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Survivability of Bacteria on Blood Glucose Testing Strips

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Abstract

Objective: Our research focus is to determine exactly how long clinically significant organisms, *Escherichia coli* 0157:H7, *Pseudomonas aeruginosa*, methicillin resistant *Staphylococcus aureus* (MRSA), and vancomycin resistant *Enterococcus faecalis* (VRE) survive on blood glucose testing strips.

Design: Four separate tubes of trypticase soy broth (TSB) were inoculated with each of the chosen test organisms and then incubated at 37°C overnight. The next day they were removed from incubation to slow down their growth. To determine the number of colony forming units (CFU) in each sample, dilutions of each organism were plated onto Mueller Hinton agar. The dilution with the most reliable colony count was used to calculate the dilution needed to create a 100,000 CFU/mL of phosphate buffered saline (pH 7.2) organism load. The blood glucose testing strips were inoculated with 10µL of inoculate at the non-electrical end of the strip and 10µL of inoculate was pipetted onto the blood collection site directly for a total of 20µL. Every day thereafter, a strip corresponding to each organism was pressed to a designated section on a CHROMagar™ plate for 30 seconds and then removed in order to replicate how long a blood glucose test strip would be handled in a clinical setting. The plate was then incubated at 37°C for 24 hours and observed for growth. Above the strip placement site, a reference sample consisting of a pure culture of each organism was swabbed onto the agar as a positive control. The phosphate buffered saline diluent served as a negative control.

Setting: This research took place in the Medical Laboratory Science Program laboratories at Salisbury University, Maryland.

Results: Each organism survived as follows: *Escherichia coli* 0157:H7, only one colony per day for days 42-45; *Pseudomonas aeruginosa*, colonies were too numerous to count for the first five days and then their number greatly declined to less than five colonies until day 11; methicillin resistant *Staphylococcus aureus* (MRSA), colonies were too numerous to count; and vancomycin resistant *Enterococcus faecalis* (VRE), colonies were also too numerous to count.

Conclusion: Even though the surfaces of a blood glucose strip are non-nutritive and desiccated, clinically significant organisms survive for many days, making these strips a potentially important source of infection when they become contaminated.

Abbreviations:

- UTI: Urinary Tract Infection
- CFU: Colony Forming Unit
- TSB: Tryptic Soy Broth
- MRSA: Methicillin Resistant *Staphylococcus aureus*
- VRE: Vancomycin Resistant *Enterococcus faecalis*
- MDR: Multi-drug resistant
- ICU: Intensive Care Unit

Index terms: Blood glucose test strip, Methicillin Resistant *Staphylococcus aureus,* Vancomycin Resistant *Enterococcus faecalis, Escherichia coli* 0157:H7 *Pseudomonas aeruginosa,* Survivability, Fomite, Hospital acquired infection, Diabetes.

Introduction

Blood glucose test strips and meters are devices regulated by the U.S. Food and Drug Administration (FDA). Diabetic patients may need to have their blood glucose tested anywhere from once a day, to seven times a day depending on the status of their health. Whether the patient is newly diagnosed with diabetes, has type 1 or type 2 diabetes, or is currently prescribed insulin determines how often blood glucose tests need to be performed. Diabetic patients that monitor their glucose from personal glucometers at home likely have a lower risk for microbial contamination of the devices, but there is a higher potential for glucometers used on multiple patients in healthcare settings to be contaminated by pathogenic organisms.

The possibility of contamination of blood glucose test strips by pathogens is a growing concern which does not currently have much quantifiable data. Blood glucose test strips are packaged in vials containing approximately twenty strips and are carried by healthcare staff to multiple patients throughout their work day. These test strips are commonly used in institutions such as hospitals, outpatient clinics, nursing homes, and schools[1]. Blood glucose test strips were not designed to be used more than one time and are manufactured with only enough enzyme for a single use. The way these blood glucose test strips are used, health care professional reaching into the vials repeatedly, creates the possibility for contamination. Health care professionals are often required to wear gloves, however if their gloves are not changed between different patients there is a possibility for the spread of bacteria.

