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Investigating The Viability Of Two *Stenotrophomonas maltophilia* Isolates After Air-drying

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ABSTRACT *Stenotrophomonas maltophilia* is a water-borne infectious bacterium that is found in both clinical (hospitals) and non-clinical environments. This human pathogen is commonly recovered from respiratory tract infections. A recent study at a hospital in Taiwan suggested that dry patient charts can serve as a vehicle of transmission of this bacterium[7]. As *S. maltophilia* is not commonly isolated from dry surfaces, this current study tested the hypothesis that this pathogen can remain viable for some time on a dry surface. This study was designed to determine how long *S. maltophilia* could remain viable after air-drying by observing culture growth from paper disks that had been inoculated with the bacterium. The data obtained indicate that *S. maltophilia* can remain viable up to two days, providing novel information regarding the amount of time that this water-associated pathogen can survive in dry conditions.

INTRODUCTION

Stenotrophomonas maltophilia is a waterborne opportunistic bacterial pathogen, and it has in recent years become recognized as a nosocomial (hospital-acquired) pathogen that has been associated with mortality rates that range from 14 to 69 % in patients with bloodstream infections[1]. This organism is a significant threat to those who have compromised immune systems, such as cancer patients and patients with cystic fibrosis. The most common infections associated with *S. maltophilia* include those of the respiratory tract (e.g. pneumonia), bloodstream, urinary tract, eye, heart, and brain[1]. This bacterium exhibits multidrug resistance due to expression of multidrug resistance pumps, and other molecular mechanisms (e.g., plasmids that house antibiotic resistant genes)[6]. This multidrug resistance is of concern when patients with an *S. maltophilia* infection need treatment.

Due to the continuing rise in drug resistance observed in *S. maltophilia*[5], it is important to gain a better understanding of the mode of transmission of this pathogen, as patients who run the highest risk of *S. maltophilia* infection will most likely spend time in a clinical setting. Isolates of *S. maltophilia* have been recovered from both clinical and non-clinical settings. In clinical settings, *S. maltophilia* has been recovered from nebulizers, central venous catheters, irrigating solutions, and on hand soap. In non-clinical settings, *S. maltophilia* has been found in soil and on plant roots, in contact lens stock solutions, on faucets, in sink drains, and in tap water[1]. In addition to the various environments that may support the growth of *S. maltophilia*, it is also efficient at adhering to moist plastic surfaces and forming bacterial films (biofilms)[1].

The transmission of *S. maltophilia* to individuals who are susceptible occurs

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through direct contact with the bacterium[1].
A recent study conducted at an intensive care unit in Taipei, Taiwan, reported that contaminated patient charts served as a sufficient vector in the transmission of this bacterium[7]. They observed that there was cross-infection with *S. maltophilia* between the patient and their respective chart.[7] The same antibiotic resistance profile as *S. maltophilia* isolates was identified on the contaminated patient charts. This transmission was likely due to lack of proper hand sanitation by the hospital personnel between the treatment of patients[7].
The objective of this current research study was to analyze the viability of *S. maltophilia* on a dry surface over time. As far as the authors are aware, to date this is the first study to examine the effect of air-drying on *S. maltophilia* viability. The hypothesis tested in this study is that isolates of *S. maltophilia* are able to survive in dry conditions for some length of time.

METHODS

The experiments were conducted using a series of qualitative and quantitative tests. Two *S. maltophilia* isolates were chosen for this study: a bloodstream isolate, X26332, and a respiratory isolate, H43306. Both were obtained from a culture collection at a teaching hospital in Chicago, IL. These two *S. maltophilia* isolates were chosen specifically because each one represents isolates that are commonly recovered from bloodstream and respiratory tract infections. Study 1 of the experiment was carried out to observe only qualitative results. Study 2 was comprised of both qualitative and quantitative analyses. The experiments were performed in triplicate with each *S. maltophilia* isolate.

Study 1: Qualitative Analysis

Overnight cultures of H43306 and X26332 with three 5 mL subcultures for each isolate were incubated for 24 h at 37 °C while shaking at 230 rpm. Each of the subcultures was added to separate sterile tubes containing 1 mL of a 10 % glycerol solution. Each tube was standardized using a McFarland Standard in order to achieve uniform cell turbidity. The cell mixture was then inoculated onto sterile filter paper disks (6 mm) with 5 μL per disk. Two time points were recorded: at time zero and at 30 minutes. The disks were allowed to air dry at room temperature. Immediately after all disks were completely dry (by visible inspection), one disk representing each of the subcultures was dropped into a separate tube that contained 5 mL of nutrient broth; this time point was considered time zero. The subsequent "disk drop" (the point at which each filter paper disk was dropped into each tube) was made 30 minutes after time zero. A negative control tube that contained 5 mL of nutrient broth and a disk inoculated with 5 μL of the 10 % glycerol solution (without *S. maltophilia*) was also made for each time point.

