace™ ATP swabs and RLU was measured using a 3M™ luminometer. All high-touch surfaces were sampled by swabbing a ~25 cm^2 area, similar to that of a RODAC plate. RLUs were obtained according to the manufacturer's specifications (3M, 2010). Based on previously published studies, the RLU benchmarks used to assess cleaning compliance were: Pass ≤ 250 RLU, Fail > 250 RLU (Lewis, 2008; Kramer, 2006). The percentage of samples that passed or failed for each type of surface is summarized in Table 2.1.

Table 2.1. Percentage of unmatched surfaces that passed or failed before and after cleaning as determined by ATP assay revealed that most covered, regularly shaped surfaces passed before and after cleaning, whereas most uncovered, irregularly shaped surfaces failed before and after cleaning.

	Pre-Cleaning					Post-Cleaning			
	Pass ≤250		Fail >250			Pass ≤250		Fail >250	
	N	%	N	%		N	%	N	%
OR Lights	5	21	19	79		10	42	14	58
Anesthesia Keyboard	2	8	22	92		11	46	13	54
Door Handle	4	17	20	83		8	33	16	67
OR Table Mattress	20	83	4	17		20	83	4	17
Back/Side Supply Table	21	91	2	9		23	96	1	4

MALDI-TOF Mass Spectrometry

Samples were prepared by sub culturing individual colonies from the RODAC plates to Blood Agar Plates (BAPs, from Hardy Diagnostics). The BAPs were incubated for 24 hours at 35°C in

order to obtain a pure culture. A single colony from this pure culture was picked from the BAP and prepared on the target plate by adding one loopful of the bacterial culture (using a 0.1µL loop) to a single well on the MALDI-TOF plate. 1µL of α-Cyano-4-hydroxycinnamic acid was immediately pipetted on to each well and dried at ambient temperature. This was repeated for the control *E. coli* bacteria in the central well of the plate. Once all of the samples had dried, the slide was then barcode scanned into the Biomerieux VITEK and processed for species identification by MALDI-TOF mass spectrometry using VITEK analysis software.

Data Analysis

All statistical analyses were performed using Microsoft Excel or SAS version 9.2. To assess whether there was a correlation between pre- and post-turnaround measurements, post-turnaround viable colony counts (CFU) were compared to ATP swab (RLU) measurements. The RLU and CFU results for cleaned surfaces were converted to log RLU and log CFU, and the geometric means of the log RLU and log CFU for each type of surface were calculated. Measurements of 0 RLU or CFU were given values of 1 to provide a log value of 0. The geometric means of the log RLUs for each type of high-touch surface were compared (Figure 2.1). Values were analyzed by surface type for log RLU before and after turnaround cleaning. Log CFU values were also summarized after turnaround cleaning in addition to log RLU reduction. Summary tables were also produced by surface type for RLU pass/fail based on a threshold value of 250 RLU and for CFU pass/fail based on a threshold value of 2.5 CFU/cm^2 (i.e., 64 CFU per RODAC plate) (Dancer, 2004; Boyce, 2001; Griffith, 2000; Mulvey, 2011). Log (RLU) values were compared for pre- and post- turnaround cleaning (Figure 2.2) and log (RLU) values were compared to log (CFU) values (Table 2.2). To compare ATP levels before and after cleaning, RLU values were converted to log RLU.

A mixed model ANOVA, with room as a random effect, was used to compare touch points for log RLU before and after cleaning and log RLU reduction (data not shown). A mixed model ANOVA, using ranked CFU values, with room as a random effect, was also used to compare touch points for log CFU after cleaning (data not shown). A t-test was used to compare covered and not covered anesthesia keyboards for log RLU before and after cleaning (data not shown), and a paired pass/fail RLU and CFU was tested using McNemar's Test (data not shown). A signed rank test of the paired differences was used to determine whether log RLU reductions were statistically different from 0.

RESULTS

The majority (92%, 22/24) of rooms had least one surface that exceeded the 250 RLU threshold after turnaround cleaning via the ATP assay. Additionally, 42% (10/24) of the rooms had at least on surface that didn't pass as clean after turnaround cleaning via the RODAC surface sample test.

Comparison of surfaces before and after turnaround cleaning

In general, whether surfaces tested cleaner after turnaround than they did before turnaround depended on the surface type. The percentages of each surface that were "clean" ($\leq$ 250 RLU) or "dirty" (>250 RLU) before and after turnaround cleaning by the ATP assay are shown in Table 2.1. Figure 2.1 shows a comparison of the geometric means of RLU pre- and post-turnaround for each surface. Statistically there are differences between the 5 surfaces types ($p < 0.0001$ Mixed Model ANOVA).

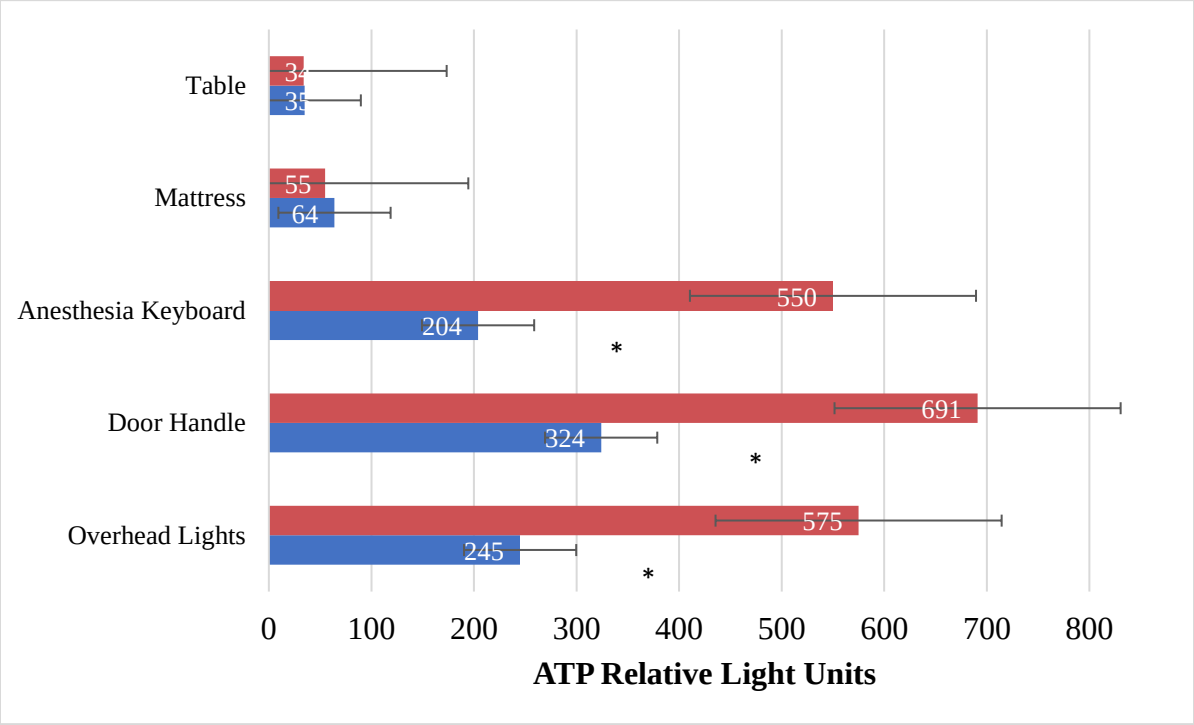

Figure 2.1. Comparison of Geometric Mean from ATP assay (geometric mean of RLU) pre- (red) and post- (blue) cleaning for each surface studied. Log RLU reductions were statistically different (*) from 0 for the overhead lights ($p = 0.017$), anesthesia keyboards ($p < 0.0001$) and door handles ($p = 0.017$), but not for the OR table mattress ($p = 0.68$) or the side supply table ($p = 0.35$).

Table 2.2. Concordance analysis for Pass or Fail results from ATP assay and RODAC assay on samples taken from different surfaces after cleaning. Samples were considered fail by ATP assay if > 250 RLU and pass if ≤ 250 RLU (Lewis, 2008). Samples were considered to have failed by the RODAC assay if > 2.5 CFU/cm^2 and passed if ≤ 2.5 CFU/cm^2 (3M, 2010).

		Results from RODAC Assay (CFU)									
		Overhead Lights		Anesthesia Keyboard		Door Handle		Mattress		Table	
		Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail	Pass	Fail
Results from ATP Assay (RLU)	Pass	9	1	9	2	7	1	20	0	23	0
	Fail	13	1	9	4	15	1	4	0	1	0
	Total	22	2	18	6	22	2	24	0	24	0

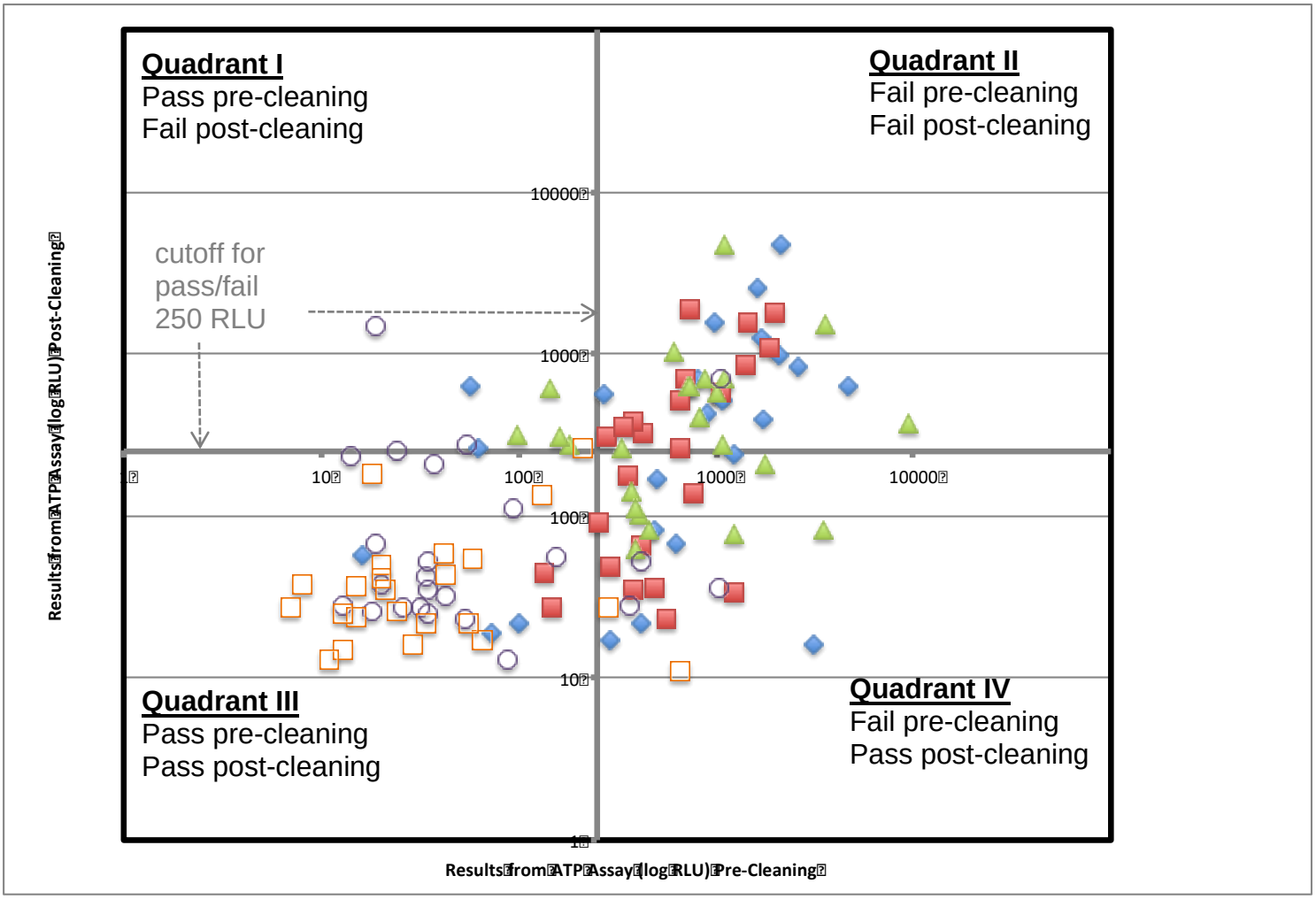

Figure 2.2. Comparison of Pass/Fail results from ATP Assay (Log (RLU)) before and after cleaning = samples from overhead lights; = Anesthesia keyboard; = Door Handle (irregularly shaped, uncovered surfaces); = Mattress; = Side Table (regularly shaped, covered surfaces). Data for cases where CFU = 0 are not shown. Cutoff value is used as origin (250, 250).

