Bioaerosols in the Slaughter Department of Processing Factories for Farmed Salmon.

Bacteria and Endotoxins

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Preface

This study was planned in collaboration with two of the major farmed salmon companies in Norway. The quality departments at both factories and I, as their occupational hygienist, have regularly discussed exposure to bioaerosols and the lack of information about this issue.

The study would not have been possible without access to a microbiology laboratory. I am very grateful to the Institute of Marine Research, Bergen who made this possible

And many thanks to my dedicated and knowledgeable supervisors:

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- Cecilie Smith Svanevik; Co-supervisor. Senior Researcher, Institute of Marine Research, Bergen.

- Bjørg Eli Hollund; Co-supervisor; Occupational Hygienist, Ph.D. Occupational Medicine, Haukeland University Hospital.

And I want to give a special thank you and acknowledgement to Marit, Eva, and Elisabeth from the two companies. Thank you so very much for financial support, help, and information.

Abbreviations

ACGIH: American Conference of Governmental Industrial Hygienists

AIHA: American Industrial Hygiene Association

ALOA agar: Agar Listeria Ottavani & Agosti

CFU: Colony Forming Units

COVID 19: Corona Virus Disease 2019

DECOS: Dutch Expert Committee on Occupational Safety

DNA: Deoxyribonucleic Acid

ELISA: Enzyme-Linked Immunosorbent Assay

EU/m3: Endotoxin Units pr. Quadrat Meter

GDP: Gross Domestic Product

GVPC agar: Glycine Vancomycin Polymyxin Cycloheximide agar

H_2S: Hydrogen Sulfide

IgE: Immune Globulin E

IL: Interleukin

IMR: Institute of Marine Research

LAL: Limulus Amebocyte Lysate

LOD: Limit of Detection

LOQ: Limit of Quantitation

LPS: Lipopolysaccharide

LPSN: List of Prokaryotic Names Withstanding Nomenclature

M^3/h: Cubic Meter pr. Hour

MALDI-TOF-MS: Matrix-Assisted Laser Desorption/Ionization-Time of Flight

MAMP's: Microbe-Associated Molecular Patterns

µm: Micrometer

mm: Millimeter

NaCl: Sodium Chloride

NOEL: No Observed Effect Level

NOK: Norwegian Kroner

ODTS: Organic Dust Toxic Syndrome

PBS: Phosphate Buffered Saline

PCD: Protein Contact Dermatitis

PD: Pancreas Disease

RH: Relative Humidity

SAV: Salmanoid Alphavirus

spp.: Species

STAMI: Statens Arbeidsmiljø Institutt

TCBS agar: Thiosulfate Citrate Bile Salts Sucrose agar

UK: United Kingdom

UNN: University Hospital of North Norway

WHO: World Health Organization

Table of Content

Abstract

Background

It is possible that pathogenic and opportunistic bacteria enter the salmon slaughterhouses with the fish and seawater and are aerosolized along the process line? The aim in this study was to obtain more knowledge about the bioaerosol composition in the working atmosphere in slaughter departments during processing of farmed salmon.

Method

Forty hours, stationary air samples were taken by an impinger sampler for five consecutive days in the bleeding, gutting, and heading areas in two slaughterhouses for farmed salmon.

Bioaerosols were examined by cultivation and enumeration of human pathogenic and opportunistic pathogenic bacteria, identification by MALDI-TOF-MS, quantification of endotoxin by Limulus Amebocyte Lysate (LAL) chromogenic method.

Result

No pathogenic bacteria were detected in the air samples. Whereas opportunistic bacteria were present in 11 of the 30 samples. The most species of bacteria and the largest bacteria count was found in the bleeding area. The bacteria count was in the range of 100 - 60 000 CFU/m^3. The endotoxin levels were in the range 1,0– 19 EU/m^3.

Conclusion

The bacteria found are typical of the marine environment thus indicating that bacteria from this environment aerosolize within the factory, especially early in the processing line, such as in the bleeding area. The measured endotoxin levels were well below the Dutch occupational limit value and is most likely not an important factor to explain any airway related health problems among the workers. However, more studies are needed to confirm the results.

Keywords

Bioaerosol, bacteria, endotoxins, slaughterhouse farmed salmon, and working environment.

Sammendrag

Bakgrunn

Er det mulig at patogene og opportunistiske bakterier kommer inn i lakseslakteriene med fisk og sjøvann og aerosoliseres langs prosesslinjen? Målet i denne studien var å skaffe mer kunnskap om bioaerosolsammensetningen i arbeidsatmosfæren i slakteavdelinger for oppdrettslaks.

Metode

Stasjonære luftprøver ble tatt som impingerprøver i fem påfølgende dager, 8 timer hver dag i bløgging-, sløying- og hodekappområdene i to slakterier for oppdrettslaks.

Bioaerosoler ble undersøkt ved dyrking og telling av humane patogene og opportunistiske patogene bakterier, identifisering ved MALDI-TOF-MS, kvantifisering av endotoksin ved Limulus Amebocyte Lysate (LAL) kromogen metode.

Resultat

Ingen humane patogene bakterier ble påvist i luftprøvene. Men det var påvist opportunistiske bakterier i 11 av de 30 prøvene. De fleste bakterieartene og det største antallet bakterier ble funnet i bløggingsområdet. Antallet bakterier var i området 100 - 60 000 CFU / m^3. Endotoksinnivåene var i området 1,0–19 EU / m^3.

Konklusjon

Bakteriene som er funnet er typiske for det marine miljøet, og indikerer dermed at bakterier fra dette miljøet aerosoliseres innen fabrikken, spesielt tidlig i prosesslinjen, for eksempel i bløggingsområdet. De målte endotoksinnivåene var godt under den nederlandske grensen, og er sannsynligvis ikke en viktig faktor for å forklare helseproblemer knyttet til luftveiene blant arbeiderne. Imidlertid er det behov for flere studier for å bekrefte resultatene.

Nøkkelord

Bioaerosol, lakseslakteri, bakterier, endotoksiner og arbeidsmiljø**.**

1 Introduction

1.1 Aquaculture

The aquaculture industry in Norway started in 1973 with the licensing law being passed by the government (Nærings- og fiskeridepartementet, 2020). Since then, the value creation for the industry has had an enormous development. In 2017, salmon and trout were sold for around NOK 65 billion, and in the same year the industry contributed with NOK 32 billion to gross domestic product (GDP). This corresponds to 1.3% of mainland GDP. In the last 5 years, growth has leveled off somewhat. This is explained by limited opportunities for capacity increase, especially given the environmental situation of the industry. But the increase in value is still considered large, this is explained by a significant increase in the price of farmed fish (Fiskeridirektoratet, 2018).

In the beginning, the fish farms were often an additional industry to other activities, and the farms were located deep in the fjords. As production has increased and technology has developed, the facilities have become larger and moved further out into the fjords and to the coast. In recent times, there has also been a focus on fish farms at sea. This has led to and will mean that the sites will be more exposed to harsh weather conditions, and the working environment will thus be more challenging (Fiskeridirektoratet, 2018).

According to a report from SINTEF (Thorvaldsen, Holmen, & Kongsvik, 2016) employees in the aquaculture industry have one of Norway's most risky occupations. In the period from 1982 to 2013, 33 people who worked in aquaculture-related activities died. Only fishermen have a more dangerous profession than those who work in aquaculture, both in terms of the risk of injury at work, and the risk of dying at work. There is cause for concern regarding the frequency and severity of accidents and development of illness at work in the industry. The technological development and the demand for high productivity exacerbates the risk of accidents and health problems. It is important that this development takes in consideration a safe working environment (Fiskeridirektoratet, 2018).

1.2 The Slaughterhouse

The slaughterhouses in Norway vary in size, from small facilities that slaughter around 5000 tons pr. year to the big ones that slaughter around 10500 tons. They mainly contain the same work operations, marked green in figure1, and might also contain operations, marked blue.

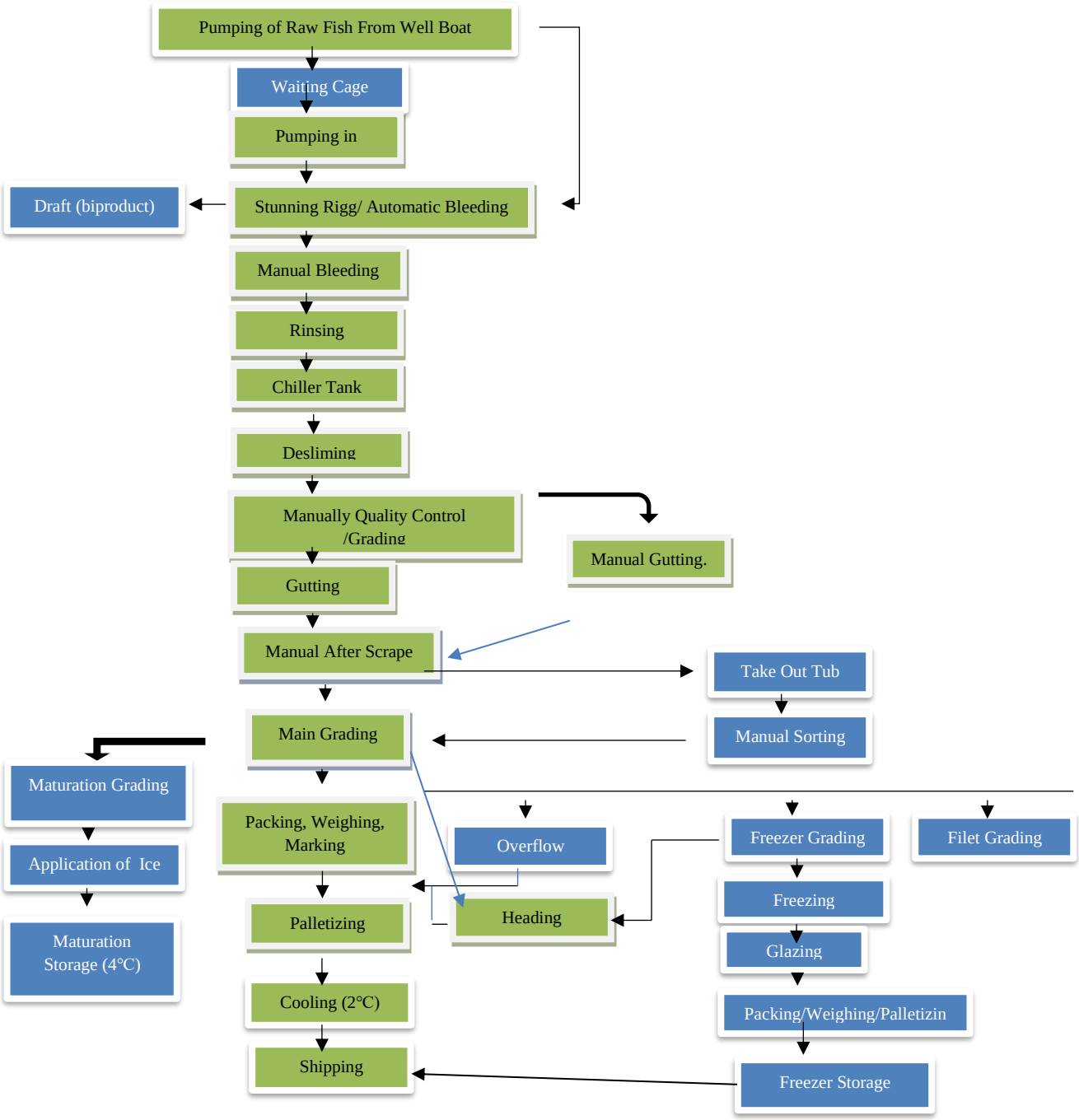

Figure 1 Shows a flowchart of the main steps in the slaughterhouse. Green boxes indicate operations mostly all factories have. Blue boxes indicate operations some factories have in addition.

Intake Fish / Stunning Rig (Figure 2 and 3). At the stunning rig, live salmon is pumped in along with seawater from a waiting cage or a well boat. Residual water runs off, and the fish is automatically anesthetized. The area is continuously wet and mostly unmanned.

Manual Stunning, Bleeding (Figure 4 and 5). The salmon proceeds on a belt for bleeding / manual follow-up. The fish are checked, and fish that were not automatic stunned are manually stunned. The fish is bled with a knife. The area is continuously wet, and the workers are exposed regularly to blood and water squirts.