Study 2: Qualitative and Quantitative Analyses

All steps necessary to prepare the disks in Study 1 of the experiment were repeated for Study 2. A total of 8 disks for each subculture were inoculated and stored in separate sterile Petri plates for the duration of the study. The "disk drop" procedure necessary for the collection of qualitative data was performed on days 0, 1, 2, and 4. In addition to the qualitative data, quantitative data were collected for Study 2. Serial dilutions of the cultures arising from the rehydrated disks were made so that the number of surviving bacterial cells (colony-forming units) could be determined.

In order to perform the serial dilutions, disks were dropped into microcentrifuge tubes containing 1 mL of nutrient broth; each disk was dropped into a separate tube. Each tube was vortexed for 1 minute, and 100 μL were removed using a micropipette and added to a subsequent tube that contained 900 μL of nutrient broth; this constituted the 10^{-1} dilution. This technique was repeated by vortexing each tube for 1 minute, and removing 100 μL in order to make the next

dilution. A total of 5 serial dilutions were performed for each subculture used in the study (10^{-1} - 10^{-5}.) On days 0 and 1, dilutions 10^{-3}, 10^{-4}, and 10^{-5} were plated for quantitative analysis; on day 4 dilutions 10^{-1}, 10^{-2}, and 10^{-3} were plated. For each dilution, 100 μL were removed by micropipette, added to a nutrient agar plate, and spread over the plate with a sterile disposable loop.

In addition to the qualitative and quantitative tests performed on day 4, a culture purity test was also carried out. This was accomplished by selecting one subculture from each isolate of *S. maltophilia*; a sterile disposable loop was used to streak over a fresh nutrient agar plate to check for the presence of isolated pure colonies of *S. maltophilia*.

RESULTS

Study 1

For disks dropped at time zero and 30 minutes, growth was observed in all subcultures for both *S. maltophilia* isolates at 24 h incubation (Figure 1).

Study 2

For disks dropped on day 0, growth of all subcultures from both isolates of *S. maltophilia* was observed at 24 h of incubation (Table 1). Colony growth was observed in every plate in the dilution series (10^{-3},10^{-4},10^{-5}) for all subcultures (Figure 3A). For disks that had been dry for one day, growth was not observed until 36 hours of incubation; although growth was positive (Table 1), there was an approximate 98 % decrease in the number of viable *S. maltophilia* X26332 (a reduction from 1.5 x 10^5 to 0.2 x 10^4 cells per mL), and H43306 (a reduction from 2.7 x 10^5 to 0.4 x 10^4 per mL) cells respectively (Figure 2). For disks that had been dry for two days, there was no visible cell culture turbidity for any of the subcultures at 24 h of incubation. At 44 h of incubation all subcultures of both isolates showed turbidity (Table 1). Culture purity was determined using a test based on similar colony morphology. This test showed that these cultures contained only *S. maltophilia* cells and no contamination (Figure 3B). For disks that had been dry for four days there was no visible growth at 48 and at 72 h incubation. (Table 1).

DISCUSSION

The results of this research study support the hypothesis that *S. maltophilia* isolates can survive in dry conditions for some length of time. Furthermore, the results of this study are in agreement with the study by Teng *et al.*[7] that reports that *S. maltophilia* remains viable after air-drying.

In Study 2, cell culture growth was delayed for each additional day of drying. There are two possible explanations for this phenomenon. The first explanation is that the process of air-drying put the bacterial cells under a considerable amount of stress; this has been observed for *Pseudomonas aeruginosa*[3], a bacterium related to *S. maltophilia*. As a result, time was needed for cells to recover prior to the re-initiation of growth. The second explanation is that the cells entered into what is known as a 'viable, but non-culturable' (VBNC) state[4]. Studies have shown that many pathogenic bacteria are capable of entering such a state[4]. These bacteria have the ability to become dormant without undergoing changes to physical cell structure; once the cells are reintroduced to appropriate conditions, VBNC microbes will resume growth[4].

Other bacterial species that have been known to enter this state include *Vibrio cholerae, Salmonella enteriditis, Shigella dysenteriae,* and *P. aeruginosa*[4]. *P. aeruginosa* is of particular interest as in the past this bacterium and *S. maltophilia* were members of the same genus[1]. *P. aeruginosa* is commonly found with *S. maltophilia* in lung infections of cystic fibrosis patients and those individuals with other lung disorders[1].