The surfaces fall into two different groups: those surfaces for which RLU started low and remained low and those surfaces for which the RLU decreased after cleaning but still typically remained above the cleanliness threshold (> 250 RLU). The surfaces for which RLU started low and remained low were flat/covered surfaces, namely the OR mattress and OR side table. By contrast, those surfaces for which the RLU decreased after cleaning were irregular/uncovered surfaces, namely the OR lights, anesthesia keyboard, and door handle. The log RLU reductions were statistically different from zero for the overhead lights ($p = 0.017$), anesthesia keyboards ($p < 0.0001$) and door handles ($p = 0.017$), but not for the OR table mattress ($p = 0.68$) or the side supply table ($p = 0.35$).

Comparison of results obtained with ATP assay to those obtained using RODAC plates

In addition, whether or not the results obtained from the ATP RLU measurements post cleaning correlated well with those obtained using standard RODAC plates depended on the surface type. We used standard cutoffs for "failed": >250 RLU in the ATP assay and > 2.5 CFU/cm^2 per surface RODAC plates (Dancer, 2004; Boyce, 2001; Griffith, 2000; Mulvey, 2011). Concordant results were observed for only 63% (75 of the 120) of the surfaces sampled. Of the 75 surfaces that were concordant between the two assays for cleanliness, 91% (68 out of 75) surfaces "passed" by both assays and only 9% (7 out of 75) "failed" by both assays. The results of the concordance analysis between the two assays for each surface type differed and are shown in Table 2.2. All of the concordantly "dirty" sites were on irregular/uncovered surfaces (1 out of 7, or 14%, for overhead lights and door handles and 5 out of 7, or 71%, in the case of the anesthesia keyboards). Statistically, the surfaces fall into two different groups: those surfaces that tended to be positive by ATP and negative by RODAC and those that were concordant between the two assays. These statistical groupings were the same as those obtained from the analysis of the ATP

assay results pre-/post-cleaning: those surfaces that were clean both before and after turnaround (the regularly shaped and covered surfaces; i.e., the patient mattress and the supply tables) also tended to show concordant results between the RODAC and ATP assays and those surfaces that were dirty both before and after turnaround (the irregularly shaped and covered surfaces; i.e., the overhead lights, anesthesia keyboards and door handles) tended to show discordant results between the RODAC and ATP assays (Table 2.2).

Comparison of results obtained from air sampling after turnaround cleaning

Active air sampling indicated a mean Total Viable Count (TVC) of 10.3 CFU/m^3 after cleaning (Range = 0-88). A threshold 10 CFU/m^3 was used to determine whether the air quality passed (≤ 10 CFU/m^3) or failed (> 10 CFU/m^3) (Knechtges, 2011). RODAC plates were utilized to identify whether there was a high level of airborne contamination in each room after cleaning. A total of 17% (4/24) of the rooms had air sample measurements that exceeded the 10 CFU/m^3 threshold. Of the 4 rooms where the air samples exceeded the threshold after cleaning, 75% (3/4) had at least one surface that exceeded 250 RLUs after turnaround cleaning and 50% (2/4) had at least one surface that exceeded 2.5 CFU/cm^2.

MALDI-TOF Analysis

MALDI-TOF was used to identify species of bacteria that were cultured from the high-touch surfaces and air using RODAC plates. Thirty bacterial species were identified at > 99% identity. All of the bacterial species identified have an environmental origin or are considered normal flora but have the ability to infect immunocompromised individuals. Interestingly, for a given room, the bacterial species identified from air samples and RODAC surface sampling were highly correlated. The most commonly identified organisms were normal flora *Staphylococcus*

and *Streptococcus* species. A Streptococcal colony isolated from an anesthesia keyboard was identified as group B (*Streptococcus agalactiae).*

DISCUSSION

To determine how the utility of ATP luminescence assays depends on the type of surface being sampled, we conducted studies looking at how concordance of ATP data with RODAC surface sampling varies with specific surface types in a hospital setting. Whereas ATP results for regularly shaped, covered surfaces (e.g., mattresses and side tables) were highly concordant with RODAC results, ATP results for irregularly shaped surfaces do not correlate well with those obtained using RODAC plates. Particularly notable was that a significant percentage (38%, 27/72) of the irregularly shaped surfaces that passed according to the ATP test failed according to the RODAC assay.

There are at least two possible reasons why greater discordance was observed between the ATP luminescence assay and the RODAC assay in the case of irregularly-shaped surfaces. One possibility is that the ATP assay yields a false positive result due to the presence of nonviable cellular ATP that is released from biological debris upon disinfection (Price, 2017). However, it is also possible that the ATP assay (which uses a swab to collect the sample) is just better suited to sampling irregular surfaces than RODAC plates due to the irregular distribution of contaminates on a surface (Kramer, 2006). ATP swab samples are likely more sensitive for irregular/uncovered services due to their ability to reach more difficult to test nooks and crannies (i.e., between computer keys and around the surface of a round overhead light handle). Prior studies have demonstrated that swab *type* effects recovery of environmental specimens, with cotton swabs holding more volume (132 μL) than rayon swabs (63 μL) (Miller, 2017). By

contrast, irregular/uncovered surfaces (door handle, anesthesia keyboard, and overhead lights) are difficult to sample with RODAC plates (Knechtges, 2011).

Implications for Infection Control and Clinical Practice

The irregular shape and increased surface area of these surfaces may make them especially prone to higher levels of contamination. Data for anesthesia keyboards with and without reusable covers suggest that putting a cover on an irregularly-shaped surface does not make it easier to clean if the cover is not discarded between uses (data not shown). These results suggest that frequently-used, irregularly-shaped surfaces could benefit from either regular, in depth, cleaning and monitoring and/or being covered with a single-use disposable cover. This is particularly true for irregularly shaped surfaces that are aged or worn. Just as clinicians don personal protective equipment during surgical procedures (e.g., gloves, eye protection, surgical masks, scrubs, hair and shoe covers), protective plastic barriers on surfaces may add an extra protective barrier to transfer of infectious agents between the clinician, surfaces and the patient (Miller, 1997; Dental Econ 2000). Temporarily covering high-touch irregular surfaces that are exposed during surgical procedures with removable plastics or adhesives and replacing these covers between patients/as part of turnaround cleaning could significantly reduce infection potential (Miller, 1997; Dental Econ 2000). Implementing additional barrier precautions should not reduce the amount of cleaning that is already taking place but should serve as an additional means of protection against the transmission of HAIs.

Limitations

A primary limitation of the study resulted from time constraints that are inherent to sampling in a clinical setting while that space is being used for treating patients. As is the case in almost every

hospital, the surgical suites were in high demand and high turnover rates are required. As a result, there is limited time available for sampling between surgeries. To ensure rigor in sampling methodologies, samples were collected by a trained Public Health Microbiologist, not by the individuals actually responsible for cleaning the rooms. This, combined with the highly variable and extended length of the surgical procedure, limited our ability to sample many rooms at different times. This limitation in turn affected our ability to achieve greater statistical power.

Suggestions for Future Studies

Studies that involve sampling of more rooms and sampling at different study sites could help to validate the results reported herein. Longer-term studies could offer a more detailed picture of clinical outcomes as they relate to the environment or particular procedures and cleaning practices on particular surfaces.

Additional studies are needed to assess whether the quantitative standards that have been developed for ATP assays in other settings are relevant to clinical environments. Quantitative ATP bioluminescence testing has long been used in the comprehensive assessment of cleanliness in the food industry using Hazard Analysis Critical Control Points (HACCP) (Powitz, 2016). However, it is not clear whether the thresholds established for the food industry are the correct ones to use in clinical settings. It is also possible that different thresholds should be used for different surfaces in clinical settings, based on characteristics such as surface shape, surface area, and touch frequency. The chosen threshold(s) should be indicative of risk to the patient for developing an infection based on their specific exposure to surfaces with high potential for contamination, especially after cleaning takes place. Therefore, additional studies and

measurement adjustments are needed in order to set threshold values on a surface-to-surface basis (Yu-Huai, 2016).

In addition, more studies are needed that explore the connection between ATP luminescence results and risk of infection. While ATP bioluminescence offer a rapid and more objective way of assessing cleanliness in the operating room over visual inspection, this method does not necessarily provide a measure of risk of infection, because it measures total bioburden and does not distinguish between pathogenic and nonpathogenic bacteria, or even live versus dead cells (Dancer, 2004; Boyce, 2001; Griffith, 2000; Mulvey, 2011). Here, we used MALDI-TOF to explore qualitatively whether pathogenic bacteria were present in our samples. These data suggest that only a small percentage of the bacteria we cultured from the operating rooms in this study were pathogenic. However, more detailed studies (e.g., using deep sequencing) could be used to more systematically explore how results from ATP bioluminescence assays in clinical settings correlates with risk of infection. Further research utilizing different methods alongside this technology in combination with hospital epidemiology reports and whole genome sequencing data may have shed light on the existing contamination. These methods may offer more advanced ways to analyze patient risks for infection or more insight about the hospital microbiome in the various wards of the hospital (Price, 2017). This type of study could also be used to set thresholds based on risk potential.

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CHAPTER 3

A Multi-Site Implementation of a Real-Time PCR Assay to predict ciprofloxacin susceptibility in *Neisseria gonorrhoeae*

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ABSTRACT

There are no commercially available Food and Drug Administration-cleared rapid tests for *Neisseria gonorrhoeae* antimicrobial susceptibility testing. This study evaluated the performance of a laboratory developed real-time polymerase chain reaction assay for genotyping the *gyrA* gene to determine antimicrobial susceptibility to ciprofloxacin. Validation and clinical performance of the *gyrA* assay was evaluated across three geographic locations (Los Angeles, San Francisco, Philadelphia). Following validation, clinical specimens were collected in Aptima Combo2® CT/NG transport medium from asymptomatic persons, who tested positive for *Neisseria gonorrhoeae* and evaluated for assay percent reportable (i.e., proportion of *N. gonorrhoeae*-positive specimens that yielded a *gyrA* genotype). The percentage of *gyrA* genotyping results differed by laboratory and specimen type. The proportion of specimens that were reportable was best for urine/genital specimens (genotyped= 76.4%; (95% confidence interval, 69.9%-82%)) followed by rectal (genotyped= 67.2% (95% confidence interval, 63.4%-70.6%)) and then pharyngeal specimens (genotyped= 36.1%, (95% confidence interval, 31.9%-40.5%)). Overall, asymptomatic patients with *N. gonorrhoeae* yielded an interpretable genotype 57.2% (784/1370) of the time, of which 480 were wildtype *gyrA*, resulting in 61% (480/784) being potentially treatable with ciprofloxacin.

INTRODUCTION

Gonococcal infections continue to pose a significant risk to global public health, resulting in an estimated 78 million cases per year (Newman et al., 2015). The Centers for Disease Control and Prevention (CDC) recognizes infection with multi-drug resistant *Neisseria gonorrhoeae* as one of the three most urgent antimicrobial resistance threats in the U.S. (CDC, 2013). Gonococcal infections are the second most commonly reported notifiable sexually transmitted infection (STI) in the U.S., second only to *Chlamydia trachomatis* (CDC, 2015). Genome plasticity and acquired antimicrobial resistance, combined with the absence of antimicrobial susceptibility tests that provide results in a clinically relevant timeframe for treatment of gonococcal infections, make antimicrobial resistance in this organism especially difficult to control (Buono, et al., 2015).

In an effort to reduce the spread of antimicrobial resistant gonococci, the World Health Organization (WHO) and the CDC regularly update the guidelines for the treatment of gonococcal infections. The most up-to-date guidance recommends dual therapy consisting of a single dose of 250 mg of intramuscular ceftriaxone and 1 g of oral azithromycin (Workowski, 2015; Bolan, 2012). The first reported *N. gonorrhoeae* case with dual therapy failure in the United Kingdom occurred in 2016 (Fifer et al., 2016). *N. gonorrhoeae* with decreased susceptibility to ceftriaxone and/or azithromycin is most prevalent in Europe and Western Asia; however, cases have been reported in the United States and Canada, as well as some countries in South America, South Africa, and Australia (Wi et al., 2017). Fluoroquinolones are well-tolerated oral antimicrobials used historically for the successful treatment of gonococcal infections. The CDC eliminated that drug class as an empirical treatment choice for *N. gonorrhoeae* infections in 2007, due to the spread of fluoroquinolone-resistant *N. gonorrhoeae*

(CDC, 2007). The most recent U.S. data indicate 19.2% of isolates of *N. gonorrhoeae* are resistant to ciprofloxacin, nationwide (Kircaldy et al., 2016). As such, the majority of gonococcal infections could theoretically be treated with oral ciprofloxacin, if the clinician knew the organism's ciprofloxacin susceptibility at the time of diagnosis.