Cooling Tanks, Bleeding Out (Figure 6). The fish moves on to the cooling tanks (helix) where the temperature is around minus 0.2 - 0.7 ℃), to bleed out. The area is unmanned. The time in the helix is determined by the temperature of the raw fish, the size of the fish, and the number of fish in the chamber. The area is wet from water and blood spills. The humidity is high in this area, and the conveyor belts and areas around are full of blood.

Desliming/Quality Control (Figure 7 and 8). The next step is desliming. The fish is rinsed with tap water or seawater (depends on the factory) before it goes to quality sorting. The fish are sorted according to external characteristics. Some fish are taken out (fish with extremely soft and white gills, fish that is fainting (dying) and dead fish). The area is continuously wet, and the area contains slime and fish shells.

Gutting (Figure 9). Gutting takes place mainly automatically (Figure 10). But also, manually, the fish are opened, and intestines, heart, and remnants of blood are removed. The area contains water, but in smaller amounts than the bleeding area. The water is in a finer mist and generates more aerosol. The manually area also contains intestinal remnants.

Grader: The fish then goes to "grader". The fish are distributed according to quality and size. The main number of fish goes to packing and transport. A small part goes directly to the heading and filleting. About 50-75% of the fish is packed (depends on the factory) and leaves the factory without filleting and beheading.

Heading (Figure 11). The area processes fish that comes directly from the factory, or/and from another factories. (Depends on the factory). The area contains ice water or/and fish particles. Some factories have something called a "Bryne" (Figure 12). This is a big container with ice water where the fish is storage before it goes to heading.

The other main processes in the slaughterhouse are:

Packing/Weighing/Marking: The fish is weighed, drop in boxes, and marked. This is mainly an automated process.

Cover/Strapping Machine: Boxes with fish are filled with ice. and lids are put on and strapped tight. The process is automated.

Palletizing: Boxes with fish are packed on pallets manually, or by a robot.

Figure 2 An example of a stunning rig. This is where the fish that is going to bleeding enters the factory and get stunned before bled.

Figure 3 An example of a stunning rig

Figure 4 Overlooking a bleeding line where the fish is manually stunned and bled.

Figure 5 Bleeding the fish manually.

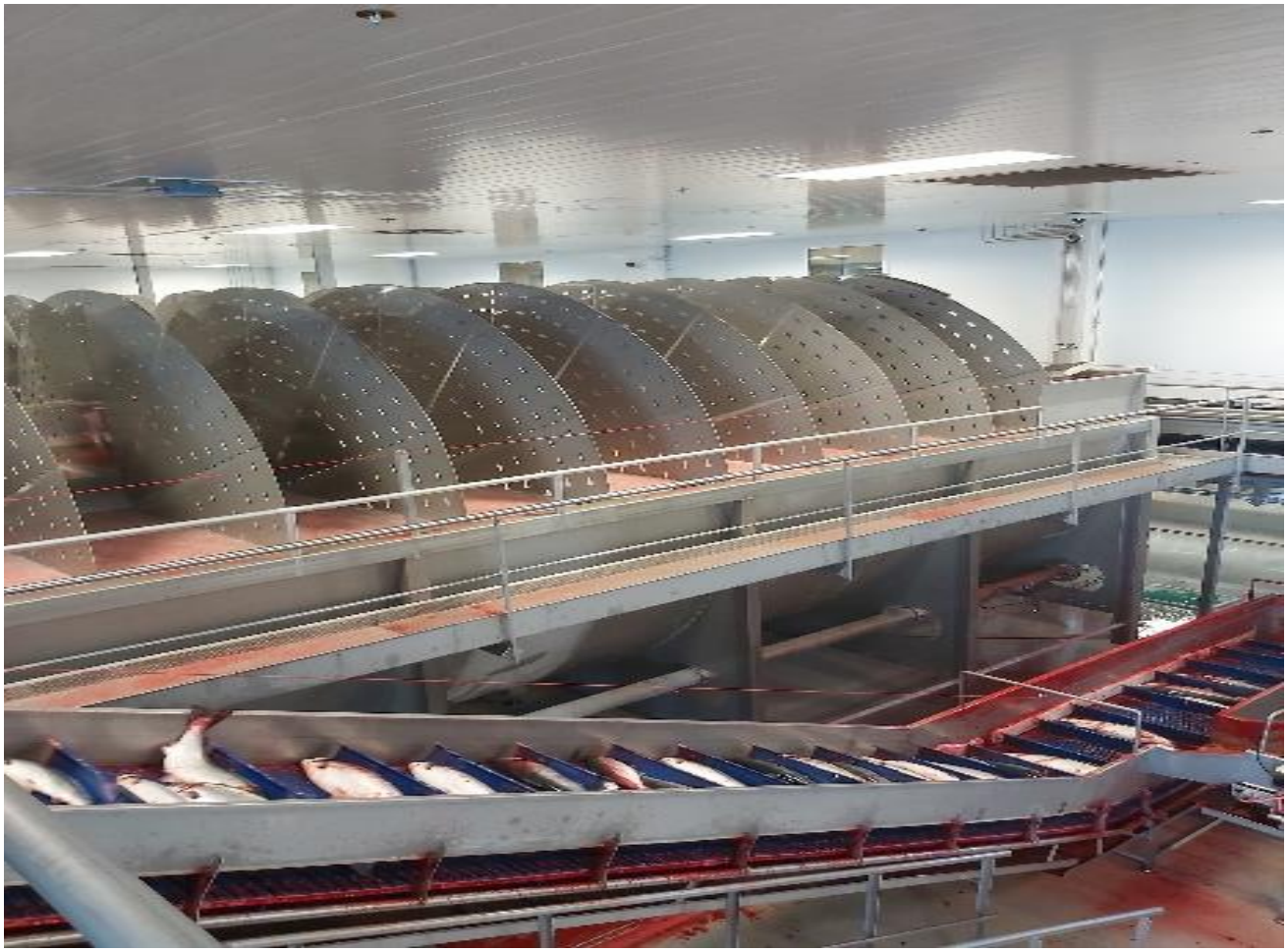

Figure 6 Example of cooling tanks where the fish lays in water and are bleeding out. In the front the conveyer belt where the fish is transported out of the bleeding area.

Figure 7 A part of the desliming area where the fish is rinsed after coming from the cooling tanks.

Figure 8 An example of a quality control area after desliming. The fish is sorted and checked before gutting.

Figure 9 An example of automated gutting machines.

Figure 10 An example of a manual gutting working station.

Figure 11 An example of a working station, heading machine.

Figure 12 An example of a storage container where the fish can be storage before heading.

1.3 Bioaerosol

1.3.1 What Are Bioaerosol.

Bioaerosol can be wet or dry. It is tiny particles of biological origin. Worlds Health Organization defines droplets as ≥ 5-10 μm aerodynamic diameter and aerosols as <5 μm (Wilson, Corbett, & Tovey, 2020). Wet and dry bioaerosols can generally spread airborne bacteria, viruses, fungi, allergens, algae, mites, pollen, toxins (endotoxins, exotoxins, mycotoxins) from microbes, and raw material/parts from animals, plants, and microbes to the surrounding environment (Bahna, 2004).

Several occupational groups may be exposed to bioaerosol, but the main groups are health personnel, laboratory personnel, agriculture workers, animal workers, the food industry workers, sewage, waste disposal and waste treatment workers, and those who work in the sawmill industry (Arbeidstilsynet, 2021 A).

Bioaerosols contain biologically active particles (Jeebay M. F., 2011). Research shows that the handling of fish during processing, such as freezing, heating, and pressurizing, can change the behavior, dose, and allergenic effect of these particles (Jeebay M. F., 2011) (Bahna, 2004) (Bernhisel-Broadbent, Strause, & Sampson, 1992) (Jeebhay, 2019). For example, fish stored on ice appears to have particles with high-molecular-weight allergens and higher IgE (Immunoglobulin E)-binding capacity than fresh fish (Jeebay M. F., 2011). This may be because formaldehyde and other natural components that develop in tissue of fish may alter the allergenicity of some proteins. (Jeebay M. F., 2011)

The type of fish, or seafood, and the part of the fish the protein originates from, can also influence the potential harmfulness of the particles (Jeebay M. F., 2011) . When handling fish, amines, digestive enzymes, skin and mucus particles, and collagen and muscle protein can be released into the air (Jeebay M. F., 2011) (Arbeidstilsynet, 2021 B). Additionally, exposure to parasites, algae, bacteria, viruses, bacterial toxins (saxitoxins, scombroid toxins), endotoxins (Gram-negative bacteria), histamines, and gases from fish decomposition, such as hydrogensulphide (H_2S), is possible (Arbeidstilsynet, 2021 B).

1.3.2 The Formation of Bioaerosol

How bioaerosol are generated are still not completely understood. But a general, simplistic explanation is that bioaerosols can be formed by breaking a surface of a biological material for instance by use of high-pressure washer, nozzles with water, wave power, suction, emptying of liquids, shower jets, cooling systems, rain droplets, and fountains. The surface can also be broken by a living being (animal/human) making strong air currents by, for example, coughing, sneezing, or vomiting (Stezenbach, 2009).

Larger drops that have a diameter of approx. 0.1 mm will precipitate reasonably quickly, but smaller droplets will evaporate before they have time to fall. This will create droplet cores or aerosol that consist of very small amounts of dry matter that can be spread over large distances (Stezenbach, 2009).

Bioaerosol can also be reintroduced to the environment, because bioaerosol that has settled on surfaces might be pick up by air movements or being kicked up when humans or animals are walking on the surface (Stezenbach, 2009).

1.3.3 Bioaerosols Spreading and Transmitting

Bioaerosol enter the atmosphere and are transported locally and globally (Smets, Moretti, Denys, & Lebeer, 2016). Bioaerosols enter the air from the land and sea (Smets, Moretti, Denys, & Lebeer, 2016) (Nunez, et al., 2016). From the ocean, bioaerosols are generated via spray from waves and bubbles. Studies show that bioaerosols from the sea mainly consists of bacteria, especially Gram-negative bacteria. The bioaerosols from the terrestrial environment contains bacteria (mainly Gram-positive), fungi, and pollen, as well as viruses (Nunez, et al., 2016).

Bioaerosols can transmit various microbes, allergens, and toxins in the atmosphere. It is believed that bioaerosols play an important role in our ecosystem and climate, and it is suggested that the different microbes, pollen, and spores are not only dispersed into the air as particles but can also form particulate communities (air biota) (Smets, Moretti, Denys, & Lebeer, 2016) (Nunez, et al., 2016).

Unlike droplets, aerosols can stay afloat for hours and spread over greater distances. They may spread from one room to another, or from one floor to another. Aerosols is like an

invisible "cloud" that just slowly settles. How far infectious droplets can travel through the air depends to a large extent on the room ventilation, whether the wind or air currents in a room move the particles from the spreading agent towards other people, the room temperature, and humidity (Xie, 2017). Additionally, the droplet size is an important factor. Small particles are believed to travel further and at a higher level than larger particles (Xie, 2017). However, it should be considered that not all microbes are present in bioaerosols as single components. Larger droplet particles may contain, for example, more virus particles. Thus, they may contain more infectious particles, but travel a shorter distance than smaller droplets with smaller amounts of virus (Guo, et al., 2021).

1.4 Bacteria

Bacteria are single-celled organisms with a cell membrane, but no organized cell nucleus or membrane-bound organelles. Bacteria can have different shapes and are usually around 1–5 μm (1 × 10−6 m) in size. They are unicellular and reproduce by simple cell division (11-13). Some bacteria can produce endospores. Spores are extremely hardy and highly resistant to external stresses (Forbes, Sahm, & Weissfeld, 2007).

Bacteria is a major component of microbes in the air. They can unlike other parts of bioaerosols, survive and complete full reproductive cycles within hours or days in the atmosphere. A yet not proven theory is that bacteria bioaerosols form communities in an atmospheric ecosystem. It is believed that water droplets from clouds and fog can provide bacteria with nourishment and protection from UV light. The bacterial groupings that are known to be abundant in aero microbial environments worldwide are Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes (Ruiz-Gil, et al., 2020).

1.4.1 Classifications

There is no official classification of bacteria. The names given are regulated by the *International Code of Nomenclature of Bacteria* (*Bacteriological Code*) and (*International Code of Nomenclature of Prokaryotes*). LPSN "List of Prokaryotic names withstanding in Nomenclature" was developed as a solution to this problem. It provides accurate information about the status of a name, synonyms, and other useful information (Parte, 2020).