Perhaps the most important question is whether VNBC cells have the ability to cause infection and disease. Studies have shown that cells in a VBNC state are able to cause disease once reintroduced to suitable conditions[4]. Therefore, as long as

nosocomial pathogens have the ability to persist on inanimate surfaces, they may serve as a continuous source of transmission to susceptible individuals[2].

A study by Kramer *et al.*[2] compiled an extensive list of nosocomial pathogens that have been isolated from inanimate surfaces in a clinical setting, along with the length of time each pathogen could persist on those surfaces. *S. maltophilia* was not included in this list. The results of this current study suggest that the list may need to be revised as *S. maltophilia* also has the ability to persist for up to 2 days on dry filter paper. To date this is the first study to observe this organism on dry paper surfaces. Observations made in this study stress the importance of educating both the public as well as those within the medical/clinical settings about the potential for the transmission of these serious bacterial pathogens either directly or indirectly to susceptible individuals.

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Table 1: Qualitative Analysis for Study 2

Isolate	Day 0	Day 1	Day 2	Day 4
H43306 Sub 1	+	+	+	_
H43306 Sub 2	+	+	+	_
H43306 Sub 3	+	+	+	_
X26332 Sub 1	+	+	+	_
X26332 Sub 2	+	+	+	_
X26332 Sub 3	+	+	+	_

Cell viability of *S. maltophilia* isolates over time on dry filter paper.
+ Indicates positive for growth; - is an indication of no growth. Three subcultures were tested for both *S. maltophilia* isolates. After 24 h, 36 h, and 44 h incubation of the disks in nutrient broth at 37ºC, growth was positive for inoculated disks that had been dry for 0 minutes (day 0), 24 h (day 1), and 48 h (day 2), respectively.

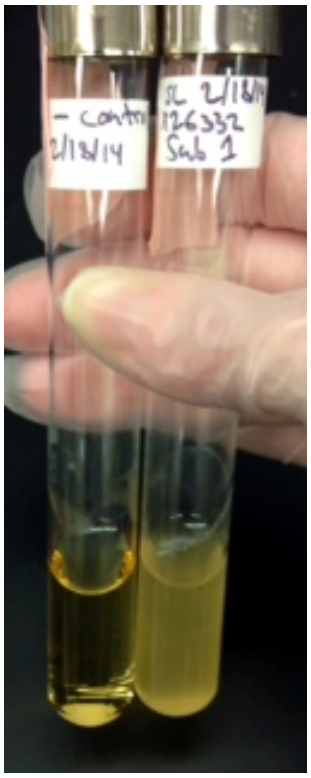

Figure 1: Qualitative results from Study 1. Figure 1 displays the indication of a positive result for the qualitative analysis portion of the experiment. The tube on the left side of the image is the negative control; the tube on the right side of the image shows the X26332 isolate, subculture 1.

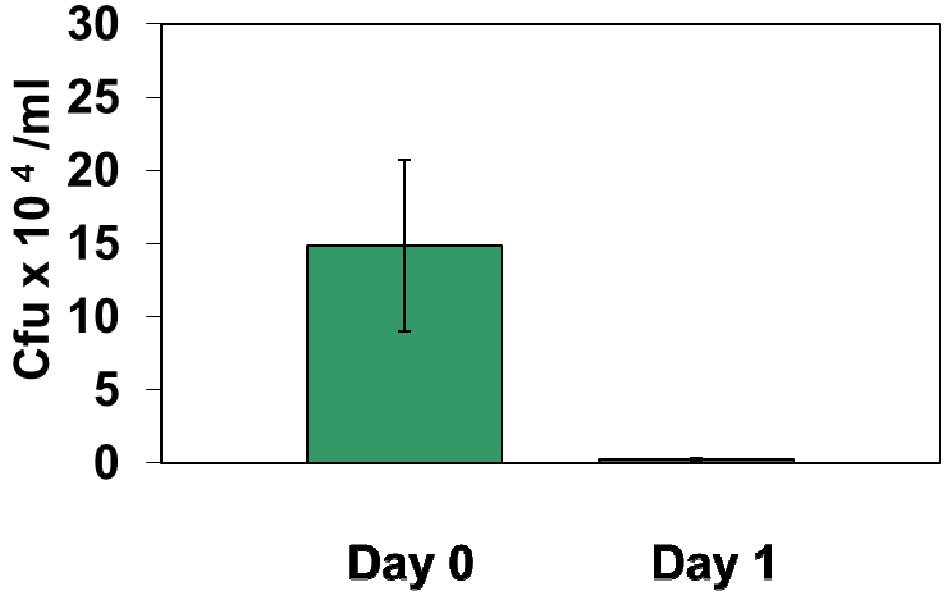

Figure 2A.
Cell viability of *S. maltophilia* X26332 after 0, 1 days on dry filter paper. Cfu, colony-forming units. Vertical bars represent standard error.

