Identity and pathogenicity of bacteria isolated from a domestic feline claw

Introduction

Microorganisms – bacteria, archaea, viruses, fungi, and protozoa – are the most genetically diverse group of organisms on Earth, particularly those bacterial species [1]. Life is hypothesized to have derived from a primordial ancestral bacterium some 3.8 million years ago, when the Earth contained little or no free oxygen and instead consisted primarily of CO$_2$ and N$_2$, in addition to smaller amounts of gases such as H$_2$, H$_2$S, and CO [2]. As the composition of the Earth’s atmosphere began to change over millennia, bacteria began to evolve and adapt to new environmental conditions. The continuing transformation of Earth has fostered the rise of an innumerable number of different species of bacteria based on metabolic pathways, cell structures and functions, and preference of conditions [3].

Perhaps the most relevant bacteria today are those with which humans interact in everyday life. Humans often overlook microbes – understandably so, given that they are impossible to see with the unassisted human eye – until they cause one to fall ill. In actuality, nearly all microbes are potential pathogens and every day such microbes invade the human body, looking for those ideal living conditions which will allow them to flourish and multiply. *Staphylococcus aureus* may enter through an elderly patient’s surgical wound, seizing the opportunity to invade and wreak havoc upon the skin and underlying musculature. A young woman having unprotected sex with a stranger may
contract *Chlamydia trachomatis* and never present clinical signs – her immune waging a quiet, asymptomatic war with the pathogen, whilst accumulating damage of the internal reproductive organs. A young child may not wash his hands after using the bathroom and unknowingly consume a side of *Escherichia coli* with his grilled cheese sandwich, resulting in a severe case of food poisoning. Bacteria are everywhere, on us and in us. Pathogens are in an eternal evolutionary arms race with hosts and are always searching for the opportunity to invade the body and cause disease [4].

The purpose of this study was to determine the identity and pathogenicity of a microbe isolated from a sample taken from a domestic feline claw. Cat scratches are a common household injury that people often allow to go entirely untreated, although bacteria from a feline claw may cause serious illness under certain conditions [5]. The experimental sample taken from the domestic feline claw yielded several bacterial colonies on a trypticase soy agar (TSA) plate, and a single colony was isolated and a pure culture was obtained for further testing.

Several tests were conducted to characterize the isolated bacterium, including: colony observation, microscopic observation, approximate growth rate, gram staining, many physiological and metabolic tests via API 20E strip, antibiotic resistance testing, erythrocyte lysis testing, growth upon mannitol salt agar (MSA), and genome analysis.

Given the location from which the bacteria were sampled from, one can predict that the isolated bacteria are obligate aerobes, which require the presence of oxygen as a final electron acceptor in the electron transport chain, and chemoorganoheterotrophs, which obtain energy from oxidation of organic compounds [4]. If the bacteria are capable of lysing erythrocytes and growing on an MSA plate (to simulate human skin),
then pathogenicity could be assumed. Antibiotic resistance would further increase the likelihood of pathogenicity.

Determining the pathogenicity of a microbe sampled from a domestic feline claw is important as it pertains to injuries – cat scratches – that are so commonplace that they often go untreated in both children and adults. The purpose of this study is to answer a question that all cat-owners, myself included, should be concerned about: are there risks of acquiring an infection specifically from feline claws, and to what extent is that pathogenicity? The results of this study may help owners decide whether or not it is worthwhile to declaw their feline friends to prevent potential infections.

**Materials and Methods**

**Gram Stain Test**

The aim of this test was to determine whether the isolated bacteria are classified as gram positive or gram negative, which will identify physiological characteristics of the bacteria. The standard Gram staining procedure was followed, which utilizes heat fixation, crystal violet, distilled water, Gram’s iodine, ethanol, and safranin. Each of these treatments was applied for a whole minute, rather than 30 seconds.
Physiological Tests

The aim of these tests was to determine various physiological characteristics of the bacteria, particularly metabolic mechanisms. Bacteria, and mineral oil where necessary, were added to each test well in an API 20E test strip and allowed to incubate at 37°C for approximately 24 hours. An oxidase test and catalase test were also done, for which the bacteria were added to the corresponding reagents and allowed to react. The oxidase test checks for the presence of cytochrome c oxidase and if they can use oxygen for energy production, while the catalase test checks for the presence of catalase, which is an enzyme that converts harmful reactive oxygen species to harmless oxygen. Bacteria were added to a fluid thioglycolate tube to show oxygen preferences.