Resistance to ciprofloxacin is the result of a highly conserved mutation in the serine 91 codon of the gyrase A (*gyrA*) gene (Tanaka, et al., 1996). Multiple studies have previously demonstrated that targeting codon 91 in *gyrA* is a highly sensitive and specific method for rapid detection of ciprofloxacin susceptibility, directly from clinical specimens positive for *N. gonorrhoeae* (Tanaka, et al., 1996; Siedner, et al., 2007; Hemarajata, et al., 2016; Allan-Blitz, et al., 2017). Herein, we describe the implementation and analytical performance of this *gyrA* genotyping assay at multiple clinical and public health laboratories. This assay has been utilized as part of a larger clinical trial (NCT02961751) evaluating the efficacy of treatment using ciprofloxacin for patients infected with strains of *N. gonorrhoeae* with a wild-type (WT) *gyrA* genotype, which are predicted to be ciprofloxacin susceptible (Siedner, et al., 2007; Allan-Blitz, et al., 2017; Hemarajata, et al., 2016).

METHODS

Overview of the study

Three testing facilities participated in this study: UCLA Clinical Microbiology Laboratory (UCLA), San Francisco Public Health Laboratory (SFPHL), and the Philadelphia Public Health Laboratory (PPHL). UCLA served as the study coordinator. UCLA generated seeded samples for the assay validation and prepared and pre-tested lots of primers, probes, and DNA controls for the testing facilities. The study was conducted in three phases: Phase 1. Consisted of validation

of the *gyrA* assay across the testing facilities; Phase 2. Consisted of clinical testing of asymptomatic patients at the facilities and Phase 3 consisted of troubleshooting experiments performed at UCLA to attempt to determine the root cause of indeterminate results.

Assay description

For all 3 phases, the *gyrA* genotype assay was used, as has been described elsewhere (Seidner, et al., 2007; Hemarajata, et al., 2016). This assay was performed using specimens that were previously determined to be *N. gonorrhoeae* positive, by commercially available nucleic acid amplification testing (NAAT), and were reflexed for *gyrA* genotyping to determine ciprofloxacin susceptibility. The following modifications were performed in this study design. At UCLA, DNA extraction was performed using a MagNA Pure 2.0 (Roche Diagnostics, Indianapolis, IN) and the MagNA Pure DNA large-volume kit. The other two facilities (SFPHL and PPHL) performed DNA extraction on a QIAcube (Qiagen, Valencia, CA), using the Qiagen QIAmp DNA Mini DNA extraction kit. In either case, 200 µL of specimen was extracted and 100 µL DNA eluted. PCR (*gyrA* genotype assay) was performed using 5 µL DNA template and 15 µL FastStart® DNA Master HybProbe mix (Hemarajata, et al., 2016) using a Roche LightCycler 480 (UCLA and PPHL) or a Roche LightCycler 2.0 (SFPHL).

Positive controls were made by extracting 200 µL of a 3.0 McFarland suspension of a MT (FQ4) and WT (FQ1) isolate on a MagNA Pure and eluting 100 µL template DNA. The negative control consisted of *Neisseria meningitidis* DNA. This species is known to cross-react with the *gyrA* primers, but not probes. A collection of other non-*N. gonorrhoeae* isolates (*Neisseria meningitidis, Neisseria sicca, Neisseria subflava, Neisseria mucosa, Neisseria cinerea, and*

Neisseria elongata) were previously evaluated and determined to not cross-react with the assay (Hemarajata, et al., 2016).

Data from all facilities were analyzed using the Meltcurve Genotyping Module in the Multi Color HybProbe Detection Format of the Lightcycler software (Hemarajata, et al., 2016). The melt temperature corresponded to the value obtained at the peak of curves, which is generated after taking the negative value of the first derivative of fluorescence generated per unit time according to Seidner et al., 2007. Samples with melt temperatures of 56°C ± 1.5°C were designated as mutant (MT) and samples with melt temperatures of 66°C ± 1.5°C were designated as wild-type (WT).

Phase I: Validation of gyrA genotype assay

Validation specimens

Accuracy, precision and limit of detection were evaluated at all three facilities using panels of contrived specimens, which were prepared at UCLA and shipped to the participating facilities. SFPHL and PPHL were blinded to the expected results for each panel. Contrived specimens were generated by first pooling *N. gonorrhoeae* negative, de-identified Aptima Combo2® CT/NG NAAT remnants by specimen type (urine, rectal, pharyngeal and genital). Twenty-two different isolates of *N. gonorrhoeae* plated for isolation on Chocolate Agar plates (Remel, Lenexa, KS) and incubated for 24 hours at 37°C in a 5% CO_2 environment. A suspension equivalent to a 3.0 McFarland was made from the colony growth in 0.85% saline (Hardy Diagnostics, Santa Ana, CA). Next, 4.5 mL aliquots of the pooled specimens were seeded with 500 µL of the individual bacterial suspensions. Ten-fold serial dilutions were prepared in pooled sample remnants to achieve expected concentrations of 10^2-10^5 CFU/ml *N. gonorrhoeae*, for

limit of detection studies. The accuracy was defined as the percent of seeded samples in the accuracy panel that were correctly identified as WT vs MT by the *gyrA* assay, based on knowledge of the genotype (at UCLA) for the isolate that was seeded into the remnant samples.

Limit of Detection Studies

The limit of detection was defined for each specimen type (urine, rectal and genital) as the lowest concentration of *N. gonorrhoeae* with a ≥ 90% detection rate for *gyrA*, which yielded an interpretable genotype based on melt curve analysis, across 10 replicates. The limit of detection (LOD) was pre-determined this way at UCLA by testing a broad range of concentrations of MT and WT *N. gonorrhoeae* of each specimen type. The UCLA-defined limit of detection was then confirmed at each facility by testing three of each specimen type seeded with MT or WT *N. gonorrhoeae* at the UCLA-determined limit of detection, 1.0 log above the limit of detection, or 2.0 log above the limit of detection for a total of 18 specimens. Because each participating laboratory used different equipment, the limit of detection was not assumed to be the same across the different facilities and was reported as the lowest concentration at which 90% of samples were correctly genotyped for each specimen type at each participating laboratory.

Accuracy studies

Accuracy panels were prepared to range from 1- 4 log above LOD. A total of 125 contrived specimens were included in the accuracy panel for each facility, and included all 22 isolates of *N. gonorrhoeae*, which were randomly selected by UCLA. Each of the secondary laboratories (PPHL and SFPHL) was required to produce 95% concordant results, to those obtained at the primary facility (UCLA), from the accuracy panel in order to satisfy the validation criteria.

Precision testing

The precision panel consisted of 15 contrived specimens seeded with a *gyrA* WT isolate in pooled specimen remnants (n = 5 each urine, rectal and genital) and 15 contrived specimens seeded with a *gyrA* MT isolate in pooled specimen remnants (n = 5 each urine, rectal and genital). *N. gonorrhoeae* was seeded at the LOD, 1 log above the LOD or 2 log above the LOD. For intra-assay precision, each laboratory tested each specimen three times within a single run. For inter-assay precision, each laboratory tested the panel of specimens across three days. Results for the precision testing were considered satisfactory if all replicates on each day tested resulted in proper identification of mutant (MT) or wild-type (WT) via melt-curve analysis.

Phase II: Clinical testing

Clinical samples were collected as part of a larger clinical trial to determine the efficacy of ciprofloxacin for the treatment of asymptomatic *N. gonorrhoeae* infections with *gyrA* serine 91 genotype (NCT02961751). The study had several inclusion and exclusion criteria including but not limited to: informed consent, age ≥ 18 years, and infection with a *gyrA* serine 91 wild-type (WT) genotype *N. gonorrhoeae* at ≥ 1 body site that is non-pharyngeal. Specimens were run at each laboratory using the validated assays to determine *gyrA* genotype.

Phase III: Evaluation of indeterminate specimens

Some of the clinical specimens did not yield a genotype. Modifiable factors that could contribute to indeterminate results were further evaluated at one laboratory (UCLA). Total DNA added to the *gyrA* reaction was evaluated by maximizing the volume of specimen extracted (400 µL vs. 200 µL used in original protocol). The same 100 µL volume of product was eluted during this larger volume extraction. For those specimens, the volume of DNA added to each RT-PCR reaction was

also increased (20 µL in a 100 µL RT-PCR reaction vs. 5 µL in a 20 µL RT-PCR reaction). Sixty-five specimens (19 pharyngeal, 21 rectal, and 25 urine/genital) that initially yielded an indeterminate result were re-evaluated using these modifications.

The potential for PCR inhibition from the Aptima Combo2® CT/NG assay specimen transport medium was also evaluated by pelleting cellular material from 40 of these 65 Aptima specimens (n = 16 rectal, n = 15 pharyngeal, and n = 9 urogenital) at 10,000 x g for 10 minutes. Pellets were then resuspended in 200 µL PCR grade water and subsequently extracted on the MagNA Pure (Roche) in parallel with 200 µL of the original specimen. These specimens were then retested using the original 20 µL *gyrA* genotyping assay.

Statistical analysis

Percent Reportable

Prevalence calculations and confidence intervals for percent reportable genotype were calculated using the "Clinical Research Calculator 1" module at VasserStats.net.

$$\%\ \text{Reportable} = \left\{ \frac{[\text{True positives}(\text{NAAT+ and } gyrA+)]}{[\text{True positives}(\text{NAAT+ and } gyrA+)]+[\text{False negatives}(\text{NAAT+ and } gyr\text{A-})]} \right\}$$

Chi-square and Fisher's exact testing

Chi-square and Fisher's exact testing was used to evaluate the proportional differences for indeterminate results between the categorical variables laboratory (UCLA, SFPHL, and PPHL) and specimen type (urine, rectal, and pharyngeal) (socscistatistics.com).

RESULTS

Results from validation of gyrA assay

Accuracy Panel

All three laboratories successfully genotyped all 125 seeded clinical specimens in the accuracy panel, resulting in 100% concordance. No very major errors (i.e., false WT) or major errors (i.e., false MT) were observed (See Supplemental Data).

Precision Panel

Intra and inter-assay precision studies yielded results 100% concordant with expected.

Limit of Detection Panel

All three of the laboratories were able to genotype specimens down to 9×10^2 CFU/mL with ≥ 90% detection of the *gyrA* results, and 100% accurate genotype for all specimens genotyped.

Application of gyrA assay to clinical samples

Launch of the assay at the three facilities was staggered, with 48 weeks of data available for UCLA, 37 for SFPHL and 20 for PPHL. A total of 1370 prospective, *N. gonorrhoeae* positive specimens were tested across the three laboratories, including 49% (667/1370) rectal, 36% (499/1370) pharyngeal, and 15% (204/1370) urine/genital specimens. The percentage of *N. gonorrhoeae* positive, specimens that yielded a successful genotype, varied considerably by specimen type (Figure 3.1). Specimens that did not successfully yield a genotype result were

considered ‘indeterminate’.

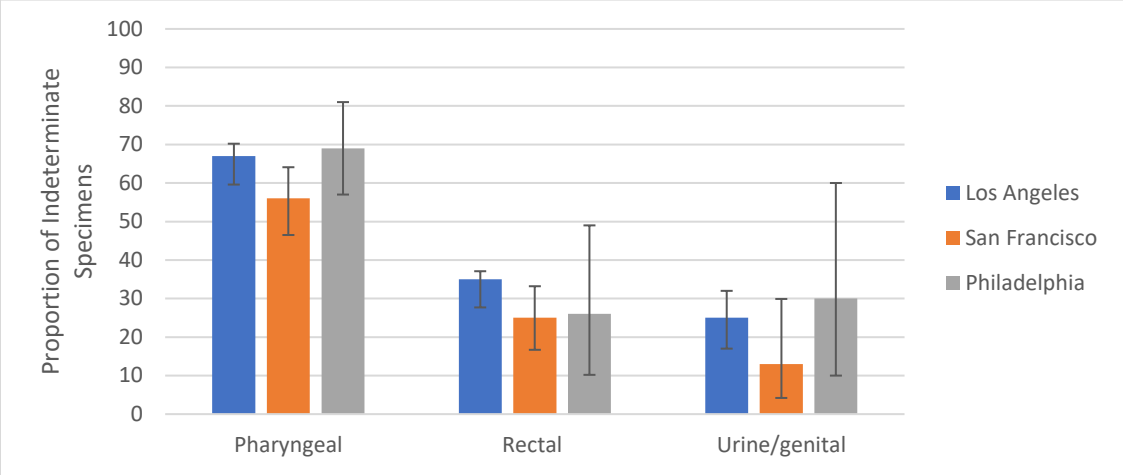

Figure 3.1. Frequency of indeterminate results for genotyping the *gyrA* gene from clinical specimen types. Error bars represent 95% confidence intervals.