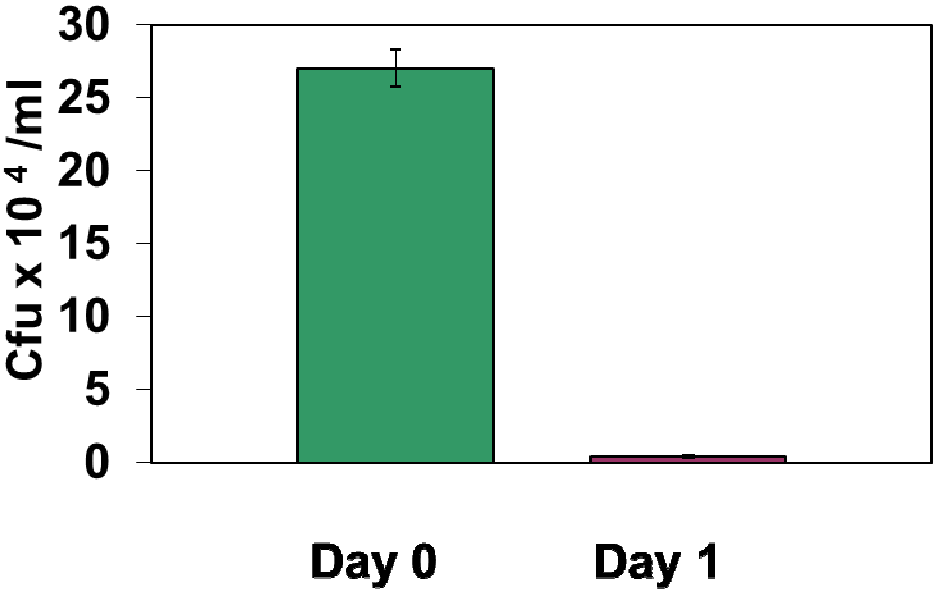

Figure 2B.
Cell viability of *S. maltophilia* H44306 after 0, 1 days on dry filter paper. Cfu, colony-forming units. Vertical bars represent standard error.

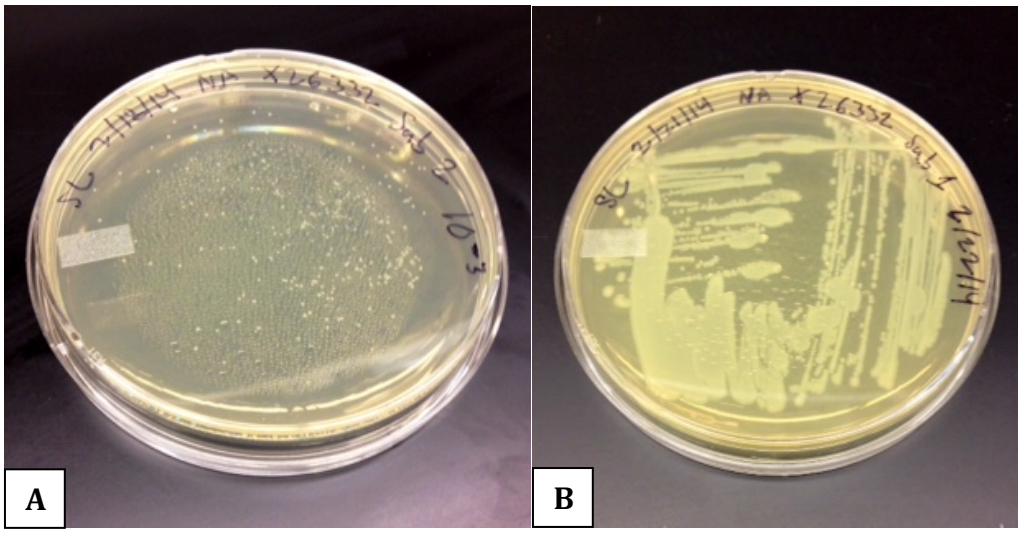

Figure 3: Quantitative Analysis and Purity Test for Study 2. **A**. demonstrates colony growth for Study 2. **B**. shows the results for the culture purity test. This test was conducted with the X26332 isolate, subculture 1.