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EXECUTIVE SUMMARY

VALIDATION OF LACTIC ACID AND PYRUVATE RAPID TESTING DEVICES AS AN EFFECTIVE APPROACH TO DECREASING THE RISE IN MICROBIAL RESISTANCE

Background: In context of escalating deaths due to drug-resistant microbes, multi-faceted approaches have been tried; however, missing is rapid microbial testing. In different aspects of routine patient encounters rapid diagnostics has proven to be an effective strategy in point-of-care decision making for clinicians – example is rapid glycemic levels.

Objective: To assess the effectiveness of the following rapid testing devices in antibiotic prescription:

- Pyruvate Rapid Testing Device. And Lactic Acid Testing Device

Methods: In a span of six months, multi-center study using a population of 400, Clinicians will be provided with testing results on either pyruvate or lactic acid devices and data will be obtained on whether antibiotics were prescribed; this will be confirmed with blood or fluid cultures.

Results: It is well documented that about 50% of antibiotics prescribed are not needed. After adopting this study, retrospective data to suggest a difference or improvement in prescribing habit would validate routine use of such equipment as one modality to solving the problem.

Conclusion: The rise of antimicrobial resistance is on leaps and bounds hence need for different approaches such as rapid bacteria testing. In this project, we seek to use rapid lactic acid and pyruvate testing devices in guiding prescribing habits; this, as one approach to the solving the problem.

Introduction

Definition: Multidrug resistant (MDR) bacteria the eminent bane of human

infection: Antimicrobial resistant bacteria are infectious organisms that have been catalyzed or have naturally metamorphosed evolutionary mechanisms capable of rendering standard care arsenal of antibiotics ineffective resulting in prolonged illness, extended hospital stays with multi-organ septicemia, infection transmission, and increased financial burden on healthcare cost. When current first-line therapies are no longer curative, the costs to human society is grave in resorting to newer generation drugs; a perilous cyclic motion that is reversing gains achieved since the discovery of penicillin in 1928.

Extent of the Problem: The flux of resistance, a current global challenge and a

national trend: Stemming from a plurality of etiologic mechanisms including biologic chemical waste dumping, care dispensing personnel, and a generation of agricultural commercialization the newer microbial resistance threats continue to emerge in rapidity with fewer new therapies. While the chronological documentation of microbial resistance is well-available it is worthwhile on this subject to note that by 2011 and up until now, most of antibiotics had become infective to one or another resistant organism. In feverish search for effective therapy against MRSA, pharmaceutical companies developed what were thought to be impenetrable potent drugs such as Linezolid in 2000. Unfortunately, and as an illustration to this ongoing plague, multiple resistance would be found against it. Resistance to the medication by Extensively Drug Resistant Tuberculosis and Staphylococcus species were uncovered in the year 2000 and 2001 respectively. Vancomycin Resistant Staphylococcus became a major issue in 2002. Daptomycin was introduced in 2003, also thought to be another top-notched drug in infection management against major bacteria. Surprisingly, in 2004/5 species of Acinetobacter and Pseudomonas known to be resistant to all antimicrobials also surfaced. Recurrent fevers,

dehydration, cachexia, line-infections and suffering that drench some patients to death when infected with multi-drug resistant MDRs bacteria leaves a haunting site for any professional in the battle against such organisms. MDR Pseudomonas alone accounts for roughly 400 deaths per year. The estimated yearly cost to the US health system is estimated at \$21 to \$34 billion US Dollars, with a projected 0.4% to 1.6% fall in gross domestic product occurring approximately twelve years ago.

Project Summary

Infectious bacteria establish a niche in human host cells and through the process of glycolysis produce measurable biomarkers that can be used as a targeted laboratory assessment in guiding antibiotic use. Since most superbug resistance has developed after antibiotic use and while appropriate prescribing habits are not completely easy all the time, additional data such as the one proposed in this study will be necessary in prescription-making. For example; it is observed that most people in the Northeast part of the country develop flu-like syndrome and some with oropharyngeal pain in November to February; symptoms mimicking streptococcal infection which if not appropriately treated could lead to rheumatic heart disease in later years. While caution with handing out antibiotics is necessary, without proper testing and ascribing such infections to common cold could also be deleterious to progress made in healthcare. We have already identified lactic acid or lactate dehydrogenase, and pyruvate as byproducts of infectious bacteria. The project will focus on consistent use of these biomarker(s) in rapid decision-making. This testing will also be relevant to all infections for example; back pain with a broad differential diagnosis such as urinary tract infection or diskitis or muscular etiology.