As observed in previous studies, the indeterminate rate from pharyngeal specimens was high, ranging from 56 – 69% of all pharyngeal specimens tested, by laboratory (Figure 3.1) (Hemarajata et al. poster, 2016). An unexpectedly high proportion of rectal swab specimens were also indeterminate, ranging from 24-35% of rectal specimens tested by laboratory. Of the urine/genital specimens tested, 13-30% were indeterminate. Therefore, the percentage of reportable specimens was the highest for urine/genital specimens, which were genotyped= 76.4% of the time; (95% confidence interval, 69.9%-82%)).

Of the specimens that could be genotyped at UCLA, 57% (n=319/557) were *gyrA* WT. At SFPHL, 72% (n=134/185) were WT and at PPHL, 64% (27/42) were WT (Table 3.1).

Table 3.1. Proportion of WT *gyrA* genotype results, by study facility and specimen type.

Specimen Type	UCLA	SFPHL	PPHL
Pharyngeal	64/110 (58%)	44/65 (67%)	3/5 (60%)

Rectal Swab	190/331 (57%)	66/94 (70%)	15/23 (65%)
Urine & Genital Swab	65/116 (56%)	24/26 (92%)	9/14 (64%)
Column Total	319/557 (57%)	134/185 (72%)	27/42 (64%)

The proportion of WT *N. gonorrhoeae* did not significantly vary by testing laboratory or anatomical source ($p = 0.551$, $\chi^2 = 3.0425$). The proportion of reportable results (WT and MT combined) also did not vary when compared by testing laboratory or anatomical source ($p = 0.694$, $\chi^2 = 2.23$). Therefore, post hoc pairwise statistical testing was not performed for reportable specimens combined.

Table 3.2. Contingency table for Chi-square analysis of indeterminate *gyrA*, by laboratory and specimen type.

Specimen Type	UCLA	SFPHL	PPHL	Row Total
Pharyngeal	227	81	11	319
Rectal Swab	180	31	8	219
Urine & Genital Swab	38	4	6	48
Column Total	445	116	25	586

Overall, the proportion of indeterminate *N. gonorrhoeae* varied significantly by testing laboratory and anatomical source ($p < 0.00001$, $\chi^2 = 28.7$). Therefore, post hoc pairwise Fisher's exact statistical testing was performed. There was a statistically significant number of indeterminate rectal and pharyngeal specimens between UCLA and SFPHL for ($p < 0.05$, $P = 0.0016$) as well as indeterminate urine and pharyngeal ($p < 0.05$, $P = 0.02$). The number of indeterminate pharyngeal and urine and indeterminate rectal and urine specimens also varied significantly between SFPHL and PPHL, respectively ($p < 0.05$, $P = 0.0013$ and $p < 0.05$, $P = 0.0221$).

Further evaluation of indeterminate specimens at UCLA

The larger volume (400 µL) DNA extraction and 100 µl *gyrA* PCR yielded a *gyrA* genotype for 10 of the 65 specimens (15%) tested by this method. Of 40 indeterminate specimens evaluated by pelleting and re-suspension, 2 rectal specimens yielded a genotype (5%).

DISCUSSION

Drug-resistant *Neisseria gonorrhoeae* continues to pose a threat to public health. The development of rapid molecular-based antimicrobial susceptibility testing can address this issue and potentially decrease resistance to antibiotics by offering more specific treatment options to physicians (Buono et al., 2015). The CDC recommends use of nucleic acid tests for the detection of *N. gonorrhoeae* infection (CDC, 2015). While exquisitely sensitive for *N. gonorrhoeae*, these assays do not provide data on antimicrobial susceptibility. As such, susceptibility data are currently only available for *N. gonorrhoeae* if a culture is performed, which is typically only conducted in the context of a suspected treatment failure. In this study, we successfully adapted a *gyrA* genotyping assay for prediction of ciprofloxacin susceptibility for use with DNA extracted

from *N. gonorrhoeae* –positive specimens across three clinical laboratories. This study demonstrates that a second-step, genotype-based approach is feasible for real-time *N. gonorrhoeae* susceptibility testing in the U.S.

Important modifications of the assay as compared to our previously reported method include extraction of DNA from specimens in Aptima Combo2® CT/NG assay specimen transport medium. Hologic holds the majority market share (> 60%) for *N. gonorrhoeae* screening in the U.S. (Hologic, 2017), and as such demonstration of the assay's feasibility with this specimen type is critical to potential down-stream adoption by laboratories in the U.S. Additionally, we demonstrate that the test can be performed equally with two different extraction platforms, the QIAcube and MagNAPure. Specimens collected in Aptima Combo2® CT/NG assay specimen transport medium are stable at room temperature for 30 days, which may allow for specimen referral to local reference laboratories that can perform the *gyrA* assay. Indeed, this approach was used in Los Angeles where clinical specimens tested at the Los Angeles Public Health Laboratory were couriered to UCLA for reflex *gyrA* testing. This central-laboratory approach may increase the economic viability of performing a second-step *gyrA* genotype test, in particular for asymptomatic patients who may not have been treated empirically for *N. gonnorhoeae* infection (Alexander, 2009).

The indeterminate frequency for the *gyrA* genotype assay was higher than initially expected (Hemarajata et al., 2016) (Figure 3.1). Nonetheless, results still conferred valuable information to clinicians about treatment options in 57.2% (784/1,370) of the specimens tested. This low rate of positivity may relate to the fact that we only tested specimens from asymptomatic patients, as symptomatic patients were treated empirically with CDC recommended therapy and excluded

from this study. In contrast, in our previous work, all-comers were tested by the *gyrA* genotype assay (Hemarajata et al., 2016). Because symptomatic patients may have higher *N. gonorrheae* load, adapting the method to a point-of-care environment may improve clinical sensitivity. Of 1370 prospective specimens tested in this study to date, a result of WT was obtained for 35% (480/1370). This suggests that treatment with ciprofloxacin might be appropriate for over a third of patients who would have been otherwise treated with dual therapy.

Of the specimens that were successfully genotyped, the assay performance was better for urine/genital specimens 77.3% (133/172) compared to rectal 65.4% (342/523) and pharyngeal specimens 34.4% (142/413). The poor performance for pharyngeal specimens is similar to that observed previously: 63.6% pharyngeal specimens that tested positive for *N. gonnorheae* in a previous study were found to be indeterminate by *gyrA* assay (Hemarajata et al., 2016). The reason for the poor performance for pharyngeal samples remains unclear. This may result because lower levels of *N. gonorrhoeae* are present in the pharynx (Alexander, 2009) or because of cross reactivity/interference due to the presence of organisms like *N. meningitidis* or other *Neisseria* commensal species (Alexander, 2009; Low and Unemo, 2017). Probit analysis was previously used to evaluate the sensitivity of the *gyrA* assay. The Probit module 90 on XLSTAT (Addinsoft, New York, NY) determined that the COBAS® 4800 CT/NT assay crossing point (*Cp*) value of ≤ 28.15 associated with $\geq 95\%$ detection of a genotype by *gyrA* genotyping assay for 100 seeded and clinical samples (Hemarajata et al., 2016). Thus, only specimens with a low COBAS® crossing point (i.e., higher bacterial load) could be genotyped by the *gyrA* assay (Hemarajata et al., 2016). Similarly, a *Cp* of ≤ 24.6 for pharyngeal swabs, ≤ 29.1 for urine, and ≤ 38.5 for rectal swabs was calculated for $\geq 95\%$ sensitivity (Hemarajata et al. poster, 2016). While the COBAS® assay is not quantitative, the lack of correlation between *Cp* and successful

amplification of *gyrA* for pharyngeal specimens may point to an inhibition rather than limit-of-detection issue.

In contrast, we previously observed only 6.7% indeterminate results using remnant DNA from the COBAS system for rectal swabs, whereas in the present study, the overall indeterminate rate for rectal swabs was much higher (25-35%, Figure 3.1). It is possible that this discrepancy between the current data and our prior observations is due to the superior sensitivity of the Hologic® transcription-mediated amplification (TMA) chemistry, which targets high-copy number 16S rRNA from *N. gonorrhoeae* (Buckley, et al, 2016, Chernesky et al., 2002), as compared to the RT-PCR chemistry targeting DNA used by the COBAS® system. Neither assay is FDA-cleared for pharyngeal swabs, as such limit of detection data are not available for that specimen type. However, comparison of the limit of detection for urine and genital specimens on the Aptima®, as compared to what is observed for the *gyrA* assay, demonstrate the expected differences of amplifying a low versus high-copy target. The analytical sensitivity of the Aptima® assay is 250 CFU/mL in urine and 362 CFU per genital swab (Hologic® package insert, 2017). Our assay has a limit of detection of 900 CFU/mL for these two specimen types. As such, it is not surprising that our genotype detection rate was lower. While we observed limited success in maximizing the total amount of DNA added to reactions, this modification resulted in a more costly test due to increased reagent volumes in the RT-PCR reaction, and increased consumables.

Overall, of all genotyped specimens, 61% (480/784) were WT, which reflects the most current CDC data from GISP on ciprofloxacin susceptibility rates for *N. gonorrhoeae* in these regions (Elizabeth Torrone, personal communication to JD Klausner). Specifically, ciprofloxacin

susceptibility rates as evaluated by the GISP program in 2016 were 70.3%, 77.3% and 54.9%, respectively for San Francisco, Philadelphia and Los Angeles (Kirkaldy, et al., 2016). These data nicely reflect the 72%, 64% and 57% WT *gyrA* genotypes initially observed in this study (Table 1). At the very least, the *gyrA* method, along with our previously published *penA* genotyping assay (Wong et. al., 2017), could be used at these public health laboratories to monitor *N. gonorrhoeae* susceptibility in real-time.

Conclusions

In summary, we demonstrate the introduction and use of a laboratory-developed, genotype-based assay for the detection of *N. gonorrhoeae* ciprofloxacin susceptibility across three laboratories. While detection rates were somewhat lower than expected, the assay yielded a genotype for a majority of specimens tested, potentially allowing more targeted therapy for more than half of these patients. Further developments of novel strategies to detect susceptibility are paramount to both the health of the public and individuals affected by gonococcal disease. Future data from this trial will evaluate the treatment outcomes for patients with WT *gyrA* results that were treated with ciprofloxacin.

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CHAPTER 4

A combination antibiogram of contemporary *Stenotrophomonas maltophilia* isolates at a multi-site tertiary care health system

ABSTRACT

Stenotrophomonas maltophilia is a multidrug-resistant opportunistic pathogen that can cause severe respiratory infections and life-threatening bacteremia in high-risk, immunocompromised patients. Few modern surveillance studies or standardized susceptibility test data are available for this organism in the clinical setting. This study investigated the prevalence of antimicrobial resistance at a single health system based on reference broth microdilution susceptibility data from 1,095 *S. maltophilia* isolates collected between 2009-2018. The antibiotics of interest were: ceftazidime (N = 1,026), ceftazidime-avibactam (N = 334), ceftolozane-tazobactam (N = 288), colistin (N = 978), levofloxacin (N = 1,033), minocycline (N = 977), tigecycline (N = 955), and trimethoprim-sulfamethoxazole (N = 1,026). Not all isolates were tested against all antibiotics, nor were all data for each antibiotic available for each year under study. Isolates were analyzed using WHONET version 5.6 from the World Health Organization (WHO). Although worldwide resistance to trimethoprim-sulfamethoxazole (TMP-SMX) is reportedly increasing, (1,004/1,027 or 98%) of all isolates tested were susceptible this drug in the present study. Similarly, (969/977 or 99% of all isolates tested were susceptible to minocycline between 2009-2018. Double coverage is often used for patient therapy, particularly for immunosuppressed patients where TMP-SMX is not a therapeutic option. Here we assessed whether drug combinations were effective against isolates of *S. maltophilia.* Twelve of the twenty-five combinations that were assessed amongst isolates, had >90% activity between 2009-2018. Using larger data sets by combining data from multiple years can provide more robust treatment guidelines for clinicians.

Specifically, this methodology can assist physicians in making more evidence-based choices at the local level when considering an empiric antibiotic therapy for their patients with *S. maltophilia*.