1.4.2 Ecology

Most bacteria can live freely, or in connection to a host to survive. Many bacteria express an enormously flexibility to adapt to living in various conditions, such as extreme cold or heat, without oxygen (anaerobic) and with oxygen (aerobic), some can live under various oxygen conditions (facultative anaerobes), or in highly acidic environments, while others must live under strict conditions (Forbes, Sahm, & Weissfeld, 2007).

Bacteria live in soil, water, air, animals, humans, and plants. They may become airborne from any of their natural habitats. Airborne bacteria, except spores, usually survive for only a short time outdoors, because of sunlight etc. The indoor environment usually contains larger amounts of bacteria than the outside environment; this because humans and animals shed bacteria to the air from their skin and respiratory tracts (Macher, Amman, Burger, Milton, & Morey, 1999).

1.4.3 Bacteria Associated with Fish and Marine Environment

The muscle tissue of healthy salmon is considered sterile when it is taken immediately from the water. Microbes are present on the outer surface, gills, and in the digestive tract. Studies show that bacteria established early during processing may be retained throughout the production chain and adversely affect the product quality and safety (Svanevik, 2015).

Studies show that the bacteria in the northern seas are dominated by gram-negative rod-shaped bacteria. *Pseudomonas* spp., *Moraxella* spp., *Acinetobacter* spp., and *Flavobacterium* spp. are the bacteria most seen in northern sea areas. *Vibrio* spp., *Aeromonas* spp., and *Micrococcus* spp. are seen less in northern sea areas, but more in warmer southern sea areas (Munn, 2011) (Gjerde, 1976).

It is rare that fish which is freshly catch from the sea contains human pathogenic bacteria (Novotny, Dvorska, A., Beran, & Pavlik, 2004) (Gjerde, 1976). Non-marine bacteria as: *Salmonella* spp. is believed to be a very small problem (Gjerde, 1976), but some suggest that certain fish might be a passive carrier of *Salmonella* spp. (Novotny, Dvorska, A., Beran, & Pavlik, 2004). Intestinal bacteria such as *E. coli, Campylobacter jejuni,* and *Klebsiella* spp. can be seen in areas with sewage contamination. *Clostridium botulinum* is seen in all sea areas around the world (Munn, 2011) (Gjerde, 1976). In addition, there is for example: *Aeromonas hydrophila, Vibrio Cholerae, V. parahaemolyticus, V. vulinificus, V. alginolyticus, Listeria*

monocytogenes, and *Mycobacterium marinum* (Novotny, Dvorska, A., Beran, & Pavlik, 2004).

Psychrophilic species are seen naturally in fish. Psychrophilic bacteria have an optimal growth temperature of 15 ℃ or lower, a maximum growth temperature of 20 ℃ or lower, and a minimum growth temperature of 0 ℃ or lower. Examples include *Shewanella* spp., *Photobacterium* spp., and *Flavobacterium* spp. (Gjerde, 1976) (Munn, 2011).

1.5 Endotoxin

Endotoxin is a part of the outer membrane in gram negative- cells and is released when the bacterial cell dissolves. Some gram-negative bacterial species such as *Escherichia coli, Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Neisseria* spp., *Haemophilus influenza, Bordetella pertussis,* and *Vibrio cholera* are known for their endotoxins. The major component of endotoxin is lipopolysaccharide (LPS). In addition to be a toxin, LPS is an important part of the membrane structure. It contributes to stabilization, and protection from certain chemical attacks (Parija, 2009).

The main components of endotoxin are: lipid A, O antigen (O polysaccharide), and the core (oligosaccharide). Antigenic nature is connected to O-antigen. Toxicity is mainly associated with the lipid A. They are stable in heat and not usually soluble. Oxidizing chemicals such as superoxide, peroxide and hypochlorite are often used to destroy endotoxins (Parija, 2009).

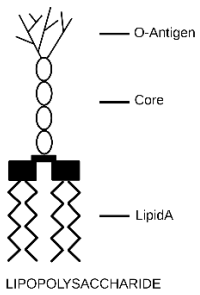

Figure 13 A schematic picture an endotoxin with the different parts, O-antigen, Core, and Lipid A. Source https://no.wikipedia.org/Endotoksin

1.6 Health Effects

1.6.1 Health Effects Related to Bacteria and Endotoxins

Health Effect Bacteria

Not all bacteria cause disease, and many species are essential to our health. Bacteria are divided into pathogenic, normal flora, and opportunistic bacteria. Pathogenic bacteria cause disease. Normal flora is a term for microorganisms that normally exist, for example, on our skin and mucous membranes without causing disease. Opportunistic bacteria can cause disease when our immune system is weakened, or when they become established in a part of the body, where it is not natural for them to be (Vorland, 2001).

The bacteria have several pathways to cause disease. Human pathogenic bacteria can be said to have three main strategies to cause disease in the body. 1. By toxins 2. Penetration of the body's defense system and 3. Fight and hide from our immune system. They may also use all three strategies (Vorland, 2001).

Toxins: There are different types of toxins. Toxins can be divided into two main categories, endotoxins, and exotoxins. An exotoxin can damage cells or by disrupt the metabolism in a host. Exotoxins are produced by both Gram-negative and Gram-positive bacteria. Exotoxins can be secreted, and they can be carried with the blood around the body; therefore, the toxic effect can affect organs far from the place where the infection is located. Exotoxins are considered more toxic than endotoxins (Vorland, 2001). Exotoxins are not known to be in the air (Macher, Amman, Burger, Milton, & Morey, 1999). Toxin alone can cause most of the clinical picture in diseases, such as cholera and diphtheria (Vorland, 2001). Endotoxins see chapter 1.8.

Fight and hide: Some bacteria, for example, have a capsule that can protect them against the body's immunity system, and thus enables them to grow in the host organism. For example, Pneumococci becomes harmless when it loses its capsule. Some bacteria can vary their surface structures frequently, so that they change quickly and "trick" the body's immune system and not be recognized. Many bacteria have been shown to be able to trigger programmed cell death in both immune and non-immune cells (apoptosis) (Vorland, 2001).

Penetrate and attach: A necessary step in colonization and invasion is the attachment of the bacterium to tissues. Bacterial adhesins can be divided into two main groups: pilus and non-pilus adhesins. Fimbria, or pilus, is hair-like structures on the bacterial surface. Fimbria allows the bacteria to attach to cells. Bacteria can also use other structures than pili to attach to cells. Some of these non-pilus adhesins bind to receptors on the leukocytes, which contribute to the uptake of the bacteria into the macrophages, but without triggering a phagocytosis response (Vorland, 2001).

Collaborate: It has been shown that some types of bacteria can cooperate. This is seen, for example, in periodontal disease. Studies show that bacterial cells recognize each other based on adhesins and receptors on the bacteria (Vorland, 2001).

Health Effects Endotoxin/LPS

LPS can induce a strong immunity response, and this can lead to symptoms as fever, vomiting, diarrhea, changes in the number of white blood cells, and high blood pressure. High values in the air might cause respiratory symptoms such as inflammation, irritation, asthma-like symptoms, and impaired lung function (Farhana & Khan, 2021).

Endotoxins are mostly released when the bacterium dies, but it is suggested that they also release small amounts when the bacteria grow. Endotoxins are less potent and less specific that exotoxins, since they do not act enzymatically (Todar, 2021)

Not all endotoxins have the same toxicity. Studies show that the composition of the O antigen varies from bacteria strains. More than 160 different O antigen structures are, for example, produced by different *E. coli* strains. The differs in the O-chain is a part of the different toxicity. Lipid A also varies among different bacterial species. It is the part of the gram-negative bacteria that stands for most of the toxicity, but its effect will differ with its structure. This means that endotoxin alone should not be used as the only measurement for the toxicity of gram-negative bacteria. Which gram-negative bacteria the endotoxin is most likely to come from, should also be part of the evaluation (Raetz & Whitfield, 2002).

In recent years, there has also been a focus on how exposure to small amounts of endotoxin in aerosol will affect the health of those who are exposed. A review article (Farokhi, Heederik, & A.M., 2018) from 2018 concludes: that there are many studies that connect low levels (< 100 EU/m^3) of airborne endotoxin to respiratory health effects. It might be that people with

atopy, or a chronic lung disease are more receptive to effects of exposure to endotoxin. But more research is needed to find the precise connection between airborne endotoxin levels and health effect (Farokhi, Heederik, & A.M., 2018).

1.6.2 Health Effects Bioaerosol

Bioaerosols are associated with several health problems; however, the exact roles of the various bioaerosol components in the development of disease remain unclear(Delort & Amato, 2018).

Many aspects of bioaerosols are involved in the development of disease, such as size, origin, concentration, allergenicity, and the ability to penetrate the respiratory tract. Host-related and environmental factors are also relevant. Environmental factors can include manual handling or automated processes, wet or dry processes (air pressure), and old or new machines. Host factors can include atopy, smoking, preexisting skin diseases, and rhinitis (Delort & Amato, 2018).

The Classification of Health Effects, Bioaerosols

Both living (viable) and non-living (non-viable) components in the bioaerosol can cause health problems.

Infections: These are caused by viable pathogenic microbes (viruses, bacteria, fungi, and amoebae (Delort & Amato, 2018) (Macher, Amman, Burger, Milton, & Morey, 1999). Several factors can influence the maintenance of infectious agents in the bioaerosol. Concentration is the main factor; however, humidity, particle density, size, and ultraviolet exposure affect the infectivity (Delort & Amato, 2018) (Macher, Amman, Burger, Milton, & Morey, 1999). For example, one study stated that influenza showed complete loss of infectivity when exposed at 30°C. This has not been shown for non-viable components (Delort & Amato, 2018).

Toxic effects and inflammation: These are caused by toxins and non-viable components. The most documented toxin is probably endotoxin; however, studies also show that other subcellular components such as sugars, lipids, and proteins can trigger the immune response. These components are part of a family of “danger signals” known as microbe-associated molecular patterns (MAMPs). On exposure to MAMPs, various signals occur that result in the

secretion of pro-inflammatory mediators. This causes attacks by leukocytes, which in turn cause an immunopathological response resulting in tissue destruction and/or impairment of organ function. (Delort & Amato, 2018).

Table 1 shows an overview over what diseases, symptoms and immunopathology that is associated with different bioaerosol components.

Table 1 An overview of etiology, symptoms, and immunopathology of bioaerosol-related diseases (Delort & Amato, 2018).

Bioaerosol component	**Respiratory disease**	**Symptoms**	**Immunopathology**
Saccharopolyspora rectivirgula	Farmer's lung	Fever, malaise, nausea, chest tightness, headache	Lymphocytosis, granuloma, lung fibrosis, high IgG titers
Endotoxins, muramic acid	Chronic bronchitis	Sputum-producing cough	Neutrophilia, tissue monocytosis and lymphocytosis
Endotoxins, mold spores, mycotoxins	Organic Dust Toxic Syndrome (ODTS)	Fever, malaise, dyspnea, chest tightness, headache	Neutrophilia, Interleukin-1 (IL-1), IL-6, IL-8, Tumor necrosis factor (TNF) cytokine production
Allergens, endotoxins, peptidoglycan, bacterial DNA	Asthma, asthma-like syndrome	Cough, chest tightness, dyspnea, wheezing	IgE, eosinophilia, chronic inflammation, bronchial hyperreactivity, tissue remodeling
Allergens, endotoxins, peptidoglycan, bacterial DNA	Allergic rhinitis	Congestion, rhinorrhea, sneeze, pruritus, nasal mucous membrane inflammation	Neutrophilia, IL-8, IL-6
Mold spores	Sick building syndrome	Congestion, pruritus, dry throat, fatigue, headache	Unknown

1.6.3 Health Effects Related to the Fish Industry.

Although most fish processing factories have become more automated, the risk of employees becoming sick or injured, remains high (Jeebay M. F., 2011). The ventilation is often unsatisfactory (Jeebay, Robins, & Lopata, 2004). The aerosols produced during production have been shown to pose a high risk of immunological sensitization, respiratory problems, non-specific bronchial hyperresponsiveness, and asthma (Bang, Larsen, Larsen, & Aasmoe, 2005) (Dahlmann-Høglund, Renstrøm, & Larsson, 2012) (Lopata, et al., 2019). Studies show that wet aerosols from processes, such as bleeding and gutting, produce a larger number of particles (respirable fraction) than dry activities, such as packing and filleting (Jeebay M. , 2011). However, dry aerosols, such as those caused by high air pressure, can be more harmful, as it is suggested that water inhibits the spread of aerosols (Jeebay M. F., 2011).

