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Case Series

An Unusual Cluster of *Roseomonas mucosa* Cases in a Hospital System: Related or Not?

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Abstract

Roseomonas mucosa is considered an opportunistic pathogen and the majority of *R. mucosa* cases are bacteremia in patients with a malignancy. Our clinical microbiology laboratory noticed an unusual trend of four *R. mucosa*-positive cultures (two blood, one abscess and one corneal swab) from four separate patients within a period of 45 days. To determine if the four isolates were related, whole genome sequencing (WGS) and single nucleotide polymorphism (SNP) analysis were performed. WGS analysis demonstrated >4000 SNPs between the isolates suggesting they were not related. Here we present a case series of four separate patients with *R. mucosa*-positive cultures, and collaboration between the clinical microbiology laboratory and state public health department to use WGS for assessing the relatedness of *R. mucosa* isolates.

Keywords: Whole genome sequencing; *Roseomonas*; Opportunistic infection

Introduction

Roseomonas is a genus of pink-pigmented, non-fermentative, Gram-negative bacilli, first described in 1993 [1]. There are several species within this genus with the most commonly reported clinical species as *Roseomonas mucosa* [2]. *R. mucosa* is considered an opportunistic pathogen, isolated from many body sources, with the majority of cases described as bacteremia in patients with a malignancy [2-4].

A pattern, or trend, of non-common bacterial organisms, such as *R. mucosa*, in the clinical microbiology laboratory can lead to suspicions of a possible outbreak occurring in the hospital system. Whole genome sequencing (WGS) can be a valuable tool in determining whether two or more isolates are related. However, the majority of clinical microbiology laboratories are not equipped with this technology and rely on their local public health department to aid in WGS-based investigation.

Here we present a case series of four separate patients with *R. mucosa*-positive cultures and how our clinical laboratory collaborated with the Minnesota Department of Health Public Health Laboratory (MDH-PHL) to assess relatedness of the isolates using WGS.

Case Presentations

In the span of 45 days, our hospital system had four separate cultures from four patients with varied collection sources that were culture-positive for *R. mucosa*. All relevant clinical information for these four patients is summarized in Table 1.

	Patient A		**Patient B**		**Patient C**		**Patient D**	
Sex	Male		Female		Male		Male	
Age	76		48		67		78	
Specimen Source	Blood		Cornea Swab		Blood		Abscess	
Reason for Admission	Generalized weakness		Not admitted-seen at an outside eye clinic		Shortness of breath, dry cough and worsening lower extremity edema		Painful lump with increased purulent drainage being observed at the drivelinesite of his LVAD	
Relevant Past Medical History	Cerebral vascular accident, seizures, and urinary incontinence		Central corneal ulcer in the left eye		Placement of a left ventricular assist device (LVAD) in 2013		Placement of LVAD in2014	
Antibiotic	**MIC (ug/mL)**	**Interpretation***	**MIC (ug/mL)**	**Interpretation***	**MIC (ug/mL)**	**Interpretation***	**MIC (ug/mL)**	**Interpretation***
Amikacin	<=16	Susceptible	NP	NP	<=16	Susceptible	<=16	Susceptible
Cefepime	>16	Resistant	NP	NP	>16	Resistant	8	Susceptible
Ceftazidime	>16	Resistant	NP	NP	>16	Resistant	>16	Resistant
Ciprofloxacin	2	Intermediate	NP	NP	<=1	Susceptible	<=1	Susceptible
Gentamicin	<=1	Susceptible	NP	NP	<=1	Susceptible	<=1	Susceptible
Levofloxacin	4	Intermediate	NP	NP	<=0.25	Susceptible	1	Susceptible
Meropenem	<=1	Susceptible	NP	NP	4	Susceptible	<=1	Susceptible
Piperacillin/Tazobactam	>64	Resistant	NP	NP	>64	Resistant	64	Intermediate
Tobramycin	<=1	Susceptible	NP	NP	<=1	Susceptible	8	Intermediate
Trimethoprim/ Sulfamethoxazole	>2/38	Resistant	NP	NP	NP	NP	NP	NP
Ertapenem	NP	NP	NP	NP	NP	NP	>=0.5	No interpretation
Antibiotic Treatment	Ciprofloxacin		Unknown		Ciprofloxacin		Meropenem and Ciprofloxacin	
*Interpretations are based on the other non-enterobacterales category in the M100 [5], as there are currently no established breakpoints for *Roseomonas* species Abbreviations: MI, Minimum Inhibitory Concentration; NP, Not Performed								

Table 1: Summary of Clinical Information, Antimicrobial Susceptibility Testing Results and Antimicrobial Therapy of Choice for Patients.

In summary, patient A was a 76-year-old male who presented to the emergency department with generalized weakness. The past medical history of this patient is significant for a cerebral vascular accident, seizures and urinary incontinence that led to the placement of a chronic suprapubic catheter. This patient was mostly wheelchair dependent and required physical assistance upon presentation. The overall presentation was unremarkable; however, an elevated white blood cell count of 13.6 $x10^9$/L (reference range: 4.5 to 11 $x10^9$/L) was found. Blood cultures were drawn upon admission and submitted to the clinical microbiology laboratory.