INTRODUCTION

Stenotrophomonas maltophilia is a multidrug-resistant, Gram-negative, non-fermentative bacillus of clinical significance. *S. maltophilia* can produce biofilms on surfaces and equipment in the clinical environment, including plastic tubing, rendering the bacteria impermeable to antimicrobial agents and resistant to host defenses (Lewis, n.d.; Nicodemo, 2007; Chang, 2015). As such, this opportunistic pathogen frequently causes bacteremia and pneumonia in the sickest hospitalized patients that require the use of central venous catheters, and mechanical ventilators (Chung, 2012). Other risk factors for *S. maltophilia* infection include malignancy, cystic fibrosis, broad spectrum antibiotic use, as well as corticosteroid or immunosuppressive therapy (Chang, 2015). *S. maltophilia* incidence is 7.4 to 37.7 patients in 10,000 at risk for opportunistic infections (Garazi, 2012). *S. maltophilia* is increasing in frequency due to the increase in antibiotic usage and the increase in immunocompromised patients in hospital populations (Garazi, 2012). The World Health Organization (WHO) has classified *S. maltophilia* as one of the leading causes of multidrug resistant infections in the hospital setting (Brooke, 2014).

In some cases, it can be difficult to distinguish between colonization and infection in immunocompromised patients that test positive for *S. maltophilia*, making treatment considerations even more challenging (Chung, 2012; Cosimi, 2016). As such, hospitalized patients with these infections have been associated with high levels of morbidity and mortality (Senol, 2002). In the United Kingdom, *S. maltophilia* is responsible for about 1,000 bloodstream

infections per year and 30% of these are fatal (McKeever, 2019). Therefore, early antibiotic selection is essential to saving the lives of those with severe *S. maltophilia* infections.

S. maltophilia isolates have historically been susceptible to trimethoprim-sulfamethoxazole (TMP-SMX), which has made this antibiotic the therapy of choice for *S. maltophilia* infections. The threat of TMP-SMX resistance was first observed in a Saudi Arabian patient in 2006 (Asma, 2006). Resistance to TMP-SMX is acquired by mobile *sul*-genes from integrons (Cosimi, 2016; Toleman, 2007). The environmentally ubiquitous nature of *S. maltophilia* confers the ability of the organism to acquire and share resistance genes in this way via horizontal gene transfer (Brooke, 2012). In 2019, an XDR strain of nosocomial *S. maltophilia,* resistant to both TMP-SMX and levofloxacin, from India (SM866:CP031058) was analyzed using whole genome sequencing (Kumar, 2019). Thus, resistant strains continue to circulate in the population.

S. maltophilia ought to be at the forefront of resistance studies due to its ability to acquire resistance via multiple mechanisms including multidrug resistance pumps, plasmids that carry resistance genes, and other gene transfer mechanisms (Brooke, 2012). When *S. maltophilia* acquires resistance against antibiotics, the resistance patterns can vary substantially between patients, environments, facilities, and over time (Brooke, 2012). This high level of possible variation makes *S. maltophilia* an important clinical organism to capture in combination therapy surveillance studies.

Antimicrobial resistant *S. maltophilia* continues to pose challenges in the clinical laboratory. While some healthcare facilities routinely conduct manual AST, like broth microdilution and disk diffusion, for *S. maltophilia,* most labs that perform AST for *S. maltophilia* use automated systems such as the BD Phoenix®, Beckman Coulter Microscan®, Thermo Fisher Scientific

Sensititre™, as well as the bioMerieux VITEK™ 2 system. While this may seem like a comprehensive list of automated AST options, broth microdilution remains the gold standard. In some cases, broth microdilution results cannot be replicated by different analysts or different days (Brasso, 2017). Therefore, if data from a standardized method cannot meet acceptance criteria on would not expect that a commercial device AST could be effectively compared to the standard method (Brasso, 2017). Furthermore, there are no FDA-cleared tests for Stenotrophomonas with breakpoints that are recognized by the US FDA for newer drugs. Finally, susceptibility breakpoints for newer agents such as tigecycline, ceftazidime-avibactam, and ceftolozane-tazobactam are not available for the organism, which poses an even greater challenge for clinical laboratories and clinicians. Currently, levofloxacin is the only fluoroquinolone with established susceptibility breakpoints for *S. maltophilia.* While moxifloxacin shows promise a as treatment *in vitro,* this may not be true *in vivo* (Cosimi, 2016). As of 2020, *S. maltophilia* antibiotic breakpoints have been established for ceftazidime, minocycline, levofloxacin, trimethoprim-sulfamethoxazole and chloramphenicol (CLSI, 2020).

Still, many healthcare facilities do not have instruments or populations to produce adequate in-house or regional-level drug susceptibility data for this organism. There are few studies published that assess the susceptibility patterns of *S. maltophilia,* and these typically involve a small number of isolates (Gajdacs, 2019). For instance, most hospitals do not test enough *S. maltophilia* isolates to provide evidence-based treatment guidance. For this reason, the use of regional antibiograms has been proposed to improve antibiotic selection and reduce mortality for potential pathogens with low prevalence (Humphries, 2017). Furthermore, these studies have not been carried out on contemporary isolates nor do these studies evaluate combination

susceptibility profiles. Ongoing assessment of the antimicrobial susceptibility of this organism can assist healthcare providers in making more informed decisions regarding the treatment options for patients with *S. maltophilia,* and hence improve Antimicrobial Stewardship Program (ASP) efforts. In larger teaching or research hospitals, AST diagnostics are paramount because of the large population of immunocompromised patients in said facilities.

Global reports suggest an increasing level of resistance to TMP-SMX. We sought to understand what the best treatment options are for *S. maltophilia* at one healthcare system. In this study, we investigated the longitudinal susceptibility profiles of antibiotics used against *S. maltophilia* for the time-period 2009-2018, to understand the antibiotic-resistance trends at one healthcare system over time. It is important to look at data longitudinally because data can vary from one geographic location to another or between hospitals. Although, global data suggest that TMP-SMX resistance may be increasing, this drug is still a fine candidate in this healthcare system.

METHODS

A total of 1,095 *Stenotrophomonas maltophilia* isolates were tested for antimicrobial susceptibility between 2009 and 2018 by the reference broth microdilution method (CLSI, 2015). The reference broth microdilution method is the ISO and FDA reference method to determine AST. While this method is the standard for AST from the regulatory stance, it is not performed in many clinical laboratories. This method is the ISO and FDA reference method to determine AST. *S. maltophilia* has no established breakpoints for ceftazidime-avibactam, ceftolozane-tazobactam, colistin or tigecycline. Therefore, the following interpretive criteria, from the non-fermenter *Pseudomonas aeruginosa,* were used in this study as a proxy for susceptibility (ceftazidime-avibactam ≤ 8, ceftolozane-tazobactam ≤ 4/4, colistin ≤ 2, and tigecycline ≤ 2).

Isolate susceptibility patterns were analyzed individually and in combination with other antibiotics. The antimicrobials selected for a combination antibiogram were ceftazidime, colistin, levofloxacin, minocycline, tigecycline and trimethoprim-sulfamethoxazole (TMP-SMX). Susceptibility combinations were included only for the years that data was available for ceftazidime-avibactam and ceftolozane-tazobactam based on when these drugs because available in the market (2015-2018 and 2016-2018, respectively). All other drug combination susceptibilities were provided for each year from 2009-2018. Isolates included in this study were those recovered from normally sterile anatomical sites (e.g., blood), as well as isolates where the physician requested testing.

WHONet 5.6 software and methods from M39, 4th Ed. were used to create antibiograms for *S. maltophilia* isolates recovered from patients in the UCLA hospital system between January 2009 through December 2018 (N = 1,095). Only one isolate per patient was used for each year in this evaluation and all patient data was stripped of patient identifiers within the WHONet system. All data included in this study was IRB exempt.

Antimicrobial susceptibility testing was performed at the time of isolation using the M07 CLSI reference broth microdilution (BMD) procedure (CLSI, 2015). Incubation took place at 35˚ C for 24 hours. The CLSI M100-S29 interpretive criteria available for *S. maltophilia* were used to determine resistance or susceptibility (CLSI, 2019).

Calculations were based on the percent susceptibility to at least one drug as not all isolates were susceptible to both drugs in a given combination. Therefore, the best combinations were identified and stratified by time. Once this exercise was complete, the percent susceptible to each drug was included in an antibiogram table and susceptibility trends between years were assessed.

The same steps were repeated for isolates from blood (N = 133). χ^2 analysis was performed using socstatistics.com.

RESULTS

Demographic information

Males accounted for most of the cases (56%, 618/1,095) followed by females (42%, 461/1,095); no gender was reported for a small percentage of individuals (1.5%, 16/1,095). Patients fell into five age categories: younger than 18 years old (13%, 143/1,095), 19-21 years old (2%, 21/1,095), 22-40 years (14%, 159/1,095), old between 41-60 years old (28%, 302/1,095), or age 61 and over (41%, 453/1,095); and most patients were middle-aged or older adults. The remaining isolates were from those with an unknown age (2%, 17/1,095). Most of the patient isolates were recovered from respiratory sources (54%, 592/1,095), followed by (other—wounds, tissue, swab, etc.) (22%, 240/1,095), blood (12%, 133/1,095), urine (8%, 93/1,095), sterile body fluids/aspirates (3%, 37/1,095).

Susceptibility data

Overall, all *S. maltophilia* isolates collected between 2009-2018 exhibited levels of susceptibility between 32%-98% to individual antibiotics (Table 4.1). Minocycline (Minimum Inhibitory Concentration (MIC $\leq$ 4, N = 977) or trimethoprim-sulfamethoxazole (MIC $\leq$ 2/38, N = 1,026) monotherapy exhibit the highest levels of susceptibility in *S. maltophilia* isolates (99% and 98%, respectively) (Table 4.1). Isolates exhibited susceptibility to tigecycline 76% of the time (N = 955) while isolates were susceptible to levofloxacin 69% of the time (N = 1,033). 43% of the isolates were susceptible to ceftazidime-avibactam (N = 334) and 45% were susceptible to

colistin (N = 978) (Table 4.1). The same isolates were infrequently susceptible to ceftazidime (N = 1,026) or ceftolozane-tazobactam (N = 288) (32% and 31%, respectively) for the same time period (Table 4.1).

Table 4.1. *Stenotrophomonas maltophilia* isolates and MIC (µg/ml) tested between 2009-2018.

Antimicrobial agent (Total isolates)	Number of Isolates with MIC values				% Susceptible
	≤ 2	>2 X ≤ 4	8	≥ 8	
Ceftazidime (1,026) *	115	105	93	711	32
Ceftazadime-avibactam (334) *⸸	45	59	40	189	43
Ceftolozane-tazobactam (288) *♯	63	25	31	169	31
Colistin (978) *	446	102	39	231	45
Levofloxacin (1,033)	710	153	93	77	69
Minocycline (977)	941	28	7	1	99
Tigecycline (955)	730	141	64	20	76
TMP-SMX (1,026)	1003	4	19	0	98

⸸ Isolates only available for testing 2015-2018
* No CLSI established breakpoint for *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* breakpoint used
♯ Isolates only available for testing years 2016-2018

The *S. maltophilia* susceptibility profiles varied from year to year (between 18%-100%) across drugs (Table 4.2). *S. maltophilia* isolates were most resistant to colistin in 2009-2010, with only (37/201, 18%) of the specimens tested exhibiting susceptibility (Table 4.2). Isolates were susceptible to minocycline 100% of the time for 4 of the years studied (2009, 2010, 2014 and 2018, N = 364) (Table 4.2). Isolates of *S. maltophilia* were only susceptible to TMP-SMX 100% of the time in 2014 and 2018 (N = 186). However, this was not a statistically significant difference. The most dramatic change in *S. maltophilia* susceptibility occurred between 2017-2018 for colistin, which changed from 76% to 52% (N = 214) and between 2016-2018 for levofloxacin, which changed from 79% to 59% (N = 201). The blood isolates followed this same susceptibility trend for these two antibiotics (Table 4.3). However, the most dramatic change in *S. maltophilia* blood isolate susceptibility occurred between 2017-2018 for colistin, which

changed from 92% to 50% (χ^2 (1, N = 40) = 0.8252, p = 0.363654) and between 2015-2018 for levofloxacin, which changed from 100% to 50% (χ^2 (2, N = 90) = 1.3803, p = 0.710169) (Table 4.3). Up until these time points, the susceptibility to these antibiotics was improving for colistin and levofloxacin since 2009 (Table 4.3). In 2015 and 2017, blood isolates were susceptible to TMP-SMX 92% of the time (χ^2 (2, N = 286) = 0.0695, p = 0.965849) and in all other years every blood isolate was susceptible to TMP-SMX. In years that *S. maltophilia* had lower susceptibility to TMP-SMX, the bacteria were more susceptible to minocycline for all isolates (Figure 4.1 and Figure 4.2).