Of the health problems associated with bioaerosols in the fishing industry, epidemiological studies indicates that rhinitis is the most common, representing ca. 5–24% of cases, which is probably an underestimation. Rhinitis is also often the first sign of an underlying allergy. Asthma accounts for approximately 2–36% of cases. The variation in the estimates varies because of countries different definitions on occupational diseases, working conditions, and what kind of allergen the worker is exposed to (Jeebay M. F., 2011).

In addition to respiratory problems, skin problems occur to those who work along the production line. The main reason for skin problems is that the skin is unprotected when handling the fish. It is then exposed to various constituents of the fish, such as amines, histamines, digestive enzymes (trypsin and pepsin), and cadaverine. All these are high-molecular-weight proteins. The skin problems are mainly contact urticaria and various types of eczema Chronic recurrent dermatitis is observed when working with fish protein, known as protein contact dermatitis (PCD). However, most cases of eczematous dermatitis, around 75%, are of an irritating nature and are caused by frequent contact with water and fish juices. Additionally, contact dermatitis is observed due to frequent contact with detergents, hand soap, and various spices added to the fish (Burdzik, Jeebhay, & Todd, 2012) (Aasmoe, L; Bang, B.; Anderson, G.; Evans, R.; Gram, I.; Løchen; M., 2005).

But there are also many other work environment factors than bioaerosol that can contribute to health problems (UNN, Arbeidsmiljø og arbeidsmedisin).

Unpublished occupational rapports, and rapports from UNN, show that the noise level is often very high in the fish processing industry. It is not uncommon for the sound level to be around 80-95 dB (A), and in addition impulse noise is seen. The impulse noise often originates from truck driving and handling of pallets and fish boxes (UNN, Arbeids og miljømedisin, 2004).

For those who work to keep the production premises clean, there will also be an exposure to chemical work environment factors. The industrial cleaners use highly toxic chemicals in their routines. Their work is characterized by hard physical work, with exposure to many different chemicals. Exposure may occur through both skin and respiratory tract (UNN, Arbeids og miljømedisin, 2004).

Other factors that can contribute negatively are night work, shift work, high performance requirements, and workers from many different cultures that might not fully understand each other. This can contribute to frustration, discrimination, and staff conflicts (UNN, Arbeids og miljømedisin, 2004).

Thermal working environment (cold) is a working environment factor that can also contribute to the respiratory problems (Bang, et al., 2005) (Conway & Husberg, 1999). The temperature is around 8-10 ℃ in the production areas. Ice water, cold fish, and sea water will contribute to the experience of being cold, in addition to work surfaces made of steel (Bang, et al., 2005).

1.7 Limits and Assessment Criteria

In Norway there is no legal limit values for microbes in the working environment. In Norway, we have" Regulations concerning action and limit values for physical and chemical agents in the working environment and classified biological agents (Regulations concerning Action and Limit values)" from the Norwegian Labor Inspection Authorities (Arbeidstilsynet, 2021 C). The regulations contain a list of classified biological factors (infection risk groups). In this list, living biological factors are classified into four infection risk groups according to the infection risk they represent (Arbeidstilsynet, 2021 C). This grouping is based on the danger associated with being exposed to these microbes rather than the exposure level (Arbeidstilsynet, 2021 C). The infection risk groups (Table 2), into which microbes are divided provide the basis for the protective measures an employer must take if workers are exposed to the given bacteria. The Norwegian Labor Inspection Authorities suggest that the level of bioaerosols should be as low as possible (Arbeidstilsynet, 2021 B).

Table 2 The infectious disease groups for bacteria and viruses according to the Norwegian Labor Inspection Authorities' "Regulations on action and limit values for physical and chemical factors in the working environment as well as infection risk groups for biological factors"

Infectious disease group	**Level of infection**	**Examples**
1	A biological factor that does not usually cause infectious disease in humans (5)	
2	A biological factor that can cause infectious disease in humans and endanger workers, is unlikely to spread to society, and effective preventive measures or treatments are generally available (5)	*Escherichia coli* *Salmonella enteritidis* *Legionella spp.* *Listeria spp.* *Vibrio spp.* Hepatitis A virus Enteric viruses Influenza virus
3	A biological factor that can cause serious infectious disease in humans and pose a serious risk to workers, although there may be a risk of spreading to society; effective preventive measures or treatment are generally available (5)	*Salmonella typhi* Hepatitis C virus Yellow fever virus
4	A biological factor that causes serious infectious disease in humans and poses a danger to workers; there can be a high risk of spread to society, and there are usually no effective preventive measures or treatment (5)	Variola virus Ebola virus Marburg virus

1.7.1 International Guidelines and Limits for Bioaerosols

According to The Norwegian Labor Inspection Authorities, international guidelines and occupational hygiene limits and criteria can be used for substances that have no set limit (Arbeidstilsynet, 2021 C).

There are several guidelines and standards from other countries and private organizations. In Norway we often use American Conference of Governmental Industrial Hygienists (ACGIH) guidelines for bioaerosol (ACGIH, 1989). These guidelines are <100 Colony Forming Units pr. cubic meter (CFU/m^3) = low exposure, 100-1000 CFU/m3 = intermediate exposure, and > 1000 CFU/m3 = high exposure. I addition the guidelines from the American Industrial Hygiene Association (AIHA) is often used (AIHA, 1986). Their guidelines are: There is no safe level of an uncontained pathogenic organism.

1.7.1 Legal limits Endotoxin

Norway does not have any legal occupational exposure limit for endotoxin in the air. The Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council recommends a health-based occupational exposure limit of 90 EU/m^3 (Health Council of the Netherlands, 2010). DECOS regards an exposure level of 90 EU/m^3 as a No Observed Effect Level (NOEL).

1.8 Research on Bioaerosols in the Fish Industry, Last Ten Years

Table 3 Overview of studies performed on bioaerosol in the fish industry last 10 years.

First Author/year	Design	Population	Exposure from	Information	Results
Exposures and Health Effects of Bioaerosols in Seafood Processing Workers - a Position Statement (Bonlokke, et al., 2019)	NA (The Fifth International Fishing Industry Safety & Health Conference)	175 participants from 20 countries	Bioaerosol	Find ways to show key findings and recommendations to the industry. Discuss future needed research.	*"Occupational hazards exist in the seafood industry; more research is needed to find good solutions to the problems."*
Hypersensitivity pneumonitis in fish processing workers diagnosed by inhalation challenge (Tjalvin, Svanes, & Bertelsen, 2018)	Case study	1 case	Salmon meat, allergen	A patient was exposed to salmon meat and afterwards examined with: Spirometry, DLCO, symptoms, physical examination, temperature and SpO2 were recorded after 30 min, and then hourly for the next 6 h. A white blood cell count was performed before and after the challenge.	"*Asthma is probably the most frequent respiratory disease associated with the fish processing industry. However, hypersensitivity pneumonitis should be considered if the patient exhibits a clinical picture with influenza-like symptoms and dyspnea.*
HMS-undersøkelsen i havbruk (Thorvaldsen, Holmen, & Kongsvik, 2016)	Questionary	447 workers	NA	Workeres own experience.	The workers like their job and the enviromnet, but there is challenges with accidents.
Respiratory symptoms, lung functions, and exhaled nitric oxide (FENO) in two types of fish processing workers: Russian trawler fishermen and Norwegian salmon industry workers (Shiryaeva O. , Aasmoe, Straume, & Bang, 2015)	Levels of fractional exhaled nitric oxide (FE_{NO}), spirometric values, prevalence of respiratory symptoms, and self-evaluated exposures	139 Norwegian salmon workers and 127 Russian trawler workers.	Cold environment , detergents, chemicals, and "contaminated indoor air"	Work on Norwegian and Russian trawlers.	*Respiratory symptoms commonly associated with obstructive airway diseases were more prevalent in salmon workers, while symptoms commonly associated with asthma and short-term effects of cold air exposure were more prevalent in trawler workers."*

Respiratory effects of bioaerosols: Exposure–response study among salmon-processing workers. (Shiryaeva, et al., 2014)	Exposure/response Personal sampling and area sampling	5 factories, 72 workers	Total protein, parvalbumin , endotoxin	Salmon factories slaughtering and filleting.	A tolerance effect during a workweek is suggested. Use of water hose is a risk process about the liberation of measured components of bioaerosols".
Exposure to Parvalbumin Allergen and Aerosols among Herring Processing Workers (Dahlman-Höglund, Renström, Fernando, & Andersson, 2013)	Personal aerosol sampling and stationary sampling.	1 factory, 40 workers	Allergen, mold, endotoxin	Herring factory	*"High exposure to herring antigen was measured during filleting work. The particles in the air around the fillet machines were mainly <0.5 µm. and the newer encapsulated machines generated fewer particles. It is important to reduce occupational exposure of workers to aerosols by improving the ventilation system, machines, and organization of work."*
Salmon allergen exposure, occupational asthma, and respiratory symptoms among salmon processing workers (Dahlmann-Høglund, Renstrøm, & Larsson, 2012)	Stationary samples And questionary	1 factory 38 workers	Allergen, mold, endotoxin	Salmon factory	*"Salmon antigen in air and found that filleting workers were most exposed. It is important to reduce aerosols by improving the ventilation system, machines, and organization of work since respiratory symptoms at work among workers were common."*
Airborne seafood allergens as a cause of occupational allergy and asthma. (Lopata & Jeebhay, 2013)	NA (Review)	NA	Seafood allergens	NA	*"This review has highlighted the importance of evaluating, identifying, and characterizing the allergens responsible for occupational seafood allergy and asthma. The insights that have been developed have the potential for promoting its application and use in various settings in the home and work environment. For seafood-processing workers, these include evaluation of the work environment, in-vitro evaluation of suspected materials, product labelling, monitoring of allergen exposure during specific inhalation allergen challenge,*

					development of exposure standards, evaluating the impact of allergen avoidance, medical surveillance of exposed workers in relation to observed sensitization patterns and symptoms, and exploring the possibility of developing immunotherapy options".
Respiratory symptoms in fish processing workers om the Adriatic coast of Croatia (Zuskin, et al., 2012)	Description of respiratory symptoms and lung function	98 workers	NA		*"These findings suggest that fish processing workers are prone to developing acute and chronic respiratory symptoms as well as to lung function changes. This calls for medical and technical preventive measures to be introduced in the work environment of the fish processing plant".*
Occupational allergy and asthma in the seafood industry– emerging issues. (Jeebay M. F., 2011)	(NA) Review	NA	Seafood allergens	NA	*"This review has identified some emerging areas and developing trends in relation to occupational allergy and asthma in the seafood industry. These relate to industrial change (linked to ecological degradation and globalization) and associated global shifts in production that form the basis for continued and increased exposure to vulnerable populations".*

The literature contains minimal information about the potential for exposure to bioaerosols and microbes in the fishing industry. In 2019, an article was published entitled, “Exposures and Health Effects of Bioaerosols in Seafood Processing Workers – A position statement”. Here, it is mentioned that the working environment in the fishing industry presents major challenges. More research on causes and measures is required, and several different professional groups must address this to develop a comprehensive understanding of the situation (Bonlokke, et al., 2019). The same article also states that allergens alone are not responsible for all respiratory tract infections. Future studies must address a wider range of potential causes, such as bacteria, glucans, and mold (Bonlokke, et al., 2019).

1.9 Rationale

Industry workers in the fish industry have a high prevalence of work-related airway symptoms. More in-depth research is required to determine why. The articles mentioned all has a focus on allergens, but bioaerosols in the fish industry can also consists of microbes (bacteria, virus, mold), algae, toxins etc. From an occupational hygiene viewpoint, it is important to evaluate the working environment, determine the sources of pollution, and suggest measures that reduce the exposure. All possible biological factors in the bioaerosol should be studied. This study aims to investigate whether bacteria from the fish and marine environment can enter the factory as bioaerosol. Such findings would help us to take appropriate steps to improve the air quality in production.