Project Narrative

On the premises of active process of energy production by bacteria using glucose to produce pyruvate and lactate as the fundamental survival mechanism for bacteria to manipulate the host cell and replicate intracellularly, we propose a consistent rapid point of care testing that will

augment current conventional use of physical examination, tachycardia, tachypnea, leukocytosis and fever in antibiotic prescription.

Performance Site, Personnel and Resources

The main project manager is Dr. Adam Au who has affiliations with Jackson Memorial Hospital. The hospital is a large academic teaching facility with both inpatient and outpatient centers. The emergency department and outpatient centers located NW 12th Avenue of Miami, are equipped with well-qualified nursing staff and personnel. In addition, I am also affiliated with Westchester General Hospital and its affiliate Fox Outpatient Medical Center. The outpatient center located in downtown Miami treats a diverse population of children, women, minority groups and would serve as an ideal sample population for the study. In the past, through house calls in Dade County Miami, I have had firsthand experience treating tourist and immigrants from South America and from various parts of the world – most of which were infectious disease-based. As a clinician on the forefront of treating common presentations such as Pharyngitis, Infective Endocarditis, Sepsis, and Cellulitis, I am aware of the problem being confronted. A former Assistant Clinical Professor at Florida International University, and with a strong research background, also current research mentor at the University of Miami Undergraduate Research program, the administrative support, statistical consulting, bioinformatics, is available in nurturing the project to its completion. These institutions have IRBs that strongly adhere to the consenting, data privacy and The NIH protection of human subjects.

Specific Aims

A large array of genetic, physiological and biochemical mechanisms are responsible for the emergence of resistance; and our frustration is that while the problem is on the rise, these generation survival end-biomarkers have not been utilized at point of care testing. For example,

it would be compelling for a house call doctor or traveling health care personnel to prescribe antibiotic for costovertebral tenderness whereas the real etiology could be neoplastic.

Specific Aim 1. A study of Routine Incorporation of Lactic Acid / Pyruvate Kinase at Point of care testing.

To substantiate an infectious process, inexpensive and rapid testing can aid in the decision process. We will determine the ability to regularly incorporate Lactate and or pyruvate at all patient visits and how that will influence prescriptions.

Specific Aim 2: Determine the effective Method of using lactate and pyruvate at patient encounters

In the triage process of patient care, the current status quo is to obtain vital signs, while the ordering of lactic acid is usually the clinicians discretion, but it is noteworthy that infectious process is always a major differential diagnosis consideration in most chief complaints in care settings. In this study, as taking vital signs we will include either of pyruvate or lactate in the triage process.

Specific Aim 3: To determine which of lactic acid and pyruvate as an effective marker of infection.

There can be false positives in using bacteria end-product biomarkers. Some non-infectious causes of lactate elevation include drug effects, seizures and malignancy. This underlies one of the major aims of this study in distinguishing which of lactate and pyruvate is a better litmus test for ongoing infection.

The Research Strategy; Significance.

