Extraction, Isolation, Identification, and Testing of a Bacterial Specimen from the Wood Center Door Handle

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Introduction

Entering into a public building is the same procedure for everybody: just grab the door by its handle and either push or pull it open to enter. We often do not pay too much thought over this simple yet seemingly innocuous act, just like we also don't think about how many people before us have affixed their hands on that surface. A door handle, like the one at the Wood Center, is one of many fomites people interact with every day at the University. According to Miriam-Webster, a fomite is an object (like a doorknob or an elevator button) that may be contaminated with infectious organisms and serve in their transmission. Many studies go in to determining what substances and find that unsuspecting everyday items like clothing, for example, can be used transport various dangerous bacterial pathogens (Frosth et al., 2018). Other diseases like hepatitis can also spread to other hosts through physical contact (Gebra et al., 2007). Fomites are also the center of new educational applications for future medical workers simply because of their impressive influence of disease spreading in places like hospitals (Yano et al., 2019). The topic of communicable diseases spreading through fomites has always been studied vastly by healthcare professionals and epidemiologists alike; one study of which found an increasing trend of effectiveness in handwashing by staff to reduce the number of

infections by *A. baumannii* since fomite-person transmission was very prevalent in these areas (Greene et. al., 2018).

I was curious as to how an object so innocent and used amply by many others could possibly harbor millions of microorganisms invisible to the naked eye especially when some have been observed to survive on common surfaces used by people every day for up to 30 days in some studies (Abad et al., 1994). Furthermore, since some bacterial species have also been shown to exhibit noticeable resistance when on hard surfaces compared to culture environments (Mitchell et al, 2018), I was curious to see what creatures dwell upon one of the most commonly used fomites on the campus.

Methods

The surface of extraction was a door handle system on the intermediate entrance on the south-western entrance of UAF's William Wood Student Center. This entrance is well-known and widely used by students and faculty on a daily basis to enter to the building making it the ideal fomite for the microbial sampling. Extraction took place at around 9:45 pm, 15 minutes before the building closed to ensure the contact by people during that day was high and that results would not be affected during janitorial cleaning when the building closed.

Using dry and sterile swabs, the outer round-surfaces of the door handles were wiped vertically up and downwards as well as around the backside where fingers can firmly grasp. After swabbing the surface, a lawn plating was done on Tryptic soy agar

(TSA), Resoner's 2A agar (R2A), and Sabouraud's agar (SA). After two days of no visible growth, I returned to the extraction site and wet additional sterile swabs with sterile water before adhering them to the surface a second time, all while plating the TSA, R2A, and SA media via a lawn method. Two days later, signs of growth in all three plates were present, and the samples were then taken to the laboratory and incubated at 37°C to maximize growth.

The isolate remained in a laboratory incubator for three days to maximize proliferation of the diverse red, white, and yellow cultures. I chose a red colony to purify for this project and made three consecutive streaks on TSA incubating each streak at 37°C until pure. After which, a slant and a culture broth were made with the purified isolate (Lab Handout 2), both of which were incubated at 37°C until sufficient growth of the pure culture was observed. Both tubes were promptly refrigerated to serve as a stock sample for various tests to come.

The pure isolate was then stained (Lab 4 Handout) for ease to view under a microscope as well as to determine if the specimen was either Gram positive or Gram negative. Gram staining was compared against *E. coli.* for the negative control and *Bacillus* for the positive control. Staining could tell us a lot about the physical nature of the isolate for Gram positive bacteria have a thick peptidoglycan cell wall that traps the dye in giving the specimen a purple color when viewed. Gram negative bacteria, on the contrary, do not have such a thick peptidoglycan cell wall and therefore do not trap as much dye

within. This gives the cells a rather light red or pink color when observed under the microscope.

Next, I extracted the DNA for genome sequencing by using a PowerSoil DNA Isolation Kit per Lab Handout 5 and then assembled the extracted genome through PATRIC (PATRIC Homework Revised). Using the assembly, I did a Kaiju taxonomy assignment and a metagenome binning on PATRIC (Lab Handout 7) to determine the species of bacteria I managed to isolate. While genome testing was underway, I made an additional lawn plating from my slant onto a TSA plate and incubated it at 37°C for later physiological testing.

After genome sequencing, I determined if my isolate could grow on differential or selective media (Lab Handout 6). Using culture from my stock slant, I inoculated my isolate onto an Eosin Methylene Blue (EMB) media plate which differentiates for enteric (intestinal) bacteria and selects for Gram negative strains and as well. An additional MacConkey (MAC) agar media was plated with the isolate too to differentiate for enteric bacteria and select for gram positive strains.