1.10 Objective and Research Question

1.10.1 Objective

Main objective: The main objective of this study is to obtain more knowledge about the bioaerosol composition in the slaughter area in the factories for processing farmed salmon.

Specific objectives: To examine the presence of pathogenic and opportunistic bacteria in bioaerosols in the slaughter area and measure the endotoxin levels in the same area.

1.10.2 Hypothesis

To answere the objectives four hypotheses and their null-hypotheses were defined.

H1: Pathogenic bacteria can be detected in air samples in the slaughterhouse.

H0: Pathogenic bacteria cannot be detected in air samples in the slaughterhouse.

H2: Opportunistic bacteria can be detected in air samples in the slaughterhouse.

H0: Opportunistic bacteria cannot be detected in air samples in the slaughterhouse.

H3: Measured concentrations of bacteria in air samples are above intermediate exposure level, according to the bioaerosol guidelines to ACGIH.

H0: Measured concentrations of bacteria in air samples are belove intermediate exposure level, according to the bioaerosol guidelines to ACGIH.

H4: Measured endotoxin levels in air samples are above the Dutch limit value.

H0: Measured endotoxin levels in air samples are under the Dutch limit value.

2 Method

2.1 Research Design

This study has a quantitative research design and is a cross-sectional study. The goal of the study is to assess possible air exposure to endotoxin, human pathogenic, and opportunistic bacteria from ocean water or fish, in the slaughtering department of processing factories for farmed salmon.

The outcome variables in this study were the presence of selected bacteria (nominal level), and the presence of endotoxin and colony forming units (ordinal level).

Contextual information such as factory design, number of fish etc. was obtained from the quality departments (Table 4). Ventilation information was obtained from the technical departments at the factories.

Table 4 Contextual information during the fieldwork

Independent Variables	**Measurement unit**	**Source of Information**	**Measuring Level**
Fish			
Amount of fish pr. day	Tons	Information obtained from factory	Ordinal level
Dead fish	Pieces	Information obtained from factory	Ordinal level
Sick fish	Pieces	Information obtained from factory	Ordinal level
Differences between factories			
Ventilation	Type of ventilation, Model, Special programs (ex. Washing)	Information obtained from factory	Nominal level
Machine models	Model, year	Information obtained from factory	Nominal level
Hygiene/washing routines	---	Information obtained from factory	Nominal level
Size/layout	---	Information obtained from factory	Nominal level
Hygiene results		Information obtained from factory	Ordinal level
Number of employees	Numbers	Information obtained from factory	Ordinal level
Shift	Day/evening/night	Information obtained from factory	Nominal level

Used of power hose	Yes/no	Visually obtained	
Sampling			
Sampling month		Noted	Nominal level
Indoor conditions			
Relative humidity	Percent	Logged at sampling period	Ordinal level
Temperature	Celsius degrees	Logged at sampling period	Ordinal level
Weather conditions			
Temperature	Celsius degrees	Local weather news	Ordinal level
Wind speed/direction	Kilometer pr. hour	Local weather news	Ordinal level
Precipitation/sun	Yes/no	Local weather news	Nominal level

2.2 Setting

The data collection has been performed in the field, under normal working conditions in two different salmon farming factories. The two factories were selected as they are similar at slaughter volume, hygiene control, washing routines, but also have some points that are different such as ventilation, waiting cages, gutting machines. See chapter 2.3.1 for further information.

The information about the factory design, number of fish etc. was obtained from the quality departments. Ventilation information was obtained from the technical departments at the factories.

2.2.1 The Factories

Building year/ size

Factory A: 2010, slaughterhouse 1821 m^2, room volume 10926 m^3.

Factory B: 2018, 851m^2+732 m^2 (Infeed/bleeding + degutting), room volume approx. 8707 m^3.

Amount of people in the slaughterhouse

Factory A: Approximately 45 on a dayshift.

Factory B: Approximately 20 on a day shift.

Waiting cage / direct intake.

Factory A: Has waiting cages outside the factory.

Factory B: Has direct intake from boat, no waiting cages outside the factory.

Infeed

Factory A: The infeed is placed higher than the workers, approximately 2 meters above the employees working station.

Factory B: The infeed is placed at the same level as the employee working station.

Helix (cooling tanks)

Same at both factories. The tanks are open, and there is no local exhaust above the tanks.

Degutting machines

Factory A: Baader 142 (16 fish/min).

Figure 14 Baader 142 (Picture from www.baader.com)

Factory B: Baader 144. Which is a newer version of 142 with a higher speed (25 fish/min, Baader 142: 16 fish/min).

Figure 15 Baader 144 (picture from www.baader.com)

Beheading machines

Factory A: Baader 434 (measured at), they also have Marel MS 2725 (line 1)

Figure 16 Baader 434 (picture from www.baader.com)

Factory B: Marel MS 2720. The major difference from Baader 434 is that the worker is standing more inside the machine.

Figure 17 Marel MS 2720 (picture from www.marcel.com)

Hygiene Control

Both factories perform daily and periodic hygiene inspections of fish, untreated seawater, equipment, and surfaces. Sampling parameters are germ count, *Legionella spp.*, *Listeria monocytogenes*, and *Enterobacteriaceae*.

The factories hygiene controls from the sampling weeks did not show any positive findings.

Ventilation

Factory A: The ventilation principle is dilution, air amount110943 m^3/h, 6.0 m^3/h/m^2, air changes/hour 1.0. No specific ventilation program for industrial washing. Recirculation air and dehumidification are used.

Factory B: Factory 2: The ventilation principle is dilution, 35000 m^3/h, air changes/hour 4.0. Special washing program for industrial washing. At «Wash» all the air is changed out. No recirculation or dehumidification, temperature approx. 20°C. At «wash + dry» air changes are at normal modus, but the air is dehumidified, temperature is approx. 20°C. Recirculation air and dehumidification are used.

The ventilation is divided into the zones that can be regulated individually. The zones are: Infeed/bleeding, degutting, packing, filet.

In summary the main differences between the factories are that Factory A is older, larger, has less air changes and more employees compared to factory B. Differences regarding the factory layouts are that the rig for intake of fish is higher than the employee's workstation at factory A. In factory B it is at the same level. Employees are closer to the floor in factory A than factory B. The production areas in factory A are more open than factory B. Factory B has separated the fillet area completely from the slaughterhouse. In factory A it is also separated, but the wall that separates the slaughterhouse from the fillet department has several openings. Also, the area between the bleeding and the heading areas is more physically separated in factory B.

2.3 Data Collection

2.3.1 Sampling Strategy

Sampling was carried out in the salmon slaughterhouses of two factories. In both factories, sampling took place for five days in one week (Monday through Friday) in the bleeding, gutting, and heading areas, one sample pr. day in each area. Thus, a total of 30 samples was collected, 15 samples from each factory, five from each area. The factories were closed Saturdays and Sundays. Because of the pandemic situation sampling was performed only in one week per factory, not for two separate weeks in different seasons (autumn and winter) as planned. The sampling period was in August.

The samples are performed stationary, not on personnel. The equipment was placed approximately 1-2 meters in breathing height from the workers.

All staff in the area was informed about the study and the measurements by the quality departments. The quality department of each factory will be informed about the results from their factory in a meeting when the study is finished.

2.3.2 Sampling Equipment

The BioSampler from SKC (SKC LTD, 2021) was used to sample bioaerosol in a liquid medium. This impinger sampler is reusable, and it can be autoclaved/sterilized. The impinger

was connected to a pump (BioLite+ Sample Pump) with a flow rate of 12.5 L/min. The sampling time was from the start of the working day to the end of the working day (8 hours). Thus, the total amount of air collected pr. sample were 6000 liters.

Figure 18 The BioSampler from SKC, picture from SKC homepage.

Performance Profile (SKC LTD, 2021):

- Flow Rate; 12.5 L/min.
- Medium used: Phosphate-buffered saline -solution (PBS).
- Method of Operation; Bioaerosol is collected into a liquid by air passes through three 0.630-mm tangential sonic nozzles that output into a swirling flow of collection liquid.
- Collection efficiency; About 100% over a broad selection of particle sizes; At 0.5 µm it decreases to around 90 %.

Relative humidity and temperature were logged throughout the working day by Tinytag (Gemini Data loggers, UK). It was not logged during industrial washing. 3 loggers were in the areas bleeding, gutting, and heading, at head height, as in the middle of the room as possible.

2.4 Laboratory Analyzes

The samples were taken directly to the in house-laboratory for cultivating for pathogenic bacteria. Agar with growth was send to the Institute of Marine Research (IMR), Bergen for confirmation by Matrix Assisted Laser Desorption Ionization- Time of flight Mass Spectrometry (MALDI-TOF-MS). The samples were also frozen immediately with approximately 25 % glycerol, at -80℃ and send to IMR for further analysis of bacteria and endotoxin.

The samples were analyzed for identification and quantification of bacteria with cultivating methods, MALDI-TOF-MS for identification of bacteria, and by LAL chromogenic method for quantification of endotoxin.

2.4.1 Detection of Human Pathogenic Bacteria

Air samples were examined for the human pathogenic bacteria *Salmonella* spp., *Legionella* spp., *Listeria* spp., *Vibrio* spp., and *E. coli*/coliform bacteria by cultivation and was performed at sampling site, at the factories' laboratories.

Detection of *Listeria* spp. was performed diluting the air samples 1:10 with ONE Broth Listeria and mixed for 30 seconds prior to incubation without stirring at 30°C for 24 ± 2 hours. After incubation, the broth was gently mixed before 10µl were inoculated onto Listeria Brilliance agar. The plates were incubated at 37°C for 24 ± 2 hours. Typical colonies were assumed presumptive *Listeria*. An alternative method was applied on frozen samples by adding 250 µl air sample to 2.25 ml. ½ Fraser broth incubated at 30 ± 1 ℃ for 25 ± 1 hours. After incubation 100 µl was spread onto Agar Listeria after Ottavani & Agosti (ALOA) and RAPID'L-mono agar and incubated at 37 ± 1 ℃ for 26 ± 1 hours. From the same broth, 1 ml was transferred to 9 ml Fraser broth and incubated at 37 ± 1 ℃ for 24 hours. After incubation of the Fraser broth, 100 µl was spread onto a second set of ALOA and RAPID'L mono agar. Incubated at 37 ± 1 ℃ for 26 ± 1 hours. Typical colonies were accounted as presumptive Listeria spp.

Detection of *Legionella* spp. was performed by inoculation of 10 µl air sample onto GVPC selective agar (OXOID) and incubation at 35-37°C (*L. anisa* for 5 days and all other organisms for 3 days). Typical colonies were assumed presumptive Legionella spp.

E. coli and coliforms were detected by inoculation of 10 µl of the air sample inoculated onto selective agar (OXOID) and incubation at 37 ℃ for 26 hours.

Detection of *Salmonella* spp. was performed by dilution of the air samples 1:10 with ONE Broth-Salmonella and incubation for 16-24 hours at 42°C. After incubation, 10µl of the broth was inoculated onto Salmonella Brilliance agar (OXOID) and incubated at 37°C for 26 hours.

Growth of both typical or non-typical colonies on any of the selective plates were sent to the microbiology lab at IMR for identification by MALDI-TOF-MS.

Vibrio spp. was detected by inoculation of 10 µl of the airsample onto TCBS selective agar (OXOID) and incubated at 35 ℃ for 24 hours.

2.4.2 Quantification of Bacteria

The number of bacteria in each sample was quantified by spreading 0.075 µl of the sample suspension, and further 0.1 µl of serial dilutions, on the surface of Marine agar and Mueller Hinton agar prior to incubation at 25 °C for 48 hours. All appearing colonies were counted.

Limit of Detection (LOD)

Limit of the detection is the smallest concentration of parameter in a sample that can be distinguish from zero. This will be 1 CFU/agar plate.

The limit of detection for air samples will vary with the amount of air used, but the CFU/m^3 can be calculated. When using an impinger the amount of air, the amount of liquid, and the amount inoculated on the growth medium must be in the calculation (Ramachandran, 2005).

The sample was diluted: 0.75 ml sample + 0.25 ml glycerol. 100 ul sample was inoculated. That means it was 0.075 ml sample from impinger in total.