Healthcare workers not only contaminate their hands after direct patient contact but also after touching inanimate surfaces and equipment in the patient area. Inadequate hand hygiene before and after entering a patient area may result in cross-transmission of pathogens and patient colonization or infection[2]. When patients have their blood glucose tested they have an open wound on their fingers which allows for the introduction of microorganisms. Poor sanitation

procedures for handling blood glucose test strips could result in cross-contamination of pathogens and patient infection

Recently, two different studies, one by Pérez-Ayala and colleagues[3] and the other by Vanhaeren and colleagues[4] were published that demonstrated blood glucose strip contamination in healthcare settings. Although both of these studies used different methods to recover and identify organisms, both found positive contamination of glucose test strips. These studies looked at glucose strip contamination risks across multiple hospital areas and multiple users.

The study performed by Pérez-Ayala and colleagues[3] found a higher percentage of contaminated strips in multi-use blood glucose vials versus individually packaged. The study cultured glucose test strips from open vials after vortexing in sodium chloride. They identified a variety of pathogenic and non-pathogenic organisms, mostly consistent with human skin contamination such as *Staphylococcus aureus*, *Staphylococcus epidermidis* strains, and methicillin-resistant *S. aureus* (MRSA).

The Vanhaeren and colleagues[4] study cultured 100µL of their suspension (test strip vortexed with 0.9% NaCl) onto Colombia colistin nalidixic acid and Drigaslki agar and they used additional selective media for possible multi-drug resistant (MDR) organisms or *Clostridium difficile* carriers. Columbia colistin nalidixic acid agar selects for Gram positive bacteria and differentiates by hemolysis patterns[5]. Drigaslki agar is used as a selective differential medium for Gram negative rods (Enterobacteriaceae and certain non-fermenters) and is inhibitory to Gram positive bacteria[6]. This study obtained similar results to the Pérez-Ayala study. Both studies found strip contamination from commensal skin organisms and MDR organisms in multiple areas of the hospital. It may seem counterintuitive that blood glucose test strips are stored in vials with risk for contamination, but it is because individual packaging of

glucose strips would generate a large amount of unnecessary waste which would also result in elevated prices[1]. High prices for glucose strips could be unfavorable for diabetic individuals who may have limited income as well as healthcare systems since diabetes is rather common.

The relevance of this research includes determining a quantifiable risk to patients for exposure to dangerous pathogens that have the potential to cause harm. Poor sanitation procedures for handling blood glucose test strips could result in cross-contamination of pathogens resulting in patient infection. There are numerous studies of inanimate objects harboring organisms in healthcare. For example, Vincenzo and colleagues[2] conducted a study to determine the degree of bacterial contamination in an Intensive Care Unit (ICU). This study examined various surfaces and equipment such as bedrails, stethoscopes, medical charts, ultrasound machines, and several others that could be contaminated by bacteria, including MDR isolates. This study found that various surfaces and equipment in the ICU were heavily contaminated with bacteria, including several MDR species. The source of this contamination was either a result of transmission of bacteria from healthcare workers' hands or by direct patient shedding of bacteria[2]. To date, only two studies have evaluated contamination of glucose testing strips, and we could find none that evaluated the length of time that organisms survive on these objects.

When it comes to the spread of healthcare-associated infection, contaminated objects are generally a concern, but since blood glucose measurement requires puncturing the patient's skin, creating an open wound, and touching the strips to the wound, any contamination on the testing strip is more likely to cause infection. The goal of the Pérez-Ayala and colleagues[3] study was to simply identify the presence of microorganisms and considered ≥2 colony forming units (CFUs) per test strip a positive result. Therefore, the amount of time a pathogenic microorganism could

survive on the test strips remains unknown. Glucose test strips contain no nutritive media to promote organism survival, so it is possible that their presence is merely transient. Our research focus was to determine exactly how long four clinically significant organisms; *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* 0157:H7, and Vancomycin Resistant *Enterococcus faecalis* (VRE), can survive on these blood glucose strips.