For physiological testing, I performed several tests per Lab Handout 8 which tested for several physiological aspects of the isolate. Amongst these were a catalase test using Hydrogen Peroxide, an oxidase test using oxidase test strips, and a fluid thioglycolate test to test aerobic qualities of the isolate. In addition, I ran a Staph API test

strip to test certain metabolic characteristics of the isolate. Standardized by the manufacturer for staphylococcus species of bacteria, my test kit tested for metabolic reactions with D-glucose, D-fructose, D-mannose, D-maltose, D-lactose (bovine origin), D-trehalose, D-mannitol, xylitol, D-melibiose, potassium nitrate, β-naphthyl phosphate, sodium pyruvate, D-raffinose, D-xylose, D-saccharose (sucrose), methyl-αD-glycopyranoside, N-acetyl-glucosamine, L-arginine, and urea.

The API test strip was then incubated at 37°C after slightly moistening the clear plastic case with distilled water to prevent dehydration and recollected the next day during open lab. Results were gathered before additional tests for VP, nitrate reduction, and ZYM could be conducted as per the Staph API Protocol. Results were then recorded, the API test strip disposed of properly into the biohazard waste receptacle, and results were entered onto BacDive for physiological analysis of the specimen.

The last analysis conducted would test for any antibiotic susceptibility. Using the culture on the TSA plate raised for physiological testing, I did an additional lawn plating on two Mueller-Hinton agar plates and then selected 8 antibiotics for my isolate for a Kirby-Bauer disc diffusion test (Lab 9 Handout). For my isolate, I chose ampicillin, clindamycin, gentamicin, oxacillin, penicillin, piperacillin, tobramycin, and vancomycin. After the inoculated media plates were divided into four quadrants, eight filter paper discs imbued with the aforementioned antibiotics were placed in their designated quadrants and then left overnight to incubate at 37°C. Ideally, the antibiotics within the

filter paper should diffuse around the discs in the media and if effective against the culture, will prevent cell proliferation with the appearance of a cleared circle around the disc where bacteria are absent. If, however, the bacteria are unaffected by the antibiotic, biofilm will grow all the way to the disc as the bacteria display resistance to the drugs imbued within the discs. Since no growth of isolate was apparent after 24 hours, the plates were left to incubate at 37°C for another day. After apparent growth was present, the diameters of the inhibition zones were summarily measured in centimeters with a ruler. Any resistance (absence of any inhibition zone) was noted.

Results

Cell Purification and Morphology

The strain extracted from the Wood Center door handles was successfully isolated and fully pure after three streaks on tryptic soy agar. It appeared pale yellow/tan white in appearance (Figure 1a). The isolate also proved to be Gram positive through Gram staining. Upon further inspection of the isolate via microscopy, the isolate appeared to be small circular with minute elongation resembling micrococcus.

Figure 1a

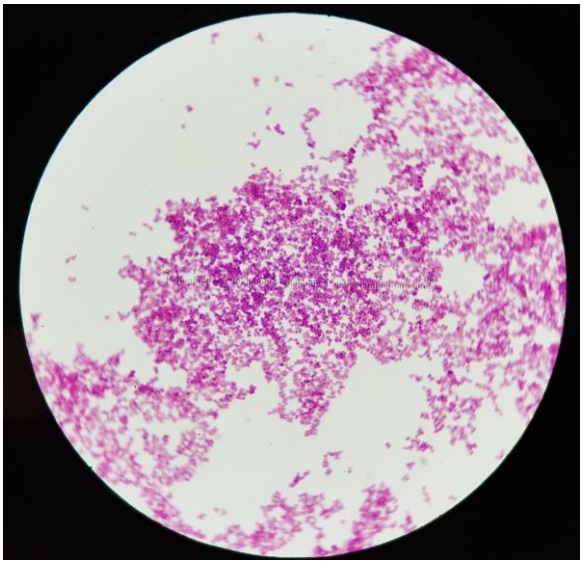

Figure 1b

Figure 1a: The third and final pure strain of culture extracted from the Wood Center door handle.
Figure 1b: Light microscopy of the pure isolate stained with Gram's iodine.