LOD = (CFU/ml sample inoculated, ml x impinger volume, ml)/ (Flow rate (L/min) x sampling time, (min) x 0.001 m3/L)

(1/0.075 ml) x 20ml / (12.5 L/min x 480 min x0.001) = 1.5/6= 44 CFU/m^3

LOD = 44 CFU/m^3

Limit of Quantitation (LOQ)

It is difficult to establish an exact value for LOQ when counting colonies. This is because 1 CFU on an agar plate is a very uncertain number that will vary when counting several parallels. It has become common to set LOQ as 10-30 times higher than LOD (Ramachandran, 2005).

2.4.3 Identification of Bacterial Colonies

Bacterial colonies obtained on solid agar were identified by applying MALDI-TOF-MS. The bacteria are preserved in a matrix on a MALDI-TOF target plate. A laser beam then releases small biomolecules (ions) from the bacterium. The amount, size and charge of released ions constitute a spectrum (Peptide Mass Fingerprint) based on time of flight. This is unique to most bacterial species. The spectrum is compared by the machine with a database of known bacterial spectra (Havforsknings instituttet, 2021).

2.4.4 Quantification of Endotoxin

The concentration of endotoxins in the obtained samples were measured by the used of Pierce^{TM} LAL Chromogenic Endotoxin Quantification Kit. LAL stands for *Limulus Amebocyte Lysate*, and it stems for horseshoe crab blood. Endotoxins' reaction with Factor C, a proenzyme found in amebocytes from the horseshoe crab *Limulus polyphemus* is used. The activity of this proenzymes activates in the presence of lipopolysaccharides (endotoxins). The method then measures the endotoxin level, by reading at 405 nm, the yellow color that is produced by the activity of this protease in the presence of a synthetic peptide substrate that releases p-nitroaniline (pNA) after proteolysis and producing a yellow color (Thermo Fisher, 2021).

By using a known standard that comes with the analysis kit, a standard curve is created. This curve is then used to determine the levels of unknown endotoxin in the samples, similar to Enzyme-Linked Immunosorbent Assay (ELISA) or total protein quantitation assays (Thermo Fisher, 2021).

A plate reader is used to analyze the results of the endotoxin analysis (LAL). This instrument uses spectrophotometry as the method.

Spectrophotometry is based on light being transmitted through a solution of a specific substance. Once the light has passed through the solution, a sensor detects how much of the light's energy the substance has absorbed. There is a difference in how much light energy different substance solutions absorb. Solutions with a high concentration of specific particles absorb more of the energy of light, than solutions with a lower concentration of the same specific particles do. At the same time, there is a difference in which part of the light spectrum

different substances/particles absorb. All substances only absorb light with certain wavelengths (Studienet, 2021).

Blank sampling is used to determine the background color of the samples. The value of this is then subtracted from all samples.

LOD

Limit of detection is stated by the manufacturer as 0.1 EU/ml. The limit of detection will vary with the air volume. The calculated LOD will be 0.3 EU/m^3.

$$\frac{0.1\frac{EU}{ml}x\ 20\ ml}{12.5\ L/\min x\ 480\ \min x\ 0.001\ L/m3}$$

2.5 Data Preparation and Statistics

Results from measurements of endotoxins and bacterial count (CFU/m^3) are presented as minimum, maximum, and median values. Graphs were prepared on Microsoft Office Excel.

2.6 Ethical Considerations

This study does not involve any measurements or direct contact with workers or other human beings. No health information was collected. Thereby the study did not need to be approved by the Norwegian “Regional Ethical Committee” for research. Nevertheless, the workers in the measured areas will be informed about results from the study. I work for the two companies, where the study is performed, as their occupational hygienist, and equipment and analysis are paid by the two companies.

3 Results

3.1 The Independent Variables, Sampling Time

The weather was stable and very similar throughout the sampling period with temperatures in the range 13,7-18,4 C, low wind speed (1,8-4,3 m/s) and no precipitation (Table 5). The main differences between the factories were higher relative humidity in factory A than in factory B. Furthermore, factory B did not have waiting cages. When it comes to differences between the sampling days, the main differences are the cages and their location. Some are closer to land than others. Cages close to land might have more and, or a different bacteria flora since they are closer to human activity and thereby possible pollution. None of the factories reported any dead fish on the sampling days.

Table 5 Summary of the independent variables noted at sampling time at factory A and B.

		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Sampling date	Factory A	24.08.20	25.08.20	26.08.20	27.08.20	28.08.20
	Factory B	31.08.20	01.09.20	02.09.20	03.09.20	04.09.20
Amount of fish pr. day	Factory A	128 tons	458.2 tons	539.6 tons	498 tons	517.5 tons
	Factory B	430 tons,	430 tons,	430 tons	430 tons	407 tons
Sick/ dead fish	Factory A	No dead fish	Melanin spots, HSMI (heart and skeletal muscle inflammation), No dead fish	HSMB. No dead fish	No dead fish	No dead fish
	Factory B	Suspicion of PD (Pancreas Disease) and SAV (Salmonid alphavirus). No dead fish	Suspicion of PD and SAV No dead fish	Suspicion of PD and SAV No dead fish	Suspicion of PD and SAV No dead fish	Suspicion of PD and SAV No dead fish
Direct/net cage	Factory A	Direct Cage A (Frøyfjorden)	Direct and net cage Cage B (Frohavet) Cage C (Frøyfjorden)	Net cage Cage D (Frøyfjorden)	Net cage Cage E (Frohavet)	Net cage Cage E Cage B

	Factory B	Direct Cage F/G (Hemnfjorden)	Direct Cage H (Trondheimsleia) Cage G	Direct Cage H	Direct Cage F	Direct Cage I (Trondheimsleia) Cage J (Trondheimsleia) Cage F
Shift	Factory A	Night (0400-1200)	Day	Day	Day	Day
	Factory B	Day (0700-1500)	Day	Day	Day	Day
Relative humidity, temperature	Factory A	90 %, 10 °CC	90 %, 11 °C	90 % 10 °C	90 %, 10°C	90 %, 11 °C
	Factory B	45 %, 11°C	45 %, 11°C	45 %, 11°C	45 %, 11 °C	45 %, 11 °C
Outside temperature at 1200	Factory A	16 °C	15. 5 °C	13.7 °C	14.8 °C	17.3 °C
	Factory B	14.6 °C	16.2 °C	18.4 °C	16.9 °C	16.4 °C
Wind speed and precipitation at 1200	Factory A	3.3 m/s, 0 mm rain	4.3 m/s, 0 mm rain	3.8 m/s, 0 mm rain	3.5 m/s, 0 mm rain	4.4 m/s o mm rain
	Factory B	2.5 m/s, 0 mm rain	1.8 m/s, 0 mm rain	1.0 m/s, 0 mm rain	2.0 m/s, 0 mm rain	3.6 m/s 0 mm rain

3.2 Identification of Bacteria

It was analyzed for the human pathogenic bacteria; *Salmonella* spp., *Legionella* sp., *Listeria* spp., *Vibrio* spp., and *E. coli* / Coliform bacteria, but none of these were detected in the air samples.

Some of the bacteria found are opportunistic (Table 6). Both gram negative rods and gram-positive coccus were detected. Area with the most different types of bacteria was the bleeding area (Table 6). *Serratia liquefaciens*, *Pseudomonas* spp., and *Micrococcus luteus* were the three bacterium types that was found the most off.

Table 6 A summary of analysis results for identification of bacteria, the bacterial count (CFU/m3), and endotoxin levels (EU/m3) in the different areas of the two factories

Factory A	**Day**	**Identification of bacteria**
Bleeding	1	No growth

	2	*Serratia liquefaciens, Pseudomonas* spp., *Micrococcus luteus, Chryseobacterium scophthalmum*
	3	*Micrococcus luteus, Pseudomonas* spp.
	4	*Serratia liquefaciens*
	5	*Micrococcus luteus*
Gutting	1	*Micrococcus luteus*
	2	No growth
	3	No growth
	4	*Micrococcus luteus*
	5	No growth
Heading	1	No growth
	2	*Serratia liquefaciens*
	3	No growth
	4	No growth
	5	*Micrococcus luteus*
Factory B		
Bleeding	1	No growth
	2	No growth
	3	*Kocuria rhizophila, Glutamicibacter bergerei, Candida guilliermondii, Pseudomonas* spp, *Micrococcus luteus. Flavobacterium frigoris, Microbacterium phyllosphaerae, Shingomonas aerolota*
	4	No growth
	5	*Micrococcus luteus, Micrococcus flavus*
Gutting	1	No growth
	2	No growth

	3	No growth
	4	No growth
	5	No growth
Heading	1	*Micrococcus luteus*
	2	No growth
	3	No growth
	4	No growth
	5	No growth

3.3 Quantification of Bacteria

Factory A had 6 out of 15 samples (40%) that were above the detection limit (LOD = 44). The number of bacteria ranged from < LOD -70000 CFU/m^3. Four of these samples with values >LOD was in the bleeding area (100-70000 CFU/m^3), while the two others were in gutting (3000 CFU/m^3) and heading (1000 CFU/m^3).

Factory B had 4 out of 15 samples (27%) that were above the detection limit. The number of bacteria ranged from 100 -60 000 CFU/m^3. Three of these samples with values >LOD was in the bleeding area (100-2900 CFU/m^3), while the last in heading (60 000 CFU/m^3).

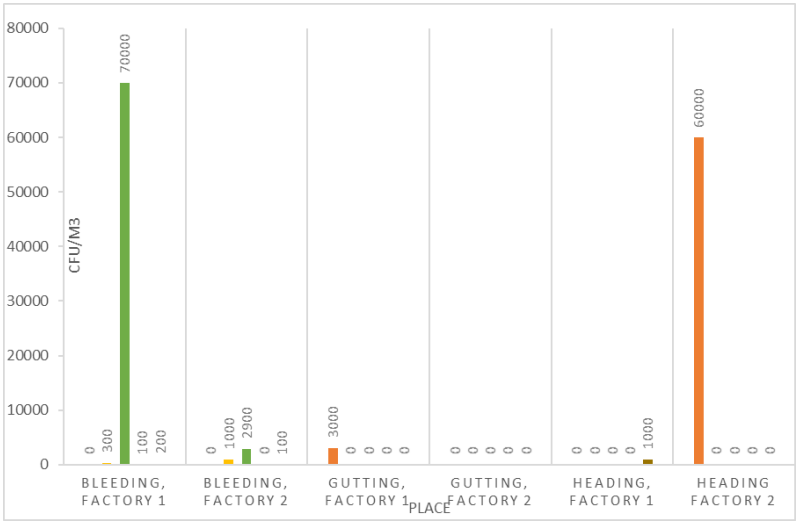

Figure 19 Results from quantification of bacteria (CFU/m^3) in air samples from the areas: Bleeding, gutting, and heading of Factory A and Factory B. Values <LOD is set as 0 in the graph.

3.4 Quantification of Endotoxin

Totally 15 of the 30 samples had an endotoxin level over the detection limit (LOD=0.3 EU/m^3) (Figure 20), with values ranging from 1,0 – 19 EU/m^3. Since 15 of the 30 samples is under the LOD, the median will be somewhere between LOD and 1. The area with the highest level of endotoxin and the most samples over LOQ (70%) were bleeding.

Factory A had 7 out of 15 samples (47%) that were above the detection limit. The endotoxin values ranged from 1.3 -19 EU/m^3. Three of these samples with values >LOQ was in the bleeding area (1.3 – 19 EU/m^3), three were in gutting (2.8 – 8.3 EU/m^3), and one in heading (7.1 EU/m^3).

Factory B had 8 out of 15 samples that were above the LOD. The CFU/m^3 value ranged from 1– 6.7 EU/m^3. Four of these samples was in the bleeding area (1,0 – 1.5 EU/m^3), and four was in heading (3,0 – 6.7 EU/m^3).