Table 4.2. Year to year change in percent susceptible isolates for individual drugs- for years 2009-2018.

Individual Drugs All Sources	2009 % S	2010 % S	2011 % S	2012 % S	2013 % S	2014 % S	2015 % S	2016 % S	2017 % S	2018 % S
	N = 95	N = 106	N = 120	N = 101	N = 121	N = 101	N = 91	N = 112	N = 132	N = 116
Ceftazidime	36	27	30	32	31	32	31	31	28	31
Ceftazidime-Avibactam *⸸							50	50	40	43
Ceftolozane-Tazobactam *♯								39	28	29
Colistin *	18	18	33	33	30	45	50	73	76	52
Levofloxacin	70	64	63	68	69	67	76	79	58	59
Minocycline	100	100	99	99	98	100	98	99	99	100
Tigecycline	77	77	77	84	73	44	77	75	84	82
Trimethoprim-Sulfamethoxazole	97	99	98	97	97	100	96	98	97	100

⸸ Isolates only available for testing 2015-2018
* No CLSI established breakpoint for *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* breakpoint substituted
♯ Isolates only available for testing years 2016-2018

Twelve out of a total of twenty-five drug combinations (48%) resulted in > 90% activity against *S. maltophilia* infection, which included every drug combined with either TMP-SMX or minocycline—the two antimicrobials with the highest susceptibility rates as monotherapies (see

Figure 4.1). Minocycline in combination with TMP-SMX was the most suitable combination for empiric dual therapy. Susceptibility to this drug combination ranged from 98-100% for all years included in this study. The susceptibility profile for tigecycline paired with TMP-SMX ranged from 97-100% for all years included in this study. This was followed by the ceftazidime/TMP-SMX combination, colistin/TMP-SMX combination or the ceftazidime-avibactam/TMP-SMX combination (96-100%). *S. maltophilia* isolates in this study were susceptible to levofloxacin or TMP-SMX 94-100% of the time. The drug combinations with the highest levels of susceptibility between 2009-2018 were exhibited by the combinations of Trimethoprim-sulfamethoxazole (TMP-SMX) paired with either colistin, minocycline or tigecycline (99%) (Table 4.4). The drug combination with the lowest level of susceptibility was exhibited by colistin when paired with ceftazidime (64%) (Table 4.4).

Table 4.3. Percentage of *S. maltophilia* isolates from blood susceptible to individual antibiotics where n is the number of blood isolates positive for *S. maltophilia* in a given year

Individual Drugs Blood Isolates only (N=133)	2009 % S (n = 16)	2010 % S (n = 14)	2011 % S (n = 12)	2012 % S (n = 14)	2013 % S (n = 13)	2014 % S (n = 14)	2015 % S (n = 12)	2016 % S (n = 13)	2017 % S (n = 12)	2018 % S (n = 13)
Ceftazidime	44	30	40	41	31	54	23	31	33	40
Ceftazidime-Avibactam							20	46	51	40
Ceftolozane-Tazobactam								35	27	30
Colistin	11	27	18	31	31	54	39	69	92	50
Levofloxacin	72	59	73	82	75	92	100	77	67	50
Minocycline	100	94	100	100	100	100	100	100	100	100
Tigecycline	72	75	78	80	75	75	100	71	90	80
Trimethoprim-Sulfamethoxazole	100	100	100	100	100	100	92	100	92	100

Table 4.4 Combined Antibiogram for 2009-2018 for *Stenotrophomonas maltophilia*, Overall % Susceptible to One or Two Antimicrobials, Includes Pediatrics and Adults (N = 1,095).

	Ceftazidime (32)	Minocycline (99)	Levofloxacin (69)	Trimethoprim-Sulfamethoxazole (98)	Tigecycline (76)	Colistin (45)
Ceftazidime (32)	—	99	77	98	7	
Minocycline (99)	99	—	97	100	—*	99
Levofloxacin (69)	77	97	—	99	83	80
Trimethoprim-Sulfamethoxazole (98)	98	100	99	—	99	99
Tigecycline (76)	87	—*	83	99	—	84
Colistin (45)	82	99	80	99	84	—

*Tigecycline is a derivative of minocycline and hence this combination is not reported

Figure 4.1. Individual antibiotic susceptibility by year for all isolates by year

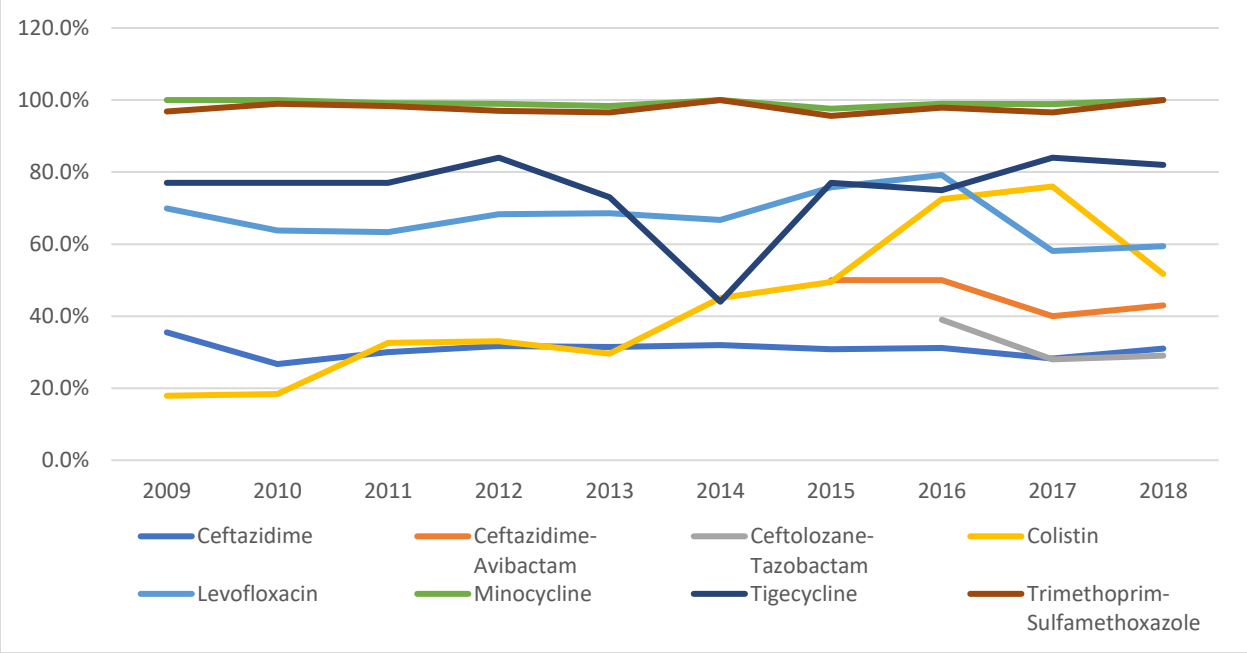
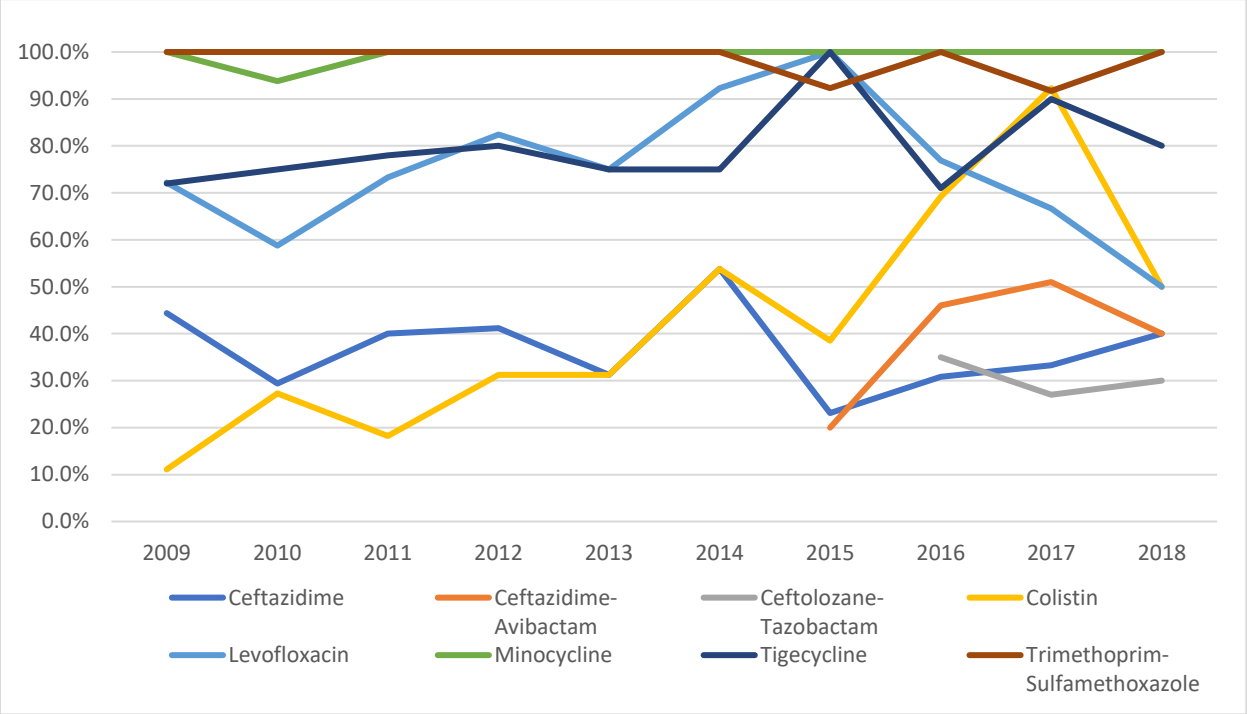


Figure 4.2. Individual antibiotic percent susceptibility by year for blood isolates



DISCUSSION

Results from this study indicate that the best treatment options for *S. maltophilia* for this facility during the period 2009-2018 were TMP-SMX and minocycline because these antibiotics had the best susceptibility profiles for the *S. maltophilia* isolates included in this study. In years that had decreased susceptibility profiles for TMP-SMX, minocycline was 100% susceptible for all isolates. The susceptibility profile for each drug combined with TMP-SMX have improved over time and suggest that these are still effective combination treatment options.

This bacterium is classically described as trimethoprim-sulfamethoxazole (TMP-SMX) susceptible (Cosimi, 2016). While, TMP-SMX is still an efficacious option for empirical therapy against *S. maltophilia*, minocycline should be considered as an alternative or backup treatment in

order to ensure 100% coverage. When analyzed as a single data set, only 2% of all *S. maltophilia* specimens collected were resistant to TMP-SMX. Thus, TMP-SMX may not be the best choice for treatment in every case, which may be especially true in those who cannot tolerate the drug or in more complex medical situations to be considered on an individual basis. Further hypothesis-driven research could be done to elicit similar findings at various institutions for comparison. While other combination treatments should still be considered for *S. maltophilia* empirical combination therapy, variable resistance and individual antimicrobial susceptibility data for individual patients should be considered before making treatment decisions in cases that are not life threatening. All observations from this study emphasize the need to continue surveilling *S. maltophilia* for the emergence and spread of antibiotic resistant isolates from inside and outside the hospital (Brooke, 2012).

LIMITATIONS

This data was retrospective in nature and accounted for specimens at one healthcare system. The relevance of antimicrobial susceptibility interpretive criteria changes over time and a larger data set may provide a deeper understanding of antimicrobial susceptibility testing (AST) trends over time and space. Chart reviews were not performed; therefore, the severity of the patient's condition or the possibility of polymicrobial infection was not accounted for. Additionally, there was no case follow up assessing patient outcomes as they related to treatment. Isolates from patients with cystic fibrosis may have been included in this analysis. Cystic fibrosis isolates often exhibit higher levels of resistance and may have skewed the average numbers generated for antibiograms (Bosso, 2006). Every isolate was not tested for each drug susceptibility pattern each year. Twenty-two infections were excluded from the larger dataset due to repeated

infections at the same body site at dates that followed an initial infection. Colonization was not differentiated from true infection for non-sterile bodily sites.