Materials and Methods

- *Pseudomonas aeruginosa*: ATCC 27853
- Methicillin Resistant *Staphylococcus aureus* (MRSA): ATCC 43300
- *Escherichia coli* 0157:H7: clinical isolate
- Vancomycin Resistant *Enterococcus faecalis* (VRE): ATCC 51299
- Tryptic Soy Broth (TSB): BBL™ Becton Dickinson, Cockeysville, MD (2mL culture tubes)
- Chemicals used for Phosphate Buffered Saline (pH 7.2)- Fisher Scientific
    - Dipotassium Hydrogen Phosphate (0.00047g/L)
    - Potassium Dihydrogen Phosphate (0.282g/L)
    - Sodium Chloride (8.5g/L)
- Remel Mueller Hinton Agar, Hanover Park, IL
- CHROMagar™ Orientation MH, Kentwood, MI

- Accuchek "Comfort Curve", Roche Diagnostics, Indianapolis, IN Blood Glucose Testing Strips

The four organisms chosen for this research are all clinically significant pathogenic bacteria that are commonly isolated in hospital acquired infections. MRSA is found as a normal commensal organism on the skin, but whenever there is a cut or break in the skin it can cause infection. MRSA is very transmissible and can be spread through direct contact with a person who has the bacteria. *E. coli* 0157:H7 and VRE are both pathogenic bowel flora and can cause severe, acute hemorrhagic diarrhea and abdominal cramps, severe urinary tract infections (UTI's) or blood stream infections. *Pseudomonas aeruginosa* is an environmental pathogen that is a common cause of pneumonia and wound infections in hospitalized patients and or/ patients with weakened immune systems. For our research we also wanted to work with two Gram negative organisms, with at least one being a non-fermenter, and two Gram positive organisms in order to be representative of some of the major groups of microorganisms.

Figure 1 Inoculation Locations: The red circles indicate the inoculation point on the test strip. The lower red circle (A) is on the area of the strip where the patient's blood sample is drawn into

the strip (A) to be detected by the electrode (B) which inserts into the glucometer. The higher red circle (C) was the second inoculation point on the test strip and is where the strip is typically grabbed to remove it from the vial.

Colony Counts

We inoculated four separate tubes of TSB with each of our test organisms and then let them grow at 37°C overnight. We removed them from incubation to slow the growth. In order to determine the dilution required to obtain a 100,000 CFU/mL organism load we first made serial dilutions from a TSB culture in the following increments: 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, 1:200,000, 1:400,000, and 1:800,000 using phosphate buffered saline (pH 7.2) as a diluent. We then used a 0.001mL calibrated loop to create streak plates of the dilutions on Mueller Hinton plates for colony counting purposes. These plates were then incubated at 37°C for 24 hours. After this incubation period the colonies for the dilutions of each individual organism were counted. The dilutions with the most reliable colony count, where the colonies were nicely separated and able to be accurately counted, for each organism were used to calculate the dilution needed to create a 100,000 CFU/mL organism load.

Inoculation and Observation

The blood glucose testing strips were inoculated with 10μL of inoculate at the non-electrical end of the strip and 10μL of inoculate was pipetted onto the blood collection site directly for a total of 20μL (See Figure 1). A total of 45 strips were inoculated to determine survival time and allowed to sit at room temperature. Every day thereafter for 45 days, a strip corresponding to each organism was pressed to a designated section on a CHROMagar™ plate for 30 seconds (touch plate/prep method) in order to replicate how long a blood glucose test strip

would be handled in a clinical setting. The plate was then incubated at 37°C for 24 hours and observed for growth. Each organism grows as a different color on the CHROMagar™ medium; yellow for MRSA, blue for VRE, pink/mauve for *E. coli*, and green for *Pseudomonas aeruginosa*. This color specificity for each organism allows for prevention of misidentification from contaminant organisms. Any observed contaminants grew as colors different from the tested organisms such as white, or bright yellow. A line was drawn across the plate and marked to indicate the placement of each inoculated strip. Above where each strip was inoculated onto the agar, a reference sample consisting of a pure culture of each organism was swabbed onto the agar as a positive control (See Figure 2). This allowed us to determine the extent of growth/survival monitored over time at each inoculated site after strip removal. The positive controls were used to confirm that the colors were behaving correctly with the CHROMagar. The negative control was a spot on the bottom of the plate of uninoculated phosphate buffered saline (pH 7.2).