Genome Analysis

Analyzing the genome of the isolated bacteria will allow for identification of the family, genus, or species, which in turn will provide the information needed to further research the bacteria isolated. DNA was extracted from the isolated bacteria using PowerSoil DNA Isolation Kit, and was sequenced in the UAF core lab. The whole genome was then run through BaseSpace – a database containing microbial DNA sequences – for comparison.
Antibiotic Resistance Test

The aim of this test was to determine whether the bacteria are resistant to modern antibiotics. If positive, then it can be inferred that the bacteria may potentially cause an infection in humans which some antibiotics are unable to treat. A TSA plate was inoculated with bacteria suspended in solution by a saturated swab to create a bacterial lawn upon the plate, then small discs containing a specific antibiotic were added to each quadrant of the plate. The plate was allowed to incubate at 37°C for a week.

Mannitol Salt Agar Test

Since the salt agar has similar conditions to that of human skin, the results of this test will allow inference upon the viability of the bacteria and their ability to grow and thrive on human skin. The bacteria were cultured on the MSA plate and incubated at 37°C for a week.

Blood Agar Test

Since the blood agar is infused with erythrocytes, the results of this test will allow inference upon the hemolytic effect of the bacteria – whether these bacteria could potentially infect and lyse blood cells. The bacteria were cultured on the BA plate at 37°C for a week.
Results

Gram Stain Test

Microscopy revealed small, pink diplococci resulting from the Gram stain test. The isolated bacteria are gram negative.

Physiological Tests

The results of the API test strip were inconclusive. The oxidase test was positive and the catalase test was negative. The fluid thioglycolate test was also inconclusive, since bacteria did not grow despite multiple re-inoculations. This test was repeated a couple weeks later, but still did not yield any bacterial growth in the tube.

Genome Analysis

Genome analysis yielded inconclusive results as well. 51% of the sample was unidentified and the other 49% was composed of DNA from over 20 different bacterial strains, differing even on the class level.

Antibiotic Resistance Test

Similar to the fluid thioglycolate test, this test also did not yield any bacterial growth upon the plate. Antibiotic resistance could not be evaluated.
Mannitol Salt Agar Test

The bacteria grew upon the plate but no fermentation occurred – the agar remained red and no yellow spots were observed.

Blood Agar Test

The bacteria grew upon the plate but no erythrocyte lysis occurred – the agar remained intact.

Discussion

Given that many of the tests completed thus far have yielded inconclusive results, it is impossible to identify this bacterium accurately. These bacteria are gram-negative diplococci and are very tiny. Based solely on morphology, I can speculate that this strain may be a species from the Neisseria genus. Neisseria species are aerobic, strongly oxidase-positive, have an oxidative metabolism, are susceptible to drying, and are fastidious growers [4]. The isolated bacteria are also oxidase-positive, which supports the idea that they may be a species of Neisseria, yet the isolated bacteria are not fastidious growers like Neisseria. Neisseria are typically catalase-positive as well, but the isolated bacteria are catalase-negative. Several Neisseria species can be found in aquatic environments worldwide and tend to be non-pathogenic (with the exception of Neisseria meningitidis and Neisseria gonorrhoeae, which cause meningitis and
gonorrhea, respectively) [6]. Domestic felines are known to escape from home and traverse various environments, including wet ones, and may bring home a non-pathogenic aquatic bacteria such as *Neisseria*.

Genome analysis resulted in over 20 different species matches, some strains from entirely different classes of bacteria, yet these results only account for 49% of the sample. The other 51% of the sample was considered to be “unknown” – the target bacterial DNA may have been in this portion of the sample which remains unidentified. In any case, the results of the genome analysis are not reliable and are not taken into consideration in the identification of this bacterium.

The API 20E test did not yield conclusive results, and the fluid thioglycolate test has proved inconclusive despite multiple attempts – the bacteria will not grow in the liquid agar. The antibiotic resistance tests also failed, as the bacteria refused to grow on the plates at all. I believe that these bacteria may not grow while suspended in liquid. This would account for why the fluid thioglycolate test continues to show no growth and why the antibiotic test also failed, since plate inoculation was from a suspended liquid culture.

Despite the fact that the isolated bacterium could not be accurately identified, the results of the MSA and BA inoculations indicate that this bacterium is not particularly pathogenic. The bacteria could grow on MSA, which simulates the conditions of human skin. Although the bacteria did grow on BA, the bacteria did not seem to lyse any cells. Based on this study, cat-owners needn’t be overly concerned about this particular microbe causing a devastating infection via cat scratch under normal circumstances. That being said, cat-owners should still be sure to wash their cat scratches thoroughly
with soap and warm water to prevent infection by any microbe, be it the one isolated for this study or any others that could take the opportunity to invade.
References