CONCLUSIONS

In summary, we demonstrate that the best choice for dual empiric therapy against *S. maltophilia* at this institution from 2009-2018 was TMP-SMX and minocycline. Monitoring at this facility included the use of the reference broth microdilution methodology. Many labs do not have the support or technical expertise to perform this type of manual testing. In these cases, commercially available, automated methods are used for AST of *S. maltophilia.* Unfortunately, many of the automated instruments do not have FDA clearance for breakpoints in newer classes of drugs for *S. maltophilia.* In these cases, it may be beneficial to submit isolates of concern to reference laboratories that can offer standard reference broth microdilution as an option for AST. In facilities with a high incidence rate of *S. maltophilia,* it may become increasingly important to continuously monitor the AST trends for *S. maltophilia* as global reports of TMP-SMX resistance continue to surface.

While global data suggests that *S. maltophilia* is becoming increasingly resistant to TMP-SMX, our diverse study site data suggests otherwise. However, the variability between geographic locations, environments, and patients is not to be underestimated. We live in a dynamic and rapidly changing world where resistant organisms can travel to multiple continents within a day. Thus it is important to maintain vigilance and continue to monitor this looming threat.

While TMP-SMX resistance rates were lower than expected, monitoring these trends allows us to set the stage for future monitoring and surveillance of antimicrobial resistance. The data herein can inform clinicians of a more targeted therapeutic approach for their patients. Additional

developments in AST surveillance along with novel strategies to rapidly perform AST are paramount to both the health of the public and individuals affected by multi-drug resistant organisms (MDROs).

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CHAPTER 5

Overall Conclusions and Recommendations for Future Studies and Practices

INTRODUCTION

The microbiology laboratory can play a vital role in monitoring the environment in order to enhance Antimicrobial Stewardship Program (ASP) practices. The microbiology laboratory is also responsible for selecting the appropriate analytical methods, which can assist medical personnel in determining the best available treatments for patients. Finally, the use of rapid antimicrobial susceptibility testing (AST) in the microbiology laboratory can reduce turn-around-time (TAT) to treatment and possibly even reduce the incidence of both Healthcare Associated Infections (HAIs) and Antimicrobial Resistance (AMR) in clinical settings.

SUMMARY OF KEY FINDINGS

The overarching goal of the work presented in this thesis was to evaluate the practical challenges and feasibility related to improving methods for ASPs. ASPs are an important and changing mechanism for surveillance of HAIs, understanding antimicrobial susceptibility patterns and suggesting individual-based treatment regimens. The specific organization of this work was divided into three main parts: Chapter 2, improving environmental monitoring and cleaning, Chapter 3, rapid susceptibility testing; and Chapter 4, enhancing antimicrobial susceptibility pattern surveillance for organisms that are highly drug resistant.

In Chapter 2, I discussed ways in which infection prevention staff and the clinical laboratory can provide semi-quantitative data and rapid identification of organisms from clinical surfaces of the

operating room. EPA approved cleaning agents are used in the operating room in conjunction with visual inspection for cleanliness. In our study, irregular surfaces (overhead lights, door handles, and anesthesia keyboards) harbored larger amounts of bioburden, which were detected by ATP assay both before and after cleaning. In fact, 92% (22/24) of rooms had least one surface that exceeded the 250 RLU threshold after turnaround cleaning via the ATP assay. Additionally, 42% (10/24) of the rooms had at least on surface that didn't pass as clean after turnaround cleaning via the RODAC surface sample test. To facilitate the reduction of HAIs by using this more refined methodology, we suggested the following approach:

- Replacing visual cleanliness inspections with Adenosine Triphosphate (ATP)-based detection methods to quantitatively measure bioburden on high touch surfaces in operating rooms.
- Developing and validating methods to use ATP for individual surfaces/surface types.
- Utilizing Rapid Microbiological Methods (RMMs) such as Matrix Assisted Laser Deionization Time of Flight (MALDI-TOF) to expeditiously identify environmental contamination.
- Developing methods for utilizing and evaluating single use covers for irregularly-shaped surfaces that may introduce more risk to patients.

In Chapter 3, we evaluated the utility of a real-time *gyrA* polymerase chain reaction (PCR) protocol to predict ciprofloxacin susceptibility in *N. gonorrhoeae* in place of traditional culture methods. The performance of the assay was evaluated at several laboratory sites, using various equipment, to understand how these approaches might best assist clinicians in selecting alternative treatments for their patients infected with gonorrhea. Several of the individual tests

were incapable of being genotyped and classified as indeterminate results. To further investigate this anomaly, we tested the following methods:

- High speed centrifugation before extraction
- Use of a Deoxyribonucleic acid (DNA) clean-up kit
- A nested PCR procedure

We found that this testing algorithm provides a rapid and reliable means to detect ciprofloxacin susceptibility in *N. gonorrhoeae* that can improve antimicrobial stewardship. The *gyrA* assay reduces the turn-around time required to determine an effective treatment by eliminating the need for culture-based AST.

In Chapter 4, we presented a combined antibiogram that includes longitudinal (2009-2018) antimicrobial resistance trends, for the highly drug resistant organism *Stenotrophomonas maltophilia,* within a single healthcare system. Recent literature cites an increase in the number of cases that are resistant to the empirical trimethoprim-sulfamethoxazole (TPM-SMX) treatment.

A few of the interesting findings from this study include:

- Only 2% of all isolates were resistant to TMP-SMX.
- *S. maltophilia* is most susceptible to TMP-SMX and minocycline.
- Enhanced antibiograms and surveillance data can provide more extensive prescribing information to clinicians so that a more tailored choice for empirical treatment can be made before AST results are reported.

LOOKING TO THE FUTURE: MOVING TOWARD A MORE EFFECTIVE APPROACHES FOR ANTIMICROBIAL STEWARDSHIP PROGRAMS (ASPs)

There is an unmet need to improve ASPs. Standardized programmatic methods do not exist across the disciplines of environmental cleaning, routine antimicrobial susceptibility testing, and treatment regimens. This lack of harmonization leads to inconsistent cleaning practices, routine test results, and therapeutic treatment in clinical settings, especially between different clinical facilities. Clinical laboratories can assist in developing and implementing methods to improve ASPs by offering enhanced testing algorithms to better control HAIs and Antimicrobial Resistance (AMR). The methods investigated herein yield a new perspective on a few ways that ASPs can be improved in the future. The overarching lessons learned from these studies include:

- Environmental/surface monitoring and surveillance can be ramped up to include quantitative and microbiological methodologies.
- Rapid, non-culture-based PCR methods can be used to predict AMR.
- Enhanced antibiograms for Multidrug Resistant Organisms (MDROs) can help to improve and tailor treatments for individual patients with complex infections.

Overall, we are in the formative days of continuous improvement for ASPs. There are multiple modalities within the healthcare environment that can be used to drive the efficiency of antimicrobial stewardship. The following methods are parts of a coordinated process that can be utilized to improve the quality and measurement of antimicrobial stewardship over time.

Real-time semi-quantitative monitoring and surveillance of environmental contamination

ATP bioburden monitoring can be used as an effective means to improve the quality and cleanliness of the healthcare environment. While ATP may not be capable of detecting the full

extent of a contamination event or issue, the technology serves as a launching pad for investigative root cause research for hospital epidemiology and microbiology teams. The scope of bioburden monitoring in food safety and the biopharmaceutical industry is extensive. To help facilitate the transition to a more effective approach for cleaning hospital surfaces, we propose the following:

- Further science-based research method development for cleaning practices related to surface variation;
- Building a hospital microbiome library using Rapid Microbiological Methods (RMMs) like MALDI-TOF or next-generation sequencing;
- Choosing the appropriate EPA approve cleaners that are appropriate to each surface type;
- Continuous improvement of cleaning methodologies and cleaning stewardship for various surfaces and wards of the hospital.

Rapid AST for detecting antibiotic resistant *Neisseria gonorrhoeae*

Drug resistant *Neisseria gonorrhoeae* is an urgent public health threat that demands the development of rapid AST at the point of care. Our reliance on the recommended combined drug therapy only serves to increase drug resistance via evolutionary selective pressure. Additionally, if we are to stem the tide of drug resistant *Neisseria gonorrhoeae,* we must reduce our reliance on the lengthy, traditional culture-based methods. Rapid, point of care AST is critical for disease management, surveillance and ASPs (Khazaei, 2018). In our multisite validation study, in Los Angeles, San Francisco and Philadelphia, we found that 480 patients resulted in wildtype *gyrA* genotypes. Thus, 61% (480/784) of the genotyped cases were treatable with ciprofloxacin. Given this, we recommend the following:

- Loosening regulatory hurdles to translate innovation into practice to protect public health;

- Further research and develop similar approaches for other multi-drug resistant organisms;
- Deploy a nationwide culture-independent *gyrA* testing program in public health, laboratories to provide patient level data to clinicians in a time sensitive manner. (This was accomplished in 2019 in the United Kingdom with *Resistance*Plus® by SpeeDx);
- Continuous improvement of ASPs via individualized treatment with the correct antimicrobials.

Enhanced surveillance methods for multi-drug resistant organisms (MDROs)

As we progress into the 21st century, we must be vigilant of the global risks that threaten our health. The evolution selection pressure and environmentally ubiquitous nature of *Stenotrophomonas maltophilia* poses a unique threat to those with an immunocompromised health status. If we are not careful to monitor the drug susceptibility patterns of MDROs and prescribe accordingly, then these MDROs will continue to surpass our ability defend ourselves with antibiotics. While trimethoprim-sulfamethoxazole (TMP-SMX) remains a useful antimicrobial for the treatment of *S. maltophilia* in our study, we recommend the following:

- Enhance surveillance for *S. maltophilia* in the clinical environment and in patients;
- Expanding antibiograms to include more antibiotics for *Stenotrophomonas maltophilia*;
- Developing a public health awareness campaign about the dangers related to the drug resistance patterns of *S. maltophilia*.

Rapid Microbial Methods (RMMs) to protect public health

RMMs are in high demand due to the progressive nature of antibiotic resistance. Conventional culture-based methods have lengthy incubation times. As such, the time to AST reporting may

lead to improper empirical prescribing decisions. Reducing the turn-around time for AST results, via RMM can improve science-based clinical decisions and reduce antimicrobial resistance.

Improving Rapid Microbiological Methods for improved patient-based treatment delivery

The implementation of automated, near-real-time microbial identification and susceptibility testing could propel us into a future where empirical treatment is a part of the distant past. Phenotypic technologies (e.g., microfluidic-based bacterial culture and digital cellular imaging) and molecular (e.g., multiplex PCR, nanotechnologies, and sequencing) methods can be used as standalone systems or paired in the future to improve turn-around-time and reduce our dependence on empirical antibiotic treatments. If clinicians prescribe according to new AST algorithms the improvement to patient-based treatment delivery and public health is possible. Although many of these platforms are currently in technical development, regulatory hurdles prohibit the adoption of methods without exhaustive validation, so the systems have yet to be cleared or approved by the Food and Drug Administration (FDA). The far-reaching goal of AST is near real-time monitoring of antibiotic resistance in order to improve the clinical outcome of patients. It is possible to envision a future where bacterial infections can be diagnosed in near real-time and reduce our reliance on empirical antibiotic therapies. Improving rapid AST methodologies can improve treatment and personalize medicine of the future.

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APPENDIX A Supporting Information

Table A.1 List of recovered organisms from operating room

Organism	Door Handle	Anesthesia Keyboard	Overhead Light	Mattress	Side Table	Air Sample
Staphylococcus epidermidis	X	X		X	X	X
Staphylococcus aureus			X			
Staphylococcus capitis	X	X	X		X	X
Corynebacterium tuberculostearicum	X	X	X	X	X	X
Staphylococcus hominis	X	X	X		X	
Micrococcus luteus	X		X			X
Staphylococcus lugdunensis					X	X
Staphylococcus auricularis	X	X				
Bacillus firmus			X			X
Paenibacillus spp.		X				
Staphylococcus warneri	X	X	X	X	X	
Dermabacter hominis					X	
Corynebacterium jeikieum						X
Brevibundimonas diminuta				X		
Enterococcus faecalis						X
Kytococcus sedentarius			X			
Staphylococcus caprae						X
Streptococcus salivarius						X
Staphylococcus haemolyticus		X	X	X		X
Streptococcus agalactiae		X				
Proprionibacterium avidum	X					
Streptococcus suis	X					
Mycobacterium africanum						X
Neisseria mucosa						X
Lactobacillus jensenii						X

Pectobacterium cartovorum	X	X				
Streptococcus parasanguinis		X				
Gordonia terrae				X		
Corynebacterium coylae	X					
Bacillus circulans		X				
Pseudomonas luteola		X				
Corynebacterium mucifaciens		X				
Staphylococcus cohnii						X
Staphylococcus simulans				X		

Table A.2 Combined Antibiogram for 2014-2018 *Stenotrophomonas maltophilia*, % Susceptible to One or Two Antimicrobials. Includes both pediatric and adult patients.