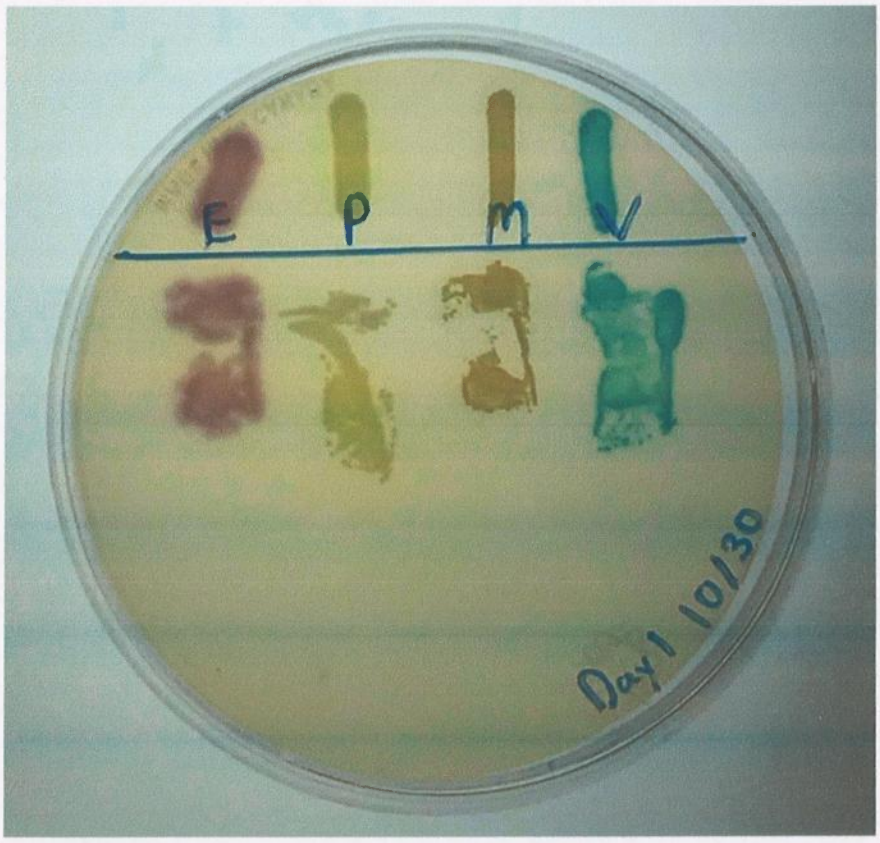

Figure 2 Inoculated CHROMagar Plate: The organisms shown in order are *E. coli* 0157:H7 (E), *P. aeruginosa* (P), MRSA (M), and VRE (V). The growth above the line are the positive controls and the growth inferior the line is from the inoculated test strips. The two different areas of the strip that were inoculated is most clearly seen with the *E. coli* and MRSA strips.