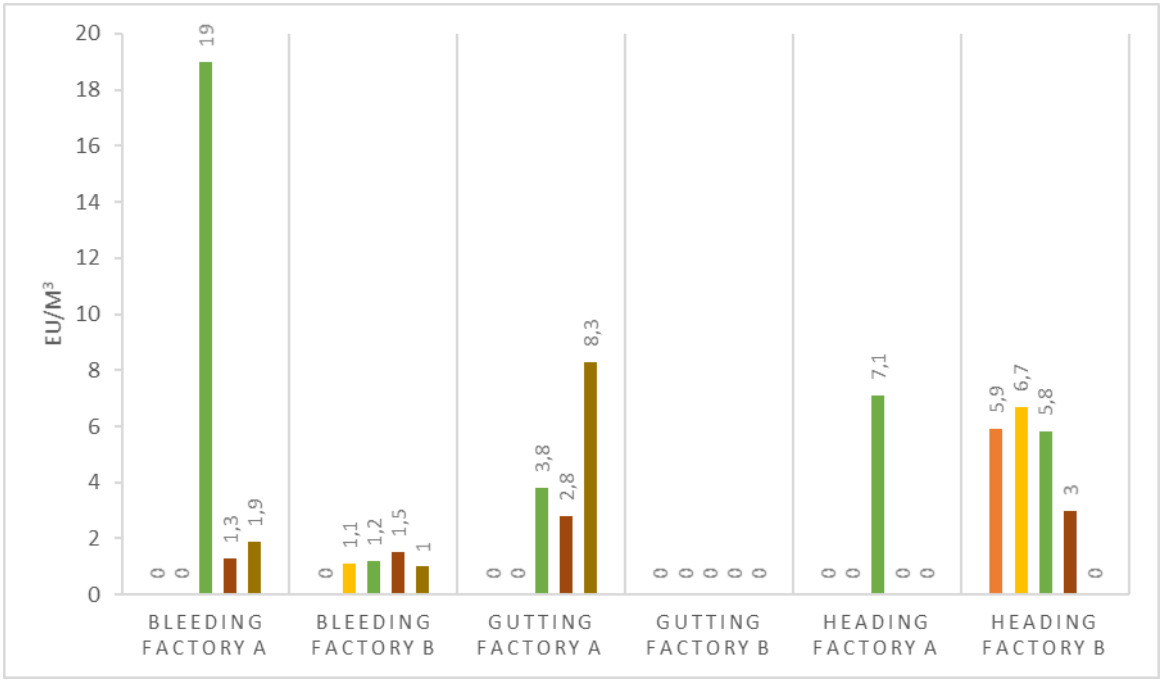

Figure 20 Endotoxin concentrations (EU/m^3) in air samples from the areas: Bleeding, gutting, and heading in factory A and factory B. Values < LOD is set as 0 in the graph.

4 Discussion

4.1 Result Discussion

In the air samples from the two salmon factories no pathogenic bacteria were detected. However, different species of opportunistic bacteria were identified. The bleeding areas were the locations where the most different types of bacteria were detected. The concentrations of bacteria were in some samples above intermediate exposure level, when compared to the bioaerosol guidelines to ACGIH. The measured endotoxin levels were low when compared to the Dutch health-based limit value.

Identification of Human Pathogenic Bacteria

No pathogenic bacteria were detected in the air samples. The bacteria detected, live naturally in the marine environment, so the measurements can be considered logical since one can assume that they come from fish and water.

Studies show that *Salmonella* spp. is generally not considered a problem in seawater (Gjerde, 1976). *E. coli* can occur, if the fish or seawater comes from an area with sewage contamination (Colin, 2011) (Gjerde, 1976). Contamination from the workers might also be a reason, but it seems very unlikely, given the detailed hygiene routines in the factories. The hygiene results from the factories own control did not show any findings of human pathogenic bacteria.

Listeria spp. and *Vibrio* spp. are bacteria that are a part of the sea environment (Colin, 2011) (Gjerde, 1976). *Listeria* will probably be the human pathogenic bacterium that is most likely to be found. *Listeria* has been reported to cause problems inside the factories. This because the bacterium can multiply in fridge temperatures, it is related to the formation of biofilm and are hard to eliminate once it is established (Leong, Alvarez-Ordonez, & Jordan, 2014) *Vibrio* spp. is mostly associated with the shellfish (Heng, et al., 2017).

Opportunistic bacteria were detected in the air in this study, such as *Serratia liquefaciens* and *Pseudomonas* spp (Table 6). These bacteria are known to be associated with the marine environment. *Serratia liquefaciens* is a gram- negative rod. The bacterium is found in both aerobic and anaerobic environments. It is capable of colonizing on soil, water, plants, and the

digestive tracts of fish, and humans. In human the bacteria can cause different infections, such as urinary tract infections, sepsis, pneumonia etc (Climaco, 2018). *Pseudomonas spp*. is a gram-negative rod, and it is found in soil and water. The bacteria thrive best in humid environments. *Pseudomonas* spp. can be the course of a wide range of infections (FHI, 2021).

Opportunistic bacteria usually do not cause disease in healthy people, but if the immune system is weakened for some reason, then they can cause health problems ((Forbes, Sahm, & Weisfeld, 2007). The bleeding area was the area with the most different species of bacteria detected.

Although no human pathogenic bacteria were found in the air in this study, it cannot be ruled out that there may be a risk, but the probability of finding any will in general be small. The species of bacteria found, will depend on the water quality in the area where the cage is located, the quality of the water on the well boat, the hygiene routines on board the well boats, and the hygiene at the factories.

Quantification of Bacteria in Air Samples

According to ACGIH's recommendations for bioaerosol values (ACGIH, 1989), the number of bacteria measured (CFU/m^3) was within the range medium (100-1000 CFU/m^3) to high (>1000 CFU/m^3) several days in the sampling period. Especially the bleeding areas in both factories have several days with medium to high bacterial numbers. There is a difference in the number of bacteria measured between the bleeding areas and the other two areas that were measured (gutting and heading). The results may indicate that there are more bacteria in the air in the bleeding areas, than in the other two areas. This finding seems logical, as it is at the bleeding area, which is the start of the processing line, there will be the greatest chance of exposure to seawater and whole fish, that may have microbes on the surface from the sea. Further down the process line, the fish will be rinsed, frozen, skinned etc. and the risk of the fish carrying microbes will be less. Why the measured number of bacteria are so different from day to day is difficult to explain. It may have something to do with the cage locality where the fish comes from. The fish is living in different cages located in different places in the sea, some are offshore, and others are closer to the cost, and hence the water quality can differ in these areas. The water that comes along with the fish into the factory could therefore have different water quality, expecting that fish from cages along the coast have higher risk of contaminated water compared to cages further from land. A second explanation could be that

the number of fish slaughtered on Day 3 were higher than the number of fish the other examined days. If the bacteria are introduced by the fish, more fish would increase the number of bacteria.

Quantification of Endotoxin in Air Samples

The measured endotoxin levels were low when compared to the Dutch legal limit value of 90 EU /m^3 (Health Council of the Netherlands, 2010), which is considered as a health-based limit value. One measurement in the bleeding area, day 3, factory A, stood out with a high level of endotoxin than the rest of the samples. This sample also had a high bacterial count (70 000 CFU/m^3). The cause of this might have the same explanations as stated above, that is location of cage, water quality, and the number of fish slaughtered can influence the concentration of bacteria. More fish could lead to more bacteria and then more endotoxin.

Further, it is not surprising to find a certain level of endotoxin in the air since seawater contains mainly gram-negative bacteria and gram-negative bacteria were detected (Nunez, et al., 2016).

The endotoxin results are consistent with other studies in slaughter areas for fish. The exposure to endotoxins among Northern Norwegian workers in the slaughter area of five factories ranged from 0.3 -29 EU/m^3. (Shiryaeva, et al., 2013) Another study of endotoxin exposure in the North of Norway comprising samples from 23 workers in salmon slaughterhouses showed results that ranged from 0.6-36 EU/m^3. (Bang, Larsen, Larsen, & Aasmoe, 2005) The endotoxin results from a study in Sweden performed on 3 workers in a salmon factory showed values between 1.6-7.1 EU/m^3 (Dahlmann-Høglund, Renstrøm, & Larsson, 2012). Thus, the results from all these studies suggest that the exposure levels to endotoxins in the fish industry in general can be considered low. The measured endotoxin values in this study can be considered very low.

4.2 Method discussion

4.2.1 Sampling Strategy

Stationary method with active sampling was used in the project. The goal in this project was to screen the background levels, find the source of pollution, and for that stationary sampling is considered suitable. Another factor is that impingers for bioaerosol sampling with 8-hours sampling time also only exist as stationary sampler. Stationary samples will usually underestimate the employee's personal exposure because many activities that involve exposure are not considered when the sampler is placed stationary in the work environment. But in those cases where the pollution is homogeneously distributed without special sources, stationary measurements will be able to give a satisfactory estimate of the air concentration in the room. If the test results are to be compared with legal limits, the samples should be taken as personal samples (STAMI, 2011).

Active sampling was used in this project. The advantage of active sampling is control over how large air volumes have been sampled, and often the sampling time also will be shorter with active sampling, since a pump can draw large amounts of air through a sampling unit in a short time. Passive sampling often requires less equipment and can be easier to handle. During active sampling, the air is drawn through an adsorbent medium where the pollutants are collected, and usually, a pump is used to draw the air through the medium, while in passive sampling no pumps are used. (STAMI, 2011).

4.2.1 Sampling Equipment

The goal was to cultivate the bacteria in the samples and do analysis for endotoxins. The literature recommends a sampling method with liquid, an impinger. Filter samples can also be used, but then the collected particles must be removed from the filter. This requires an extra step, which can cause particle loss. Microbes can easily also dry out on a filter and die. That means that the sample will not be eligible for cultivating (Mainelis, 2019).

The challenges with impingers are the loss of fluid, the choice of velocity, and the destruction of the particles. If the speed is not high enough, the larger particles will not be collected. But at high speed there is a chance that the liquid used will evaporate. High speed may also cause the particles / microorganisms collected to be destroyed, and they will therefore will not be

able to be cultured afterwards. This will give a result that is false negative. However, it has been shown that the impinger used in the present study has a very low destruction of microorganisms compared to traditional impingers. It is considered the "reference impinger" in bioaerosol studies (Mainelis, 2019).

Due to evaporation of liquid in the impinger it must be refilled with liquid regularly, or a liquid that does not evaporate must be selected. In this study the liquid was regularly checked and refilled. Various types of liquid can be used in impingers such as deionized water, autoclaved water, mineral oil, NaCl-solutions, or phosphate buffer saline with or without surfactants (Mainelis, 2019). Phosphate Buffered Saline (PBS) was used in this study as it is often the preferred medium when sampling bacteria because of its a neutral pH (Mainelis, 2019).

4.2.2 Analyses

Cultivating methods was used to detect and enumerate bacteria. This because, to assess the risk of infection, cultivation methods should be used, since microorganisms must be alive to cause infections (Forbes, Sahm, & Weisfeld, 2007). Some bacteria thrive better in a liquid growth agar than on a solid growth agar (Forbes, Sahm, & Weisfeld, 2007). The samples for bacterial analyzes were therefore inoculated on both types of culture media.

Culture-based methods are one of the most used methods for detecting bacteria. Source of error might be 1) The method only detects bacteria alive (Macher, Amman, Burger, Milton, & Morey, 1999). 2) Not all bacteria are able to grow on a growth media (Macher, Amman, Burger, Milton, & Morey, 1999). 3) There is not necessarily a connection between the type of bacteria that is most abundant and the type that manages to grow on a media (Macher, Amman, Burger, Milton, & Morey, 1999). 4) The sampling method may also affect the bacterial growth potential. This is especially evident in sampling methods where the bacteria can dry out, such as the use of filters. But it can also happen with the use of impingers (Macher, Amman, Burger, Milton, & Morey, 1999).

MALDI-TOF-MS was used to confirm the bacteria found. The main source of error for this method are that the absence of certain bacteria species in the reference library. If the reference library in the machine does not have a certain species, the machine will give an identity close to the organism or identify the organism. This can be a problem since organisms that are

closely related can have different risk levels (MALDI-TOF Mass Spectrometry for Microorganism Identification, 2015).

LAL-chromogenic method was used to analyze for endotoxin: The endotoxin in the method comes from a purified strain of *Escherichia coli*. This is an endotoxin that is extremely purified and free of most detectable contaminants (such as proteins). An endotoxin in the environment is not purified. It can together with lipopolysaccharide, cellular membrane proteins, and phospholipids take the form of a macromolecular complexes which are shed by gram-negative bacteria during growth and death. The means, that there will be a big difference between the purified endotoxins in the assay and the natural endotoxin sampled. Further, it is the Lipid A in the endotoxin that activate the lysate, but not all the lipid A may be available because the lipid A part of the endotoxin can form aggregates that are not fully dispersed. This means the method may detected to little endotoxin and thereby underestimated. The toxicity of different endotoxins also differs, depending on the biological activity of the Lipid-A molecule for different bacterial species (Sandler, 2017).