Patient B was a 49-year-old female who was not seen at our hospital, but presented to an outside eye clinic for a central corneal ulcer in her left eye. A cornea swab of the ulcer was submitted to the clinical microbiology laboratory for culture.

Patient C was a 67-year-old male admitted to the hospital with symptoms of shortness of breath, dry cough and worsening lower extremity edema. His past medical history was notable for placement of a left ventricular assist device (LVAD) in 2013 after a failed coronary artery bypass graft. He was on chronic intravenous (IV) vancomycin for an LVAD infection with *Corynebacterium* and IV fluconazole for a previous *Candida* infection. Blood

cultures were drawn upon admission.

Patient D was a 78-year-old male admitted to the hospital due to a painful lump with increased purulent drainage observed at the driveline site of his LVAD placed in 2014 following a non-ischemic cardiomyopathy event. This driveline infection was first noted eight months earlier with ongoing intermittent drainage but no major abscess formation observed. This abscess was imaged with computed tomography (CT) at an outside hospital and measured 8.2 x 4.1 x 2 cm. The abscess was drained in an operating room and the drainage was sent to the laboratory for culture. No organisms were seen with the initial Gram stain.

In each case, small, pinpoint, pale-pink colony growth was noticed on the culture plates after approximately 24 hours of incubation. After 48 hours of incubation, additional mucoid pink colonies were observed on both blood agar (BAP) (Figure 1A) and MacConkey agar plates (Figure 1B). Gram stain of the colonies revealed small Gram-negative rods (Figure 1C) with identification as *R. mucosa* using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility testing (AST) was performed on the cultures from patients A, C, and D (Table 1). AST for the three isolates was performed using broth microdilution on the Vitek® 2 (Biomerieux). There are currently no established breakpoints for the interpretation of susceptible, intermediate or resistant for *R. mucosa*. However, the M100 published by the Clinical and Laboratory Standards Institute (CLSI) does have a reporting category for other non-Enterobacterales [5], which we used for reporting the interpretations for these *R. mucosa* isolates (Table 1).

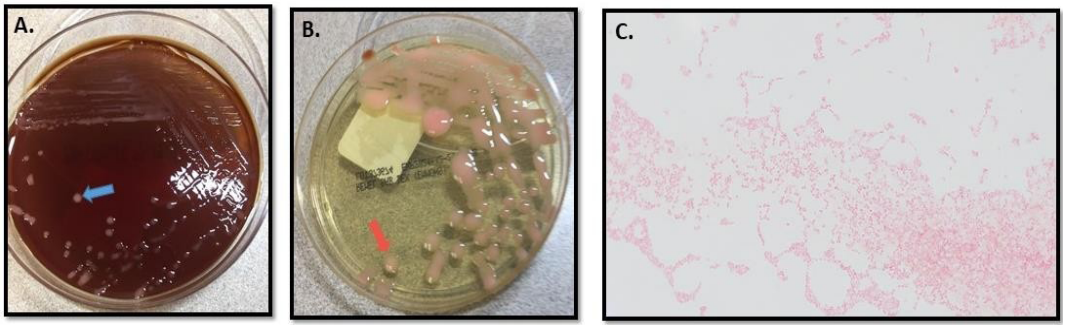

Figure 1: Representative images of *Roseomonas mucosa*- A: 48-hour growth of *R. mucosa* on BAP. Mucoid pink colony (Blue Arrow) **B:** 48-hour growth of *R. mucosa* on MacConkey agar plate. Mucoid pink colony (Red Arrow) **C:** Gram stain image of *R. mucosa* from colony growth.

Due to having four separate patients with *R. mucosa*-positive cultures in our hospital system within a short period of time, the isolates were submitted to the MDH-PHL for WGS analysis including relatedness assessment. In brief, DNA was extracted using the QIAamp DNA Mini QIAcube kit (QIAGEN) followed by sequencing library preparation with the Illumina DNA Prep method and sequencing using Illumina Miseq™ v2 (2x250 PE) chemistry. Sequencing results met quality control metrics including cluster density, percent of bases greater than Q30, and 60x sequencing depth. Whole genome single nucleotide polymorphism (SNP) analysis was performed using the CFSAN SNP Pipeline [6] as part of the Dryad 3.0.0 (http://github.com/wslh-bio/dryad/) workflow with the isolate from patient C serving as the reference genome. The core genome size for all four genomes was 1161066-bp, which equaled 84.32% of the mapping reference genome. Analysis of the SNP matrix demonstrated >4000 SNPs among all four isolates (Figure 2).