Results

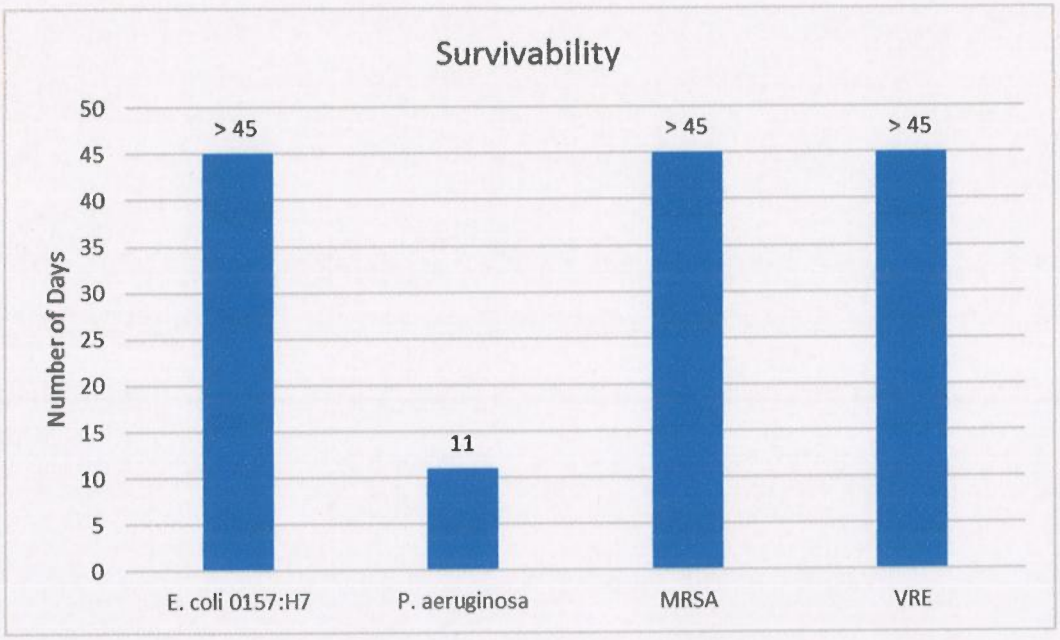

Figure 3 Organism Survival Time: The Gram- negative organisms, *E. coli* 0157:H7 and *P. aeruginosa*, had survival times of greater than 45 days and 11 days, respectively. It should be noted, however that for the last 4 days, only one colony of *E. coli* grew. The Gram- positive organism's, MRSA and VRE, both had survival times greater than 45 days, and growth on day 45 was still significant. From these results we can conclude that *E. coli* 0157:H7, MRSA, and VRE are very resilient and able to survive without any provided nutrients almost a month and half while *P. aeruginosa* only survived for about a week and half.