N = 534	Ceftazidime (31)	Minocycline (99)	Levofloxacin (69)	Trimethoprim-Sulfamethoxazole (98)	Tigecycline (75)	Colistin (60)
Ceftazidime (31)	—	95	78	98	81	75
Minocycline (99)	95	—	97	99	—	98
Levofloxacin (69)	78	97	—	99	82	89
Trimethoprim-Sulfamethoxazole (98)	98	99	99	—	99	99
Tigecycline (75)	81	—	82	99	—	90
Colistin (60)	75	98	89	99	90	—

Table A.3 Combined *Stenotrophomonas maltophilia* Antibiogram for individual years 2009-2018

Drug / Drug Combination	2009 % S	2010 % S	2011 % S	2012 % S	2013 % S	2014 % S	2015 % S	2016 % S	2017 % S	2018 % S
TMP/SMX / Minocycline	98	100	100	100	99	99	99	99	99	100
TMP/SMX / Levofloxacin	97	99	99	98	94	98	98	99	98	100
TMP/SMX / Ceftazidime	96	98	99	99	96	98	97	97	98	100
TMP/SMX / Tigecycline	98	100	99	97	98	98	98	99	99	100
TMP/SMX / Colistin	96	100	100	97	97	98	99	99	100	100
TMP/SMX / Ceftazidime-Avibactam							96	99	98	100
TMP/SMX / Ceftolozane-Tazobactam								99	98	100
Minocycline / Levofloxacin	95	97	99	97	98	98	95	97	96	99
Minocycline / Ceftazidime	94	95	98	98	97	96	91	96	95	98
Minocycline / Colistin	95	94	97	95	97	97	98	98	98	98
Minocycline / Ceftazidime-Avibactam							91	97	94	98
Minocycline / Ceftolozane-Tazobactam								96	95	98
Levofloxacin / Ceftazidime	80	74	73	80	83	82	78	83	73	72
Levofloxacin / Tigecycline	82	81	83	85	84	75	81	87	80	83
Levofloxacin / Colistin	74	71	74	74	77	81	91	95	92	81
Levofloxacin / Ceftazidime-Avibactam							79	85	76	76
Levofloxacin / Ceftolozane-Tazobactam								82	70	71
Ceftazidime / Tigecycline	84	82	81	85	83	74	76	80	85	84
Ceftazidime / Colistin	51	45	48	57	56	67	73	80	86	66
Ceftazidime / Ceftolozane-Tazobactam								47	38	33
Tigecycline / Colistin	79	78	79	76	77	82	91	91	95	89
Tigecycline / Ceftazidime-Avibactam							77	86	87	89
Tigecycline / Ceftolozane-Tazobactam								80	83	84
Colistin / Ceftazidime-Avibactam							64	88	90	72
Colistin / Ceftolozane-Tazobactam								79	86	65

Table A.4 MIC breakpoints used for each antibiotic

Antibiotic	Breakpoints used
Minocycline	<= 4
Levofloxacin	<= 2
Ceftazidime	<= 8
Trimethoprim-Sulfamethoxazole	<= 2/38
Tigecycline *	<= 2
Colistin ǂ	<= 2
Ceftazidime-Avibactam ǂ	<= 8/4
Ceftolozane-Tazobactam ǂ	<= 4/4

Figure A.1 Trimethoprim-sulfamethoxazole combinations

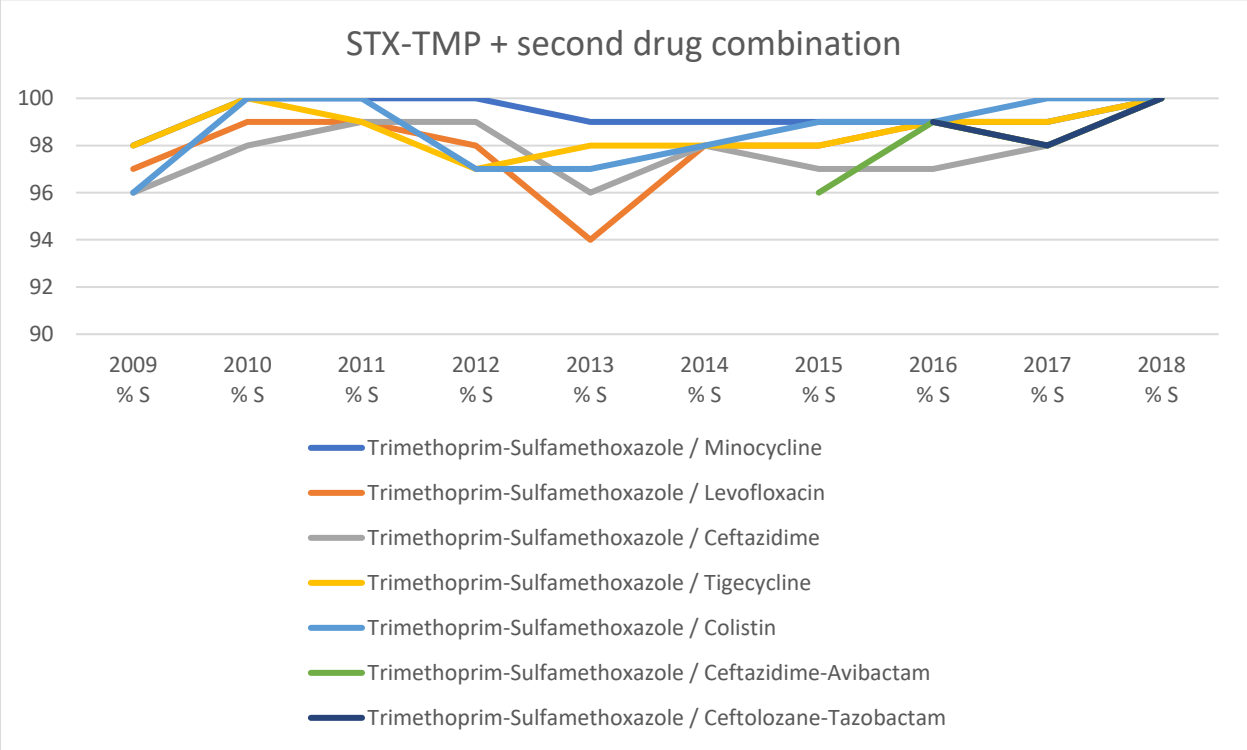

Figure A.2. Minocycline combinations

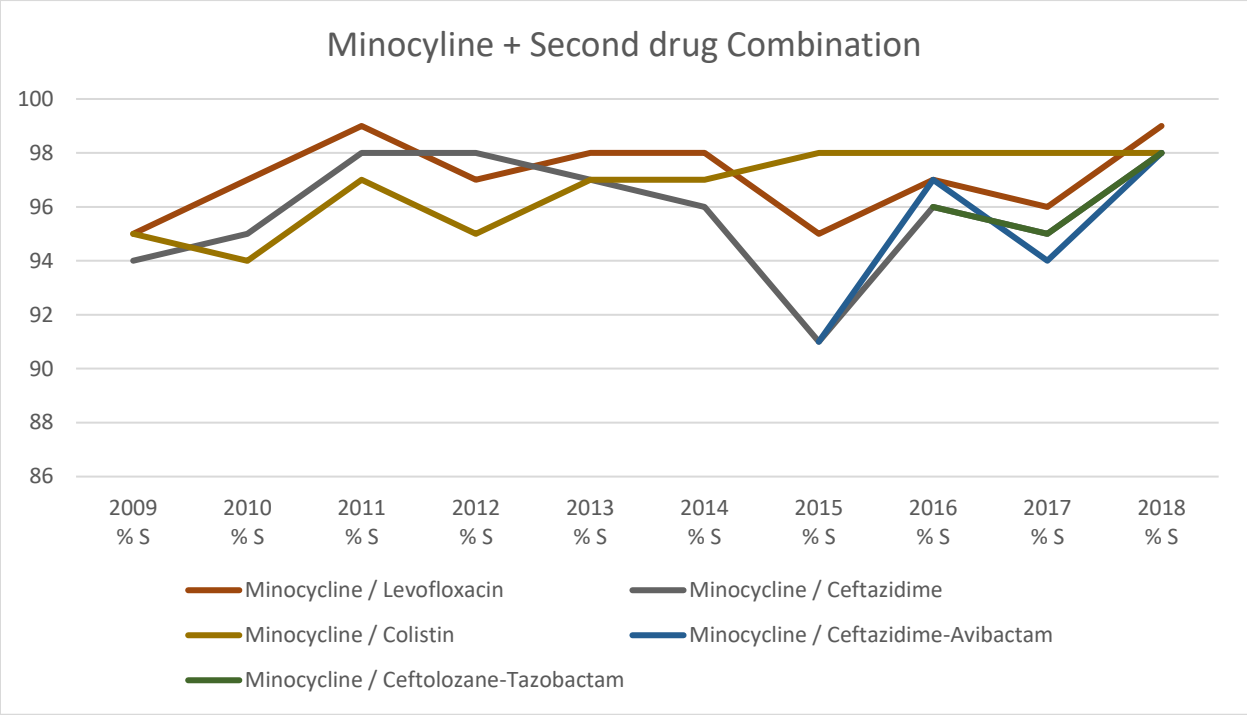

Table A.5 Year to Year Change in Percent Susceptible for Combinations of Drugs for 2009-2018.

Drug / Drug Combination (Total N=1,095)	2009 Δ % S N = 95	2010 Δ % S N = 106	2011 Δ % S N = 120	2012 Δ % S N = 101	2013 Δ % S N = 121	2014 Δ % S N = 101	2015 Δ % S N = 91	2016 Δ % S N = 112	2017 Δ % S N = 132	2018 Δ % S N = 116
TMP/SMX / Minocycline	98	+2	0	0	-1	0	0	0	0	+1
TMP/SMX / Levofloxacin	97	+2	0	-1	-4	+4	0	+1	-1	+2
TMP/SMX / Ceftazidime	96	+2	+1	0	-4	+2	-1	0	+1	+2
TMP/SMX / Tigecycline	98	+2	-1	-2	+1	0	0	+1	0	+1
TMP/SMX / Colistin	96	+4	0	-3	0	+1	+1	0	+1	0
TMP/SMX / Ceftazidime-Avibactam							96	+3	-1	+2
TMP/SMX / Ceftolozane-Tazobactam								99	-1	+2
Minocycline / Levofloxacin	95	-2	+2	-2	+1	0	-3	+2	-1	+3
Minocycline / Ceftazidime	94	+1	+3	0	-1	-1	-5	+5	-1	+3
Minocycline / Colistin	95	-1	+3	-2	+2	0	+1	0	0	0
Minocycline / Ceftazidime-Avibactam							91	+6	-2	+4
Minocycline / Ceftolozane-Tazobactam								96	-1	+3
Levofloxacin / Ceftazidime	80	-6	-3	+7	+3	-1	-4	+5	-10	-1
Levofloxacin / Tigecycline	82	-1	+2	+3	-1	-9	+6	+6	+3	+3
Levofloxacin / Colistin	74	-3	+3	0	+3	+4	+10	+4	-3	-11
Levofloxacin / Ceftazidime-Avibactam							79	+6	-9	0
Levofloxacin / Ceftolozane-Tazobactam								82	-12	+1
Ceftazidime / Tigecycline	84	-2	-1	+4	-2	-9	+2	+4	+5	-1

Ceftazidime / Colistin	51	-6	+2	+9	-1	+11	+6	+7	+6	-20
Ceftazidime / Ceftolozane-Tazobactam								47	-9	-5
Tigecycline / Colistin	79	-1	+1	-3	+1	+5	+9	0	-4	-6
Tigecycline / Ceftazidime-Avibactam							77	+9	+1	+2
Tigecycline / Ceftolozane-Tazobactam								80	+3	+1
Colistin / Ceftazidime-Avibactam							64	+24	+2	-18
Colistin / Ceftolozane-Tazobactam								79	+7	-21

Table A.6 2009-2018 Cumulative Antibiogram, Blood Isolates Only

N = 133	Ceftazidime	Minocycline	Levofloxacin	Trimethoprim-Sulfamethoxazole	Tigecycline	Colistin
Ceftazidime	—	100	83	99	90	70
Minocycline	100	—	100	100	—	100
Levofloxacin	83	100	—	99	89	86
Trimethoprim-Sulfamethoxazole	99	100	99	—	100	99
Tigecycline	90	—	89	100	—	88
Colistin	70	100	86	99	88	—