	Patient C	Patient D	Patient A	Patient B
Patient C	-	5493	4650	5374
Patient D	5493	-	6282	6384
Patient A	4650	6282	-	5456
Patient B	5374	6384	5456	-

Figure 2: SNP matrix comparing *R. mucosa* isolates from all four patients. Whole genome SNP analysis performed using the CFSANSNP Pipeline [6] as part of the Dryad 3.0.0 (http://github.com/wslh-bio/dryad/) workflow with the isolate from patient C serving as the reference genome.

Discussion

We present a case series of four patients in our hospital system with *R. mucosa*-positive cultures within a short time period. WGS and SNP analysis performed by the MDH-PHL suggests that the four *R. mucosa* isolates were not related to one another since there were >4000 SNPs between isolates (Table 1). The number of SNP differences needed to demonstrate relatedness of two bacterial organisms is not established and varies between different bacterial species [7]. There are many publications on *Mycobacterium tuberculosis* that do not agree on the minimum number of SNPs [7-9]. For example, Clark et al. 2013 states that ≤ 50 SNPs indicates relatedness [8], while Walker et al. 2013 sets the lower limit as ≤ 6 SNPs [9]. The same can be true of other bacterial organisms, such as methicillin-resistant *Staphylococcus aureus* [10] and Shiga-toxin-producing *Escherichia coli* O157: H7 [11]. More studies and data are needed to determine if there is a defined number of SNPs that indicates relatedness, and whether the value can be universally applied to all bacterial genera. Currently, literature suggests ≤ 20 SNPs is supportive of organism relatedness.

Supporting the molecular WGS SNP data, an epidemiologic investigation showed that these four patients did not overlap in their time at the hospital or clinic. The three patients who were admitted to the same hospital for their infections were not treated or seen in the same hospital locations. There was no overlap in providers, nursing staff or equipment used to treat the patients. A characteristic common among patients with *Roseomonas* infections is the placement of a central venous catheter [2]. Interestingly, patients C and D both had LVAD placements within the previous decade. The LVAD placements of patients C and D were placed a year apart from one another and at different hospitals within our health system (Table 1). Patients A and C both presented with bacteremia, which is currently the predominant infection associated with *Roseomonas* [2]. There are no reports of *R. mucosa* being the cause of a corneal ulcer as seen with patient B; however, several case studies have reported *Roseomonas* species as the cause of endophthalmitis [12,13] and keratitis [14,15]. It must be noted that in three of the four cases, *R. mucosa* was not the only organism cultured. All four patients were treated successfully and had good outcomes (Table 1). There is evidence to suggest that *Roseomonas* species are considered pathogens of low pathogenicity, and this is especially true in immunocompromised individuals [2].

To date, there have been no studies performed to determine the virulence of *Roseomonas* species and no genomic studies to designate any virulence genes. One can hypothesize that since the colonies are mucoid (Figure 1), there is large production of exopolysaccharide by *Roseomonas* species enhancing biofilm formation on various devices in a patient. More in-depth and basic science studies need to be performed to determine if this hypothesis has merit. Recent studies have supported skin microbiota as the main reservoir of *R. mucosa*, previously assumed to be an environmental pathogen [16]. Using 16S rRNA gene analysis, phylogeny, pulsed-field gel electrophoresis, and surveys in diverse metagenomics databases concluded that *R. mucosa* is the main human-associated species, and that opportunistic infections due to this species are related to patient skin microbiota. In contrast, some strains of other *Roseomonas* species isolated from patients with cystic fibrosis were related to environmental clades [9]**.**

AST was performed on three out of the four isolates. *Roseomonas* species are reported to have high resistance to penicillin, cephalosporins, and piperacillin/tazobactam [2]. There are currently no established antimicrobial susceptibility breakpoints for any *Roseomonas* species through the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or CLSI, but these resistance trends were seen in previous case reports [2]. Our results conformed with published results demonstrating all three *R. mucosa* isolates had MIC values <=1 or 4 ug/mL to meropenem and similar low MIC values to the aminoglycosides which suggest that they are susceptible to these antimicrobials (Table 1). The three isolates had MIC values >64 ug/mL for piperacillin/tazobactam and MIC values of >16 ug/mL to most of the cephalosporins (Table 1). These high MIC values suggested that the *R. mucosa* isolates were resistant to these antimicrobials. Again, the varying susceptibility patterns between the three isolates of *R. mucosa* supported the molecular WGS SNP data that these three isolates were likely unrelated.

In this case series, we presented the first example of using WGS SNP analysis to determine if four isolates of *R. mucosa,* from four patients, were related to one another. Our work with the MDH-PHL strongly suggested that the four *R. mucosa* isolates were not related and, instead, represented an unusual trend that was observed in the culture results of our patients. This work also highlighted the importance of a strong relationship between a hospital system microbiology laboratory and their local or state health department in order to rule out a possible common source outbreak situation.

Ethical Guidelines: There are no funding sources to report. We are in accordance with our Institutional Review Board, since publishing a case report does not fall under human research and does not require the full approval process.

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