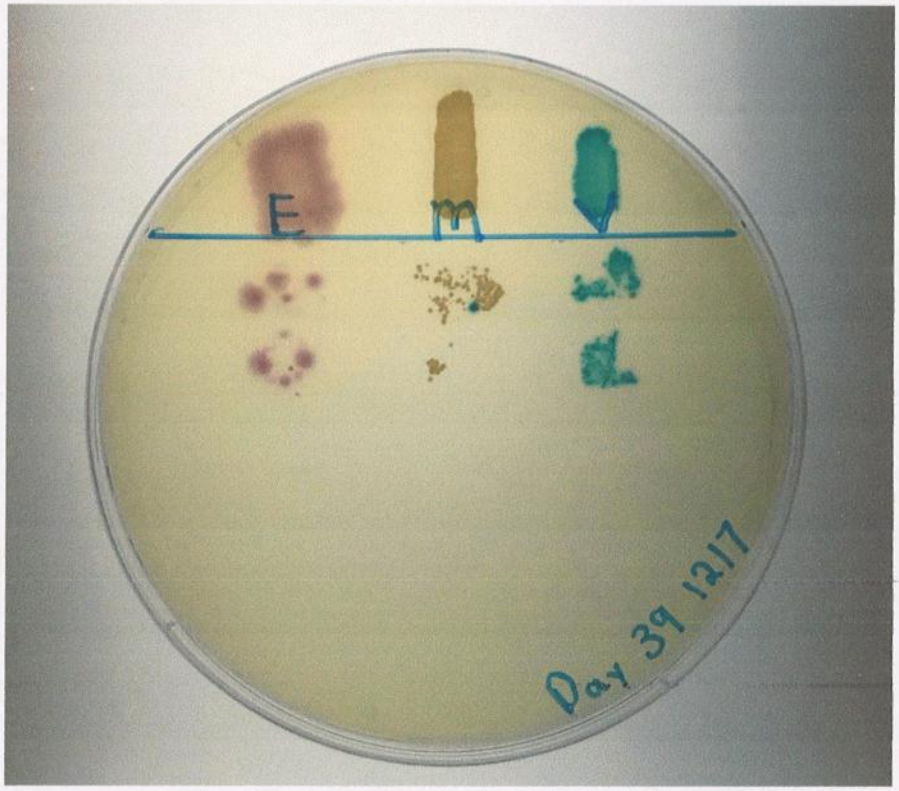

Figure 3 Inoculated CHROMagar plate from Day 39 survivability: This plate shows distinct areas of growth that correlate to the two different inoculation areas on the test strip. This separation however, was not distinct enough 11 days of *P. aeruginosa* survival to draw conclusions regarding differential survivability of *P. aeruginosa* at the two inoculation sites.

Discussion

Although our search was not exhaustive, we were unable to find the infectious dose for our chosen organisms, so we decided to use an organism load of 100,000 CFUs/mL which is the level of bacteria considered clinically significant for a UTI. 100,000 CFUs/mL is considered significant bacteriuria and is very specific for the diagnosis of a true infection. The concentration of organism that human beings could reasonably be expected to introduce onto a glucose strip is the amount of organism they are shedding as normal flora or from an infection. We cannot determine an exact number for the number of microbes a person is shedding because it varies

between each individual. For patients using the glucose strips, the source of microbes is unwashed hands, so the most reasonable source of these microbes is from people going to the bathroom. Hence, a number was chosen that is associated with true infections and provided a level for standardization. A standard inoculum for studies regarding this type of work has not been published, so we needed to choose an inoculum that was the most realistic worst-case scenario.

We inoculated two different places on the test strips with 10μL (20μL total on each strip) in order to see if the organisms would grow better on the end of the strip, where the user would typically grasp the strip when removing it from the vial, or the area where the blood drop is drawn into the strip. We specifically chose 10μL because it was sufficient enough to show results as well as fit onto the designated area of the strip (see Figure 1). In addition, 10μL is barely discernable as a drop, so it could be reasonably missed as a contaminant on the hands. We placed the strips in contact with the CHROMagar plate for 30 seconds as a realistic risk assessment for diabetic patients having their glucose tested in a healthcare setting with these strips. The touch plate method allows us to visualize the organisms' growth and therefore their ability to survive on the strips over time. We used phosphate buffered saline to prepare organism suspensions as it is pH neutral and not osmotically damaging to organisms while also being non-nutritive. Sources of contamination (skin, urine, feces, etc.) would also not be typically nutritive, (although diabetic urine can technically be nutritive), and we did not want any enhancement of growth from our diluent.

The touch plate method for organism inoculation is a realistic way to replicate the transfer of organisms to a glucose test strip by touching. The previously mentioned study by Vanhaeren and colleagues[4] recovered their microorganisms by placing the glucose test strips in sodium chloride

and vortexing for thirty seconds to then plate the inoculum onto agar. This is a more aggressive method for removal or microorganisms which likely overestimates the amount of bacteria that would transfer by a simple touch.

Even though a 100,000 CFU/mL is a clinically possible organism load, it is realistically higher than the amount of normal flora that would be shed from healthy individuals. The next phase of this research would be to test the longevity of these clinically significant microorganisms with lower organism loads, as well as testing the survivability of other organisms. In addition, we could repeat this study inoculating only one site on the strip so that we can get reliable data as to whether the survivability on the two sites is different.

Our results indicate that there is a clear risk of glucose strip contamination with the four tested microorganisms. There is a possibility that individually packaged strips may be necessary in health care settings in order to minimize these risks. There has been no direct evidence of infections definitively linked to glucose strips, which leads to the question of whether or not glucose test strips were overlooked as the cause of infection in a patient since there are so many other modes of transmission for infection within the hospital setting.

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