Genome Sequencing and Phylogeny

Metagenome binning provided by Patric identified the strain as *Kocuria palustris clonal* population after reading a total length of 2,933,535 base pairs and analyzing 109 contiguous pairs. Patric did not find any additional species to compare against in similarity as a possible match. Additionally, contigs provided to Kaiju resulted with phylum identified as *Kocuria palustris* as the most abundant species of bacteria identified in the isolate out of other similar micrococci with runner-up species being *Microbacterium sp.* (10%), *Micrococcus lylae* (2%), *Kocuris flava* (2%), *Kytococcus sp.* (2%), and others (Figure 3). Additionally, Patric found 2 antibiotic resistance genes within its own database and 32 within the DrugBank database. These genes were not specified, nor did Patric or DrugBank describe which antibiotics the isolate was resistant to.

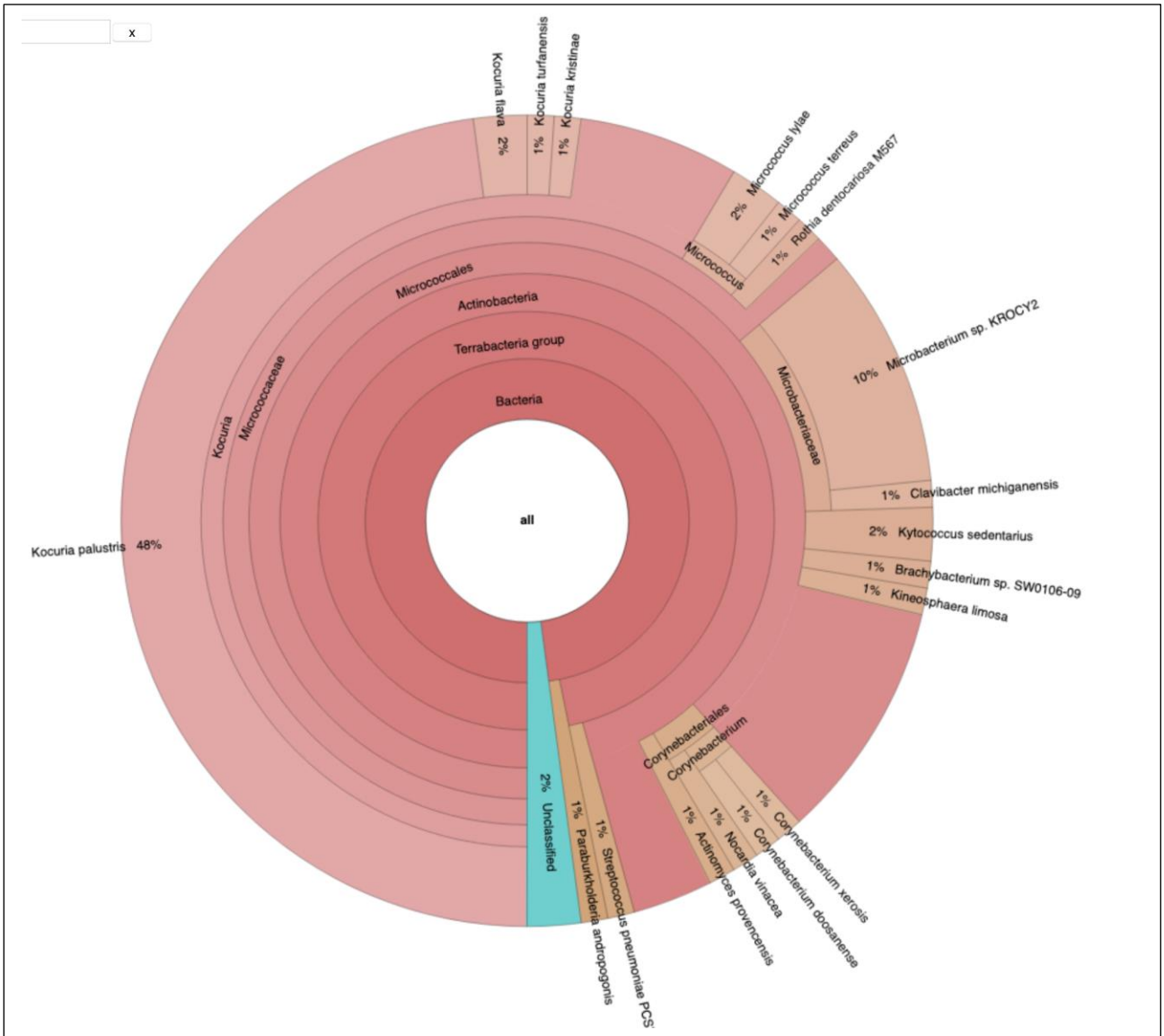

FIGURE 4:
Krona projection display of phylum identified in isolate to be majorly Kocuria palustris.