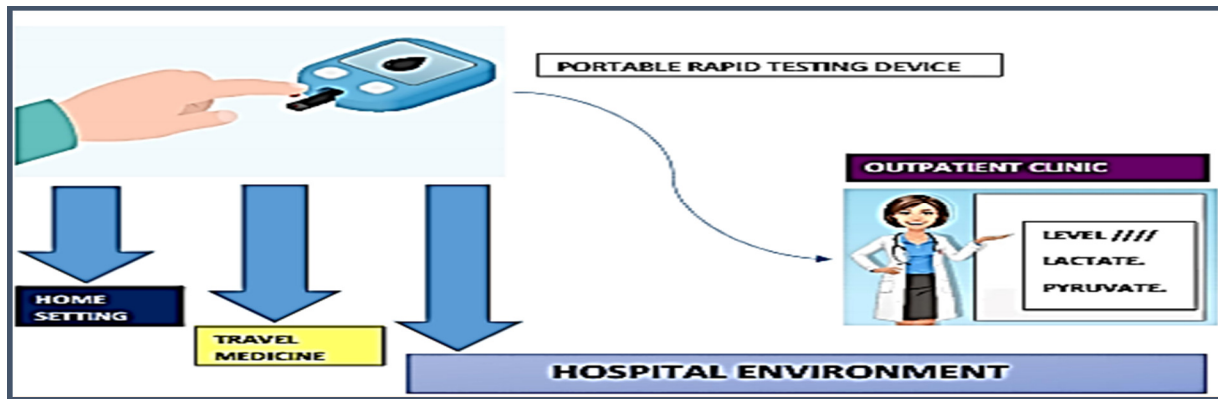
The complex pathophysiology of the current rapid increasing resistance prevalence is multifactorial. Random mutations leading to modified catalyst, proto -or quasi intrinsic

resistant genes, antibiotic -catalyzing process, degradation/r genes in the environment with the involvement of soil bacteria as well as the role of plasmids, phages, all well-known machinery to resistance. Lactate is a biochemical end-product produced from the reduction of pyruvate by lactate dehydrogenase. In normal adults, lactate accounts for less than 2 mmol/L. However, in tissue hypoxia, and hypo- perfusion as in acute bacterial infection illness, the curve is shifted to production of more pyruvate and lactate. Many anthropogenic activities are implicit in creating the selection pressure for bacterial resistance such as mass release of antibiotics into soil, large biocide disposal toxic waste, house -hold sanitizers, and inadvertent inappropriate antibiotic prescribing. A problem on a global scale, some of the measures that have been proposed are: aggressive national and global surveillance of antibiotic use with immediate interventions, proper collection of data with use of antibiotics in all relevant sectors, antibiotic- free farming, proper disposition of pharmaceutical waste, and limiting the use of last-resort antibiotics unless very necessary in complex refractory infection in humans. In spite, it simply has not been feasible to routinely use the above-mentioned biomarkers as effective tools in guiding antibiotic prescription decision- making at point of care. Here we propose an effective method that will halt reducing the efficacy of antibiotics. Once we have identified its effectiveness, we will adapt the device that will be completely usable on a large scale. As a long-term goal, we envision to support the global adaption of such a device in applicable areas of the world where resistance is rampant.

Innovation

In bacterial infection, the assimilation reaction required for growth and maintenance involves the use of one molecule of glucose converted to glucose 6 phosphate and via ATP is converted to fructose 6 phosphate. Via multiple intermediate products and pathways Pyruvate enters the TCA cycle. Pyruvate has 5 potential end products; the focus of our rapid point of care testing in

this project is lactic acid. Although the technology is available in detecting levels of these biomarkers, their application in daily clinical use has yet to be fully investigated.



APPROACH: APPLICABILITY OF LACTATE AND PYRUVATE DEVICES IN VARIOUS PRACTICE ENVIRONMENTS.

In our initial assessment, Sergio Monteriro et al. offers a comparison of two main portable analyzers and supports their use. In testing, a finger prick of 1 ml of blood is placed on a lactate test strip. In the prior studies, while one device completed the analysis in 60 seconds by measuring lactate directly, the other device converts lactate to pyruvate and produces the assay results by color change measured by a wavelength of 520nm. Other studies have confirmed the sensitivity of such devices. In our calibration, a standardized solution of lactate would be used periodically to ensure accuracy of each measurement. Results could directly be transmitted to the doctor's view of the patient's record in a secured fashion which would also be tested.

Conclusion

Empiric antibiotics not only cause emergence of microbial resistance, but also *Clostridium difficile* infection, and nephrotoxicity. Ultimately the decision to administer antibiotic is one that is made by the clinician in the integration of multiple data such as physical exam at point of care. Rapid testing devices such as the one proposed in this study will provide an essential additional information in guiding doctors when it comes to making such crucial decisions.

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