4.3 General Limitations and Strengths

Internal Validity

The results must be interpreted with caution, as the measurement period, due to the COVID-19 pandemic, was reduced to only one time period (August/September) instead of taking samples also in winter (January/February), as originally planned. Ideally, measurements should have been performed at all seasons, as there may be differences in sea temperature and thus microbes in the water. The factories also have more than one shift pr. day, so all shifts should have been measured to give a more detailed result. Shift later in the day, may have an accumulation of bioaerosol. The variation in number of bacteria were large from day to day, even when the production was stable, more samples might have given a better understanding of this issue. Several measurements would also have provided opportunities to look at relationships between different variables (production, weather, season, etc.) and exposure.

One of the strengths of this study is that measurements were performed by taking full-shift samples during the working days that represent normal daily activity. This gives an indication of the potential exposure for workers during a workday. Another strength of this study is that the air samples were analyzed at a laboratory with knowledge on marine microbiology. The

laboratory is accredited according to ISO 17025 It is also a strength that the study included two different factories. This makes the results more general and relevant.

One challenge with the equipment was its dependency on electricity. The pumps cannot run on battery but must have power from a socket. This had to be considered when placing the equipment. The use of extension cords made it possible to place the equipment in satisfactory places. A slaughterhouse has high humidity, a lot of water spills and fish particles. This is not an ideal environment for measuring equipment.

External Validity

Within Norway's borders the findings can probably be generalized to other salmon slaughterhouses. My experience as an occupational hygienist indicates that most salmon slaughterhouses in Norway have mainly the same structure, essentially the same equipment, the same work and hygiene routines, and the same washing routines. Elsewhere in the world, there may be other solutions in the slaughterhouses due to other climatic conditions, bacteria content, technical limitations etc., which means that care should be taken to generalize the results from this study to other countries.

4.4 Recommended Further Work.

More research is needed on bioaerosols, both in general and specifically aimed at the aquaculture industry. In general, more research is needed on how the various components of the bioaerosol affect each other. Can different components in the bioaerosol have synergistic or inhibitory effect on each other? And what will be the overall health effects on employees, when the bioaerosol exposure is seen in connection with the other work environment factors, to which employees are exposed during the working day?

As mentioned under limitation and strengths, a study like this should be performed again but with a larger number of tests, it should be looked upon possible season, production, and shift variations. In addition, more factories should be tested.

Further, to be able to create optimal ventilation solutions for the salmon slaughterhouses, studies should be carried out on how the bioaerosols travel in the factory. What factors increase and inhibits their travel?

More research is also needed on the various constituents of the bioaerosol that can be naturally found in such industries, such as viruses, algae, and bacteria. More research and focus are needed on lay-outs, materials, and equipment used to build the factories, so that exposure level to bioaerosol, and other working factor is held as low as possible. It should also be considered whether one should go more in depth in the various areas in the factories. The challenges are most likely not the same in all areas.

And last, but not least, more research on exposure to microorganisms and the development of symptoms and disease are needed. Some knowledge has been established about the mechanisms that can biologically explain the development of disease, but the knowledge about which exposure levels and which components of the bioaerosol lead to disease is still limited.

4.5 Reflections on the Role as Researcher

The starting point for the study, was that I am genuinely interested in bioaerosols and the challenges the seafood industry has with this in the working environment. This has made me very open and eager to find ways to gain more knowledge.

My many years of experience as an occupational hygienist for the aquaculture industry means that I have gained some experience from the industry. In addition, I have a degree in bio medical laboratory science with experience from microbiology, which gives me a certain level of knowledge within the choice of and understanding of laboratory analyzes. Experience has probably also given me a good basis for finding the right measuring equipment and placement of this, so that I can achieve what is the goal of a quantitative study, to measure and obtain good data.

I have been involved in every step of the project. I was early out to find companies that I could work with, and I also applied for financial help for equipment / analyzes from the companies. Furthermore, I spent a lot of time finding the right sampling equipment and analysis material. Further, I have performed all the sampling and laboratory analyzes myself. I think all this has given me good insight into how much work there is behind a research project and how many details need to be in place.

5 Conclusion

Among the 30 air samples examined in this master work, no human pathogenic bacteria were detected. However, some opportunistic bacteria were found, all previously described from the marine environment. It can be considered plausible that bacteria from the marine environment can enter the factory and the air through fish and the seawater that comes along with the fish when it enters the factory. The concentration of bacteria varies greatly from day to day. The most different species of bacteria and the highest number of bacteria were found in the bleeding area in the facotories. This can be an indication that the bleeding area is the area where the workers will experience with the greatest exposure to bacteria from seawater and fish, but more studies are needed to confirm this.

The endotoxin levels detected were below the Dutch limit value. This can suggest that endotoxin is most likely not the most important factor to explain airway related health problems, but it cannot completely be ruled out, since some studies suggest that also small amounts of endotoxin can induce respiratory effects. More studies are needed to confirm this.

6 Recommended Measures

There are few limit values for bioaerosols and their components. The Norwegian Labor Inspection Authority's recommendations states: For substances that are suspected of posing a health risk, but where there is insufficient knowledge of the health hazard, measures that remove or reduce exposure as much as possible, are particularly important (Arbeidstilsynet, 2021).

Although one factory has more efficient ventilation, the results indicate that general ventilation is not enough to obtain satisfactory control of the bioaerosol. This is most likely related to the fact that bioaerosols consist of very small particles that are easily transported over large distances and that can fall to surfaces and be quickly reintroduced into the air with human activity, air movements and the like. Based on the Norwegian Labor Inspection Authority's recommendations and the results of this study, it is recommended the use of local exhaust and / or processes build in. Getting better control of water consumption should also be part of the measures, but this can have a negative effect if not done in conjunction with improving ventilation, as a lower humidity could make the bioaerosol even more floating, so that it can spread even more.

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Appendix

6.1 Appendix 1Protocol laboratory analyzes.

6.1.1 Making of Marine Agar

Equipment:

- Weight:
- Agar powder: Difco 2216 Marine agar
- Autoclave: Tomy sx-700E
- Plastic petri dishes:
- Water: Distilled water, Note Millipore MilliQ
- Microbiological Safety Cabinet:

Procedure:

- Weighed 55, 1 g agar powder in the chemical safety cabinet.
- Added this to 1 liter of water.
- Mixed and cooked for 1 min.
- Autoclaved at 121 ℃ for 15 min.
- Poured into plastic 90 mm, petri dishes in microbiological safety bench, LAF-(Laminar Air Flow) bench.
- Allowed to cool down and settle.

6.1.2 Qualitative analysis Listeria

- Added 250 µl sample to 2.25 ml. ½ Fraser broth.
- Incubated at 30 ± 1 ℃ for 25 ± 1 hours.
- Transferred 100 µl samples from 1/2 Fraser broth on the media: ALOA and RAPID'L mono. Spread out with L-stick. Incubated at 37 ± 1 ℃ for 26 ± 1 hours.
- Transferred 100 µl ½ Fraser broth solution with sample to 2.25 ml Fraser Broth Incubated at 37 ± 1 ℃ for 26 ± 1 hours.
- Transferred 100 µl Fraser Broth solution with sample onto the media: ALOA and RAPID'L-mono. Spread out with a L-stick. Incubated at 37 ± 1 ℃ for 26 ± 1 hours.
- Read the result.

6.1.3 Quantitative analysis bacteria (CFU), Marine agar.

- Diluted the samples with PBS solution in rows 10^{-1}, 10^{-2}, 103, 104, 105 and 106.
- Spread out 100 µl sample on Marine agar with a L-stick.
- Incubated at 25 ± 1 ℃ and 46 ± 2 hour.
- Read the result.

6.1.4 Quantitative analysis bacteria (CFU), Mueller Hinton Agar

- Spread 100 µl sample with a L-stick on Mueller Hinton agar, one for 25℃ and one 37℃
- Incubated at 25℃ ± 1 ℃ and 37℃ ± 1 ℃ and 46 ± 2 hour.
- Read the result.

6.1.5 Purification of colonies for MALDI-TOF - MS identification

- Inoculated one colony on a Marine agar and spread it with a loop.

- Incubated at 25℃ ± 1 ℃, 46 ± 2 hour.
- Read the result.

6.1.6 Cultivation in Broth

- Added 250 µl sample to ?? ml Mueller Hinton broth.
- Incubated at 25℃ ± 1 ℃, 46 ± 2 hour.
- Read the result.

6.1.7 Listeria Identification, Brilliance Agar– One broth, OXOID

- Day 0: 1 part sample + 9 parts ONE Broth-Listeria, mixed for a minimum of 30 seconds.
- Incubated the broth without stirring at 30°C for 24 ± 2 hours.
- Day 1: Gently stirred the mix. 10µL inoculated onto agar plate and incubated at 37°C for 24 ± 2 hours.

Result on agar:

- Blue colonies with and without opaque white halos.

6.1.8 Legionella Identification, GVPC Selective Agar, OXOID

- 10 µl sample inoculate onto agar, Incubated at 35-37°C (*L. anisa* for 5 days and all other organisms for 3 days).

Result on agar:

- Greyish/white colonies

6.1.9 E. coli/Coli. Bacteria Identification, Selective Agar, OXOID

- 10 µl sample inoculated onto agar, incubated for 26 hours at 37 ℃

Result on agar:

- E. coli: pink colonies

6.1.10 Salmonella Identification, Brilliance Agar, One broth, OXOID

- Day 0: 1 part sample + 1 part ONE Broth-Salmonella Incubated for 16-24 hours at 42°C.

- Day 1: 10µl inoculated onto agar. Incubated for 26 hours at 37°C.

Result on agar:

- Salmonella: Purple colonies

- Klebsiella/Enterobacter blue colonies

6.1.11 Vibrio identification, TCBS Agar, OXOID

- 10 ul inoculated onto agar. Incubated for 24 hours at 35℃

Result on agar:

- Yellow colonies

6.2 Appendix 2 Overview Bacteria Found.

Figure 21An overview of the bacteria found, their natural habitat, and pathogenicity.

Bacteria	Normal Habitat	Pathogenicity	Reference
Serratia liquefaciens	Gram- negative rod, usually motile and contain peritrichous flagella, facultative anaerobe. Found in both aerobic and anaerobic environments.	Capable of colonizing on soil, water, plants, and the digestive tracts of fish, and humans. Considered opportunistic. Can cause urinary tract infections, bloodstream infections, sepsis, pneumonia, meningoencephalitis, and other infections	(Climaco, 2018)
Pseudomonas spp.	A psychrophilic, Gram-negative rod, motile with a polar flagellum Found in soil and water. Thrive best in humid environments. Multiply easily where moisture is found	Considered opportunistic.	(FHI, 2021)
Micrococcus luteus *Micrococcus flavus*	Gram-positive, to gram-variable, cocci. Found in soil, dust, water, and air, and human skin	Considered opportunistic. The bacterium can colonize human mouth, mucosae, oropharynx, and upper respiratory tract. particularly in hosts with compromised immune systems.	(Forbes, Sahm, & Weissfeld, 2007)
Chryseobacterium spp.	Gram-negative rod, non-motile. Widely distributed in nature	Rarely pathogen for humans but can be opportunistic for patient populations who have an indwelling vascular line or device, have existing medical comorbidities, or who are immunocompromised.	(Booth, 2007)
Kocuria rhizophila (Actinobacteria)	Gram-positive cocci. Found in soil and on human skin	Generally considered non-pathogenic.	(Forbes, Sahm, & Weissfeld, 2007)

Flavobacterium frigoris,	A psychrophilic, gram-negative rod Found in microbial mats in marine environments	Considered non-pathogenic.	(Forbes, Sahm, & Weissfeld, 2007)
Glutamicibacter bergerei	Gram-positive cocci. Found in soil and on human skin.	Considered non-pathogenic	(Forbes, Sahm, & Weissfeld, 2007)
Candida guilliermondii	A species of yeast. Normal flora of human skin.	Considered rarely opportunistic fungal pathogen.	(Forbes, Sahm, & Weissfeld, 2007)
Microbacterium phyllosphaerae	Gram -positive cocci. Found in soil and marine environments.	Considered non-pathogenic.	(Forbes, Sahm, & Weissfeld, 2007)
Sphingomonas aerolata	Gram-negative rod. Found in marine environments.	Some considered opportunistic.	(Munn, 2011)