Physiological Tests

There was no growth exhibited on the Eosin Methylene Blue (MEB) media but there was a small, thin, white presence of isolate growth on the MacConkey (MAC) agar media. Additionally, the isolate proved to be a facultative aerobe through analysis via a thioglycolate broth with prevalent growth in the oxygen rich domain and minimal

growth in the anoxic regions; moreover, the isolate tested positive for catalase but negative for oxidase. Remarkably, every metabolite tested with the API test strip was negative. No further identification of the isolate could be made when results were submitted for interpretation on BacDive.

Antibiotic Resistance

The isolate showed resistance to clindamycin with susceptibility to ampicillin containing an inhibition zone of 5 cm, gentamicin (inhibition zone of 3 cm), penicillin (inhibition zone of 4 cm), piperacillin (inhibition zone of 3.5 cm), oxacillin (inhibition zone of 4 cm), tobramycin (inhibition zone of 2.25 cm), and vancomycin (inhibition zone of 2.25 cm).

Discussion

I extracted the isolate from a fomite without expecting to find a bacterial strain that is found abundantly in water deer and reindeer milk (Li et al. 2017). Since my isolate was taken from the handle of a very popular communicable surface, I was expecting a microbe that would be more abundant on humans. In particular, the *Kocuria* strain is found mostly in water deer and reindeer (Li et al. 2017). Whereas water deer are not native to Alaska, there is a small population of reindeer nearby the campus. It is curious how I was able to find evidence of their presence far away from their residence.

The genomic data from Patric and from Kaiju both identified the isolate as *Kocuria palustris*. Furthermore, both systems presented their findings of this strain with high confidence since Patric did not identify the genes of other/similar strains of bacteria. Perhaps this was due to the diversity of the systems with PATRIC more focused with genomes and Kaiju more focused on phylogeny. Moreover, the krona projections indicate a majority of genes belonging to *Kocuria palustris* whereas the other matches projected by Kaiju involved variant species within the *Kocuria* genus overall. I therefore feel confident and persuaded with enough evidence that the isolate is predominantly *Kocuria sp.* However, uncertainty resides within the physiological tests conducted on the isolate. The catalase and oxidase tests appear nominal, plus the thioglycolate test does not make me weary about the isolate: a facultative aerobe would likely reside in the lactate of deer milk. Furthermore, this strain was sampled from a door handle exposed to an ample amount of oxygen.

All of the metabolite tests on the API test strip, however, turned up negative which raises some concern. The strip examined metabolic reactions for lactose, which is commonly found in milk. There were other sugars tested by the strip as well like glucose and sucrose. This unexpected result might indicate that the isolate has very specific dietary needs has very specific dietary needs, otherwise there might have been an error in preparing or handling the strip. Maybe there was a flaw during transfer via the aseptic method that might have fried my isolate by not allowing the transfer loop to cool properly or perhaps the strip grew dehydrated while incubating. It might be worth looking into in

future analysis by means of a retest for the Staph metabolites or maybe even a different test strip protocol altogether.

Additionally, I was surprised to see that the isolate grew white on the MacConkey agar. I would have expected the strain to turn pink since the genome testing revealed that it is found largely in lactate. After all, the MAC agar is supposed to turn the isolate pink due to lactose fermentation (Lab Handout 6). Perhaps this bacterium might simply reside in the environment prevalent with a lot of lactate but not actually consume the sugar since the lactose and glucose tests yielded negative results.

Remarkably, there were 7 out of 8 antibiotics that proved to be effective against *Kocuria sp.* This is assuring especially since studies show that symptoms of infections by the *Kocuria palastrus* include ulcers in the eyes of patients that lacked Vitamin A (Mattern et al., 2014). However, the isolate did show resistance to clindamycin. I would have expected there to be more resistance since Patric identified 34 unspecified genes for antibiotic resistance. It would also be worth investigating the identified antibiotic genes further to confirm these finding and understand the bacteria more and the hazards associated with this strain considering that it resides on the Wood Center door handle.

It would be worth sharing this information with public health specialists and school administrators that an antibiotic-resistant pathogenic strain of bacteria was successfully isolated and identified from one of their many facilities. Furthermore, it

should also be worth sharing the nutritional needs of students from the university's food provider to ensure that malnutrition from its meal programs are not compromising the students of the university that are exposed to this strain of bacteria on a relatively daily basis. Hopefully with better knowledge and awareness, those in charge of the general welfare to the university can make informed decisions that not only improve health conditions but also help to keep the students, faculty and staff of the university safe!

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