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Airborne Opportunistic Pathogens in Infrastructures: An Underrecognized and Underappreciated Hazard

Thomas Neil McManus

NorthWest Occupational Health & Safety, North Vancouver, Canada
Email: nwohs1@gmail.com

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Abstract

This article builds on a life-threatening pneumonia that developed following a visit to a worksite. Bacteria encountered in infrastructures normally require either aerobic or anaerobic conditions for survival. Design of the structures involved in handling, processing and storing of wet organic materials reflects and enforces these requirements. Facultative aerobic, opportunistic bacteria that also have developed resistance to antibiotics, in particular, *Klebsiella pneumoniae*, can thrive under both conditions. Normally these bacteria live in liquid or semi-liquid media such as sludges and biofilms and pose an especial concern to occupations exposed to them. This concern is heightened when bacteria normally present in liquids or semi-liquids become airborne in droplets or solid particles. Aerosolization occurs under processes that disrupt biofilms on surfaces or oxygenate water using compressed air and through highly turbulent flow. A scan of the literature highlight indicates a recent shift to assessing the airborne microbial environment in many types of infrastructures to bacteria capable of causing pneumonia and other diseases. Identification of infection by *Klebsiella pneumoniae* requires diagnostic tests not commonly used in these situations. *Klebsiella pneumoniae* is exhibiting increasing antibiotic resistance to the point where lung infections become untreatable. This article will review and analyze the situation involving opportunistic bacteria in the context of infrastructures and will offer approaches for eliminating or at least minimizing the threat to the health of personnel who work in or around infrastructures potentially affected by this predator.

Keywords

Airborne Transmission, Antibiotic Resistance, Facultative Anaerobe, Gram Negative, Klebsiella Pneumoniae, Opportunistic Pathogen, Vaccination, Immune System Stress

1. Information for Readers

This article provides results from work performed by investigators in support of interests and needs of practitioners in applied disciplines. The target audience is expected to be worldwide in scope and interdisciplinary, and to span a broad spectrum of interests and education. Background information provided in the article is limited only to that which has relevance to further the continuity of the discussion. What is seen as elementary to one group of readers with strong backgrounds in some areas will be seen as essential by others having no background. An article that provides advanced concepts that are also easy to read will be greatly appreciated.

2. Introduction

This article describes a situation involving a life-threatening case of pneumonia caused by bacteria normally found in the human intestine. The source was believed initially to have been a structure within the infrastructure. This article is intended to alert and inform persons whose work occurs in relevant structures, occupations and experiences about the potential for experiencing the same consequences, to identify possible mechanisms of emission and methods of control, the means to perform air sampling and culture, and to stimulate further enquiry into this situation.

The infrastructure contains many types of structures. Optimally, these are designed in a manner to optimize function. One characteristic of many of these structures is that they have wet surfaces and/or also contain standing water or water thickened by organic material or material capable of supporting growth of bacteria. Generally, this situation does not pose a problem.

The conveying, handling, and treatment of wastewater occur in environments having specific, carefully controlled properties [1] [2]. These properties create isolated aerobic and anaerobic conditions. These conditions favor growth of aerobic bacteria or anaerobic bacteria. The environment favourable to aerobic bacteria is usually lethal to anaerobic bacteria and vice versa [3]. This strategy is employed to destroy the population of anaerobic bacteria and the pathogens within it.

Previous articles in this series [4]-[6] considered the growth of bacteria in single- and dual layer systems. The layer exposed to air generally supports growth of aerobic bacteria. The layer under the surface layer of water becomes oxygen-deficient and supports growth of anaerobic bacteria. Hence, interior surfaces of structures in the infrastructure can contain a complex mix of microorganisms. These articles focused on emission of lethal levels of hydrogen sulfide (H_2S) and carbon dioxide (CO_2) following disturbance of the fluid in the anaerobic layer. The microorganisms present in the layers have received little attention.

This article considers the risk posed by the microorganisms found in the water present in infrastructures and similar environments. These microorganisms can be the same or different from those that release gases following disturbance. They can enter the body by ingestion or inhalation. Microorganisms entering by ingestion have received considerable attention over the years. Some are known to be pathogenic (disease-causing) for which treatment is generally available. The pathogenic response usually involves infection and invasive disease as well as a toxic biochemical reaction. Emerging antibiotic resistance is a major threat to the exposed population.

Preventive measures are critically important as a means of eliminating and at least minimizing the risk of infection. Preventive measures include containment to prevent contact with the water or sludge containing microorganisms. Containment can occur in fully or partially enclosed systems. Additional measures include sterilization of the water or sludge to kill the microorganisms and development of vaccines and programs to inoculate the exposed population. Where contact with wastewater containing pathogenic microorganisms can occur, additional protective measures are required. These include impervious outer clothing, gloves and protective eyewear.

Airborne transmission is a second route of exposure to pathogenic microorganisms contained in wastewater. Airborne transmission appears to have received little attention over the years. Treatment has focused primarily on microorganisms (pneumococci) that can cause pneumonia because of infection via the respiratory system and the lung. Treatment involves administration of two courses of antibiotics, one for each of two groups of disease-causing bacteria. This inoculation is also effective as a preventative measure.

Containment becomes increasingly important as a means of prevention of exposure to pathogenic airborne bacteria. Of considerable importance in this discussion are mechanisms of emission that are not confined in closed systems. These pose considerable concern where pathogens are present in the water or sludge. Exposure can occur during operation and maintenance. Any action that applies a shear force to a pseudo-plastic, non-Newtonian fluid will release trapped gases through a decrease in viscosity [6] [7].

The first mechanism is powerwashing using a high-pressure jet of water. Domestic machines can generate jets containing pressure of 13.8 MPa (2000 lb/in^2) [8]. The function of powerwashing is to break apart and remove deposits such as biofilms from surfaces and to liquefy sludges.

A parallel situation (second mechanism) can occur in animal husbandry during manure handling and storage in barns and storage structures. Aerosol production can occur during homogenization of manure induced by mechanical mixing equipment and in storage structures through operation of propellers immersed in the fluid and connected to the power take-off of a tractor. Violent agitation and production of aerosols occur when the propeller is positioned near the surface of the liquid.

The third mechanism involves turbulent flow in structures. Turbulent flow is more likely during storm events. This is an especial concern in combined systems that receive surface flow as well as sanitary flow. Flow in sewers is less aggressive during storm events because of the rounded bottom of many of the structures.

Flow in narrow structures containing flat bottoms can become violent because of the energy intrinsic in the rapid movement of the water. This turbulence is an especial concern in open-topped structures.

The fourth mechanism involves turbulence induced into the water by equipment intending to create this condition. Open-topped tanks in wastewater treatment plants pose an especial concern in this regard. These tanks contain a network of pipes located near the bottom. Emission points created by short, upward pointing pipes are topped by structures resembling shower heads that contain many holes. Compressed air escapes from the emission points and forms bubbles in the water as it rises. On reaching the surface, the bubbles burst and the air escapes into the atmosphere. Bursting of the bubbles creates aerosols containing water and suspended microorganisms.

Protective measures for use around these sources of emission include those mentioned previously as well as respirators. Respiratory protection must act against droplets and solids containing viable microorganisms as well as reproductive forms (spores, cysts). Some bacteria produce endotoxins [3]. Endotoxins are fragments of bacterial cell walls. Endotoxins cause serious respiratory issues.

3. A Life-Threatening Exposure Event Having an Unexpected Cause

The preceding discussion "sets the stage" to introduce an event that had life-threatening consequences following unexpected exposure to a bacterial agent contained in solid or liquid aerosols. The exposure event occurred during a visit by car to an office in a building located on filled land above a muddy tidal flat. Water that covers parts of the tidal flat during part of the day is known to be heavily polluted by untreated sewage. A Wastewater Treatment Plant containing open, aerated tanks is located beside the access road near the site of the meeting. The type and source of the aerosol that caused the medical event are not known.

3.1. Description of the Episode

Three to four days following the visit to the site, the victim developed a highly abnormal choking cough during a walk at a normal pace on a flat surface. The coughing stopped and started again several hours later. The victim started to cough again intermittently every few minutes. The cough persisted day and night. The cough was accompanied by chills and fever, and clear and colored sputum.

Sleep in normal positions was not possible. The victim could gain comfort only in a reclined position.

The symptoms persisted in a consistent manner and showed no sign of let-up over several days. The victim sought treatment at a local community medical facility, and was interned for several days. During the course of diagnosis, the victim received chest X-rays and blood tests and was diagnosed to have pneumonia.

The victim, now the patient, received the standard course of antibiotics for the treatment of one of the groups of bacteria that cause pneumonia. Blood tests and

X-rays confirmed the success of this component of the treatment. The patient was discharged along with a prescription for the antibiotic needed for the second component of the treatment. At the end of the treatment, as prescribed, the coughing continued. This suggested more complex involvement.

Admission occurred at a second, more advanced facility. Treatment, without success, initially followed the same strategy as used in the first facility. This strongly suggested the presence of a causative agent not addressed in the treatment protocol and blood tests used to assess progress.

The causative agent was unknown. This prompted investigation to determine the identity of this agent and the antibiotic recommended for killing it and its susceptibility to treatment. At this point, the ability to obtain success was far from certain. Sputum testing was chosen for this investigation. A sputum test involves collection of saliva for culturing. In this case, the test was successful but the identity of the microorganism was not known. This necessitated further investigation involving comparison of cultures against those of known microorganisms.

Following identification of the causative agent, consultation of databases indicated recommended antibiotics. Confirmatory tests then indicated the suitability of the recommended antibiotic against the strain of the infectious agent involved in this situation. The antibiotic chosen was different from those used to treat microorganisms that cause pneumonia. This explained the lack of success in prior treatment of the infection. Administration of this antibiotic produced almost instant positive results and recovery proceeded rapidly. Up to this point, success in this process was far from certain.

3.2. Analysis of the Episode

The following information is provided to readers to support investigation of events posing similar signs and symptoms.

A pneumonia condition resulted from exposure to aerosol droplets containing viable vegetative bacteria and possibly bacterial spores. The source of emission was not identifiable. Possible sources included aeration tanks at a wastewater treatment plant close the roadway near the destination; and a large settlement having sanitation issues located in the same area. Hence, the source of the emission that caused the event as described is not known based on information available at the time.

The antibiotic needed to combat the unknown microorganism was not present in mixture administered in the standard treatment against the microorganisms that caused the pneumonia. That is, the mixture of antibiotics administered to fight the pneumonia was incapable of acting against the infection caused by the unknown microorganism. This observation explained the lack of success in prior treatment of the infection and is the foundational reason for preparing this article. The outcome that occurred was not predictable from the information provided to doctors for the standard treatment of pneumonia

4. Background Information about Bacteria

Background information concerning bacteria is needed in order to discuss this situation further in an orderly manner that provides background sufficient to explain what occurred in the example in the preceding section and how to respond to it.

4.1. Classification of Bacteria: Gram-Positive Bacteria

A topic of fundamental importance in understanding what occurred in the episode described above is classification of bacteria. Bacteria generally are subdivisible into two main groups: those that are Gram-Positive and those that are Gram-Negative [3] [9] [10]. There are many different types of Gram-Positive and Gram-Negative bacteria. These bacteria cause a wide range of different health issues.

Microbiologists categorize Gram-Positive and Gram-Negative bacteria based on their structures and their appearance under a microscope following Gram staining [9]. (Gram was the scientist who developed the technique.)

Gram-Positive bacteria have thick cell walls and appear blue or purple under the microscope following Gram-staining [9] [11]. Gram-Positive bacteria include cocci- (spheres) and bacilli- (rod) shaped, or have branching filaments. Examples of Gram-Positive bacteria include various species of *Staphylococcus*, *Streptococcus*, *Enterococci*, *Corynebacterium diphtheriae*, and *Bacillus anthracis*. Most of these names are instantly recognizable. Toxins emitted by disease-causing, Gram-Positive bacteria include emetic toxin, diarrheal enterotoxins, or neurotoxins.

4.2. Classification of Bacteria: Gram-Negative Bacteria

Gram-Negative bacteria stain pink or red and have thin walls. Gram-negative bacteria have two membranes, one external and one internal [9] [12]. These bacteria also have a thinner peptidoglycan cell wall than Gram-Positive bacteria, which sits between their two membranes. They can be spherical-, rod-, or spiral-shaped. Examples of Gram-Negative bacteria include *Vibrio cholerae*, *Escherichia coli*, *Bartonella henselae*, *Campylobacter*, *Legionella*, *Salmonella*, and *Salmonella typhi*. Most of these names are instantly recognizable. (Note that *Klebsiella pneumoniae* is not listed in this source as an example). Disease-causing, Gram-Negative bacteria emit endotoxins following disturbance. Gram-Negative bacteria are among the most significant public health problems worldwide due to high resistance to antibiotics.

4.3. Classification of Bacteria: Respiratory Requirements

A second way to classify bacteria reflects their respiratory requirements, namely their response to oxygen in their environment [3]. These characteristics are exploited in wastewater treatment [1] and animal husbandry [2].

Obligate aerobes need oxygen because they cannot ferment or respire anaerobically. In situations where there is stratification in water or a multi-layer system, obligate aerobes congregate in the upper region where the oxygen concentration is highest. Obligate anaerobes are poisoned by oxygen and hence congregate in the lower region where there is stratification in water or a multi-layer system where the oxygen concentration is lowest. Aerotolerant anaerobes do not require oxygen as they use fermentation to make ATP (Adenosine Tri-Phosphate). Unlike obligate anaerobes, they are not poisoned by oxygen.

Facultative anaerobes make ATP by aerobic respiration if oxygen is present, but are capable of switching to fermentation when oxygen is absent [13] [14]. Facultative anaerobes can grow with or without oxygen because they can metabolize energy aerobically or anaerobically. Facultative anaerobes are able to grow in both the presence and absence of oxygen due to the expression of both aerobic and anaerobic respiratory chains using either oxygen or an alternative electron acceptor [15]. Facultative anaerobes gather mostly in the oxygenated area of water because aerobic respiration generates more ATP than fermentation.

Examples of facultatively anaerobic bacteria include *Staphylococcus* spp. [15], *Escherichia coli*, *Salmonella*, *Listeria spp.*, *Shewanella oneidensis* and *Yersinia pestis* [16] [17]. (Notice that *Klebsiella* is not mentioned in these references.)

Since facultative anaerobes can grow in both the presence and absence of oxygen, they can survive in many different environments, adapt easily to changing conditions, and thus have a selective advantage over other bacteria. As a result, most life-threatening pathogens are facultative anaerobes [18].

4.4. Classification of Bacteria: Opportunistic Infection

Opportunistic infections develop when the immune system is weakened by an infectious agent, possibly by use of antibiotics and/or by immunosuppressive drugs, or another agent. Infectious agents include pathogenic viruses, bacteria, fungi, and parasites [19]. The stress imposed on the immune system enables an otherwise uninvolved pathogen to become active beyond the normal level of activity, and potentially considerably so [20]. The two stressors may be related or unrelated to each other. Under normal conditions, such as in humans with uncompromised immune systems, an opportunistic pathogen would be less likely to cause significant harm and would typically result in a mild infection or no effect at all.

Opportunistic infections can contribute to antimicrobial resistance making these infections more severe than would occur otherwise. Some uninvolved pathogens that cause these infections possess intrinsic resistance (natural resistance) to many antibiotics while others acquire resistance over time

Overuse or misuse of antibiotics can cause the disruption of normal microbiota and lead to an opportunistic infection caused by antibiotic-resistant pathogens [16] [17]. Antibiotic-resistant pathogens gain the ability to outcompete other normally occurring bacteria.

4.5. Classification of Airborne Bacteria: Treatment of Pneumonia

Treatment of pneumonia reflects the type of bacteria, Gram-Positive or Gram-Negative against which treatment is required [21]. Antibiotics administered against Gram-Positive bacteria differ from those administered against Gram-Negative bacteria. Hence, the occurrence of facultative aerobic, opportunistic pathogens in the infection could cause extreme difficulty in detection and treatment, as explained in previous sections and occurred in the illness described in this article. The occurrence of antibiotic resistant, facultative aerobic, opportunistic pathogens in the infection could render treatment almost, if not totally impossible. That is, antibiotics, singly or in combination against bacteria that have demonstrated resistance may offer no capability to eliminate the infection.

4.6. Classification of Airborne Bacteria: Prevention of Pneumonia

The main strategy for protection against bacteria that can cause pneumonia is vaccination. Since pneumonia is a disease of the lung(s), this means that the route of entry for the causative agent(s) is airborne exposure. Protection against airborne exposure during many types of work activity using engineering controls and respirators is very difficult and very uncertain. This occurs because of the difficulty in sampling for airborne bacteria, the difficulty in preventing and detecting emissions and the lack of standards of tolerance for collective and/or individual exposure. Hence, the strategy for preventing pneumonia relies on vaccination against bacteria known to cause infection, in this case *Streptococcus pneumoniae*. Protection against infection in this manner protects against subsidiary infection by antibiotic resistant, facultative aerobic, opportunistic pathogens.

Streptococcus pneumoniae infections are a major cause of illness and death worldwide and a logical choice in decision-making to provide protection against infection and disease. The World Health Organization (WHO) [22] estimated that more than 700,000 deaths among children under 5 years of age were attributable to pneumococcal disease. The WHO and recommends this strategy.

In Canada, Invasive Pneumococcal Disease (IPD is most common among the very young, adults 65 years of age and older and adults and persons at increased risk due to underlying medical, social, behavioral, or environmental risk factors. The distribution of IPD cases by serotype (strain) of bacteria varies by age group, risk factors and geographic region [23]. *S. pneumoniae* is transmitted by direct contact and respiratory droplets or indirect contact with respiratory secretions of infected or colonized persons. The incubation period for IPD has not been clearly defined and may be as short as 1 to 3 days.

Not mentioned in the document or not mentioned clearly are considerations concerning unusual “lifestyles”. These are describable as “lifestyles” posing risk of exposure to bacteria that cause IPD. These ‘lifestyles’ include travel for business or pleasure to destinations where wastewater treatment is non-existent or delivered at a primitive level. The destinations mentioned typically include cities having combined sewer systems (wastewater + surface water). Storm events can overwhelm these systems and divert raw sewage into waters at beaches and other tourist attractions located near water’s edge. Visitors often or usually are completely unaware of the risks posed by these exposures. Additional sources of exposure include passage through poorly ventilated terminal buildings in airports; and large-scale sporting events and entertainment; and the confined quarters in aircraft cabins and passenger cars on trains and mass public transit.

Another "lifestyle" that poses elevated risk of exposure for which protection is necessary is the type of work performed by workers exposed or potentially exposed to animal waste and wastewater. Wastewater causing potential or actual exposure to bacteria often contains an aerobic and an anaerobic layer. As mentioned, facultative anaerobes capable of causing infectious disease can occur in both layers.

Vaccination against *Streptococcus pneumonia* (Pneu-C) can occur in two ways: no previous vaccination or 'catch-up' for individuals vaccinated to lower levels of protection [24]. Vaccine-naïve adults aged 65 and older and adults under 65 with risk factors for Invasive Pneumococcal Disease should receive 1-dose of Pneu-C-20 or Pneu-C-21 vaccine. These classes of vaccine refer to breadth of coverage against serotypes of bacteria. (C-20 refers to protection against 20 classes of bacteria.) The higher the number refers to a broader level of protection. Pneu-C-23, offers the broadest breadth of protection (23 serotypes) but has limited lifetime (5 years or less). Serotypes also have subgroups.

Catch-up vaccination for persons vaccinated to lower Pneu-C levels aim to provide increased pneumococcal serotype coverage in individuals previously vaccinated with lower valent vaccines [24]. Regardless of pneumococcal vaccination history with Pneu-C-13 or Pneu-C-15 or Pneu-P-23, one dose of Pneu-C-20 or Pneu-C-21 is recommended for adults with IPD risk factors. (Medical doctors are familiar with these terms)

5. *Klebsiella pneumoniae,* a Vicious Predator

Medical testing described in a previous section identified *Klebsiella* as the bacteria that potentiated the pneumonia. The information above provides the "what", the "when" and several possibilities for the "how" and the "where". Still to answer is the "why". Plausibility in answers to these questions provides the basis for determining the significance of the exposure and the response, and its application to work occurring in similar circumstances worldwide.

5.1. Occurrence

Klebsiella spp. (spp. = species) occur worldwide, particularly in tropical and subtropical regions, and are ubiquitous, in forests, and in vegetation, soil and water [25] [26]. It naturally occurs in the soi land the mucosal membranes of host animal species including humans [9]. *K. pneumoniae* increases crop yields in agricultural conditions [27]. Other host animal species include mammals (horses, bovines, rhesus and squirrel monkeys, guinea pigs, muskrats, lemurs, and bats); aquatic animals (elephant seals, California sea lions, and harbor seals); reptiles (snakes, crocodiles, and American alligators); birds; insects; and plants (banana, rice sugar cane and maize) [25] [26]. Beyond human carriers, isolation of

Klebsiella pneumoniae has occurred from various environmental sources..

5.2. Types of Infection

Infection caused by *Klebsiella* is broadly divisible into two classifications: pneumonia and other infections [28]. Although they are common pathogens for community-acquired pneumonias and bacteremias (bacteria in the blood stream), a slight majority (56%) of the infections are hospital-acquired. *K. pneumoniae* is most pathogenic to humans among all *Klebsiella* spp. [27] Although, the number of infections is lower than some other pathogens, infections by *Klebsiella* spp. demonstrate substantial morbidity and mortality. *K. pneumoniae* occurs naturally in the nasopharynx and intestinal tract of humans. It is one of the leading causes of community-acquired pneumonia and has a death rate around 50%, even following antimicrobial therapy [28].

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium [27]. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli [28]. *Klebsiella pneumoniae* also is an opportunistic pathogen [27].

In 2019, there were 192,530 global deaths attributed to resistant strains of *Klebsiella pneumoniae* out of 1,264,514 deaths in total due to antibiotic resistance caused by various agents [28]. Deaths due to *Klebsiella pneumoniae* were second only to those due to *E. coli* (219,100) under the same circumstances. There is an important distinction in these numbers. Few deaths due to antibiotic resistance of *E. coli* occurred in the lung. *Klebsiella pneumoniae* infect the lung. Hence, antibiotic-resistant *Klebsiella pneumoniae* is by far the most serious predator. Widespread awareness and knowledge among potentially susceptible populations is desperately needed.

Klebsiella pneumoniae is resistant to almost all available antimicrobial agents singly or in combination. and infections with *Klebsiella pneumoniae* have caused high rates of morbidity and mortality, in particular among persons with prolonged hospitalization and those critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters) [29]-[31].

5.3. Workplace Exposure Monitoring for *Klebsiella pneumoniae*

Workplace exposure monitoring for airborne bacteria and emissions from potential and actual sources is currently undergoing an evolution.

Lee *et al.* [32] monitored exposure to H_2S and endotoxins (fragments from cell walls of Gram-Negative bacteria) in four multi-unit Wastewater Treatment Plants (WWTPs). (Endotoxins cause serious respiratory distress.) They observed that endotoxin levels were not significantly affected by type of process unit while levels of H_2S levels were affected by the process unit and the type of activity occurring. Søstrand *et al.* [33] and Austigard *et al.* [34] focused solely on H_2S.

The focus solely on individual species of airborne bacteria appears to be considerably more recent. Yang *et al.* [35] collected air samples from various units in a typical WWTP. Community compositions of airborne bacteria were identified by high-throughput sequencing technique. The main emission sources of airborne bacteria were treatment facilities with aeration by mechanical agitation located indoors. For treatment facilities located indoors, higher percentages of airborne bacteria were associated with wastewater and sludge, while more airborne bacteria originated from the ambient air for outdoor installations. Opportunistic pathogens including *Micrococcus*, *Bacteroides*, *Chryseobacterium*, *Pseudomonas*, and *Acinetobacter*, were detected in airborne bacteria. *Klebsiella* was not reported in this study.

Poopedi *et al.* [36] sampled for airborne bacteria above aeration tanks at two Wastewater Treatment Plants that use different aeration modes. Samples were analysed using the Illumina MiSeq platform. Thirty-six potential airborne bacterial pathogens were identified in the air samples. *Bacillus*, *Enterococcus*, *Clostridium*, *Streptococcus*, *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Bacteroides fragilis*, *Acinetobacter baumannii*, and *Escherichia/Shigella* dominated the results. *Klebsiella* was also detected but not at a level of dominance. Bioaerosols from mechanical aeration tanks (72%, 26/36) had a relatively higher diversity of airborne bacterial pathogens than diffused aeration tanks (17%, 6/36). In addition, most of the identified airborne bacterial pathogens were Gram-Negative bacteria. Opportunistic pathogens were also present.

These authors commented that since the 1970s, WWTP workers have commonly reported symptoms ('sewage worker's syndrome'), including fatigue, unexplained tiredness, headache, respiratory diseases, and fever, among others [37] [38]. More importantly, research found a correlation between sewage worker's syndrome and bioaerosol emission from WWTPs. Poopedi *et al.* [36] concluded that a comprehensive identification of pathogenic bacteria in bioaerosols from WWTPs is crucial to determining causal agents associated with health outcomes among workers, and also for providing a basis for preventing health risks.

Other studies have identified other opportunistic bacterial pathogens in bioaerosols originating from WWTPs. These include *Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Klebsiella*, *Mycobacterium*, and *Enterococcus* among others [39]-[43]. These references identified various factors, including source of the wastewater, aeration technology, inactivation rate of the bacteria, the amount of aerosolised material, meteorological conditions (e.g., temperature, sunlight, wind), and humidity influenced the composition, load, dispersion, and survival of the aerosolised bacteria [43].

In a recent study [44], *Klebsiella pneumoniae* was suspended in synthetic saliva in a nebulizer and nebulized for 5 min into an aerosol chamber and further prolonged in the aerosolization phase by mixing for a further 15 min under four different conditions: 20˚C, 50% relative humidity (RH); 20˚C, 80% RH; 30˚C, 50% RH; and 30˚C, 80% RH. Samples were collected at 0, 5 min, and 15 min and then subjected to survival analysis and comparative transcriptomic analysis in order to help elucidate the underlying mechanisms of airborne survival. Survival analysis showed that a higher humidity and lower temperature were favorable for the airborne survival of *Klebsiella pneumonia*. The effect of RH was more remarkable at 20˚C than that at 30˚C.

5.4. Air Sampling for *Klebsiella pneumoniae*

Air sampling for *Klebsiella pneumoniae* is purpose-oriented. Air sampling targets all bacterial cells in air or viable (live) cells only, depending on the sampling device. Culturing of viable cells provides a means of identification because colonies are visible to the unaided eye. The simplest option that provides a means of identification as well as quantitative output involves the use of agar plates and a device that captures them onto the plate in a separated and distributed manner.

The device used to position the plate into a separated airstream (a grid of small openings in a circular pattern) is an impactor. Small portable devices are available in the marketplace. Plate diameter ranges from 50 mm to 100 mm. In operation, a fan or pump inside the device draws the air to be sampled into the openings in the cover positioned above the plate. The cover of the 50 mm unit has 200 holes. Impaction of the air onto the surface of the agar creates a circular pattern of dimples (small depressions on the surface). Viable cells in droplets or particulates will germinate under appropriate conditions of temperature and humidity. Individual cells multiply to form colonies. Examination of the colonies for ability to grow, color, shape, and surface features enables identification and sampling to produce new colonies for further testing. The latter provide the ability to determine susceptibility to and resistance against individual antibiotics and antibiotic mixtures.

The composition of the agar on the plate creates conditions hospitable for growth or inhospitable conditions against growth [45]. Colony formation and ultimately identification depend on growth. Agar formulations that are indiscriminate permit growth dependent on other factors including, temperature, humidity and light. Selectivity can be introduced through addition of antibiotics to inhibit growth of some bacteria without affecting others. Other additives can affect the shape of the colony, thus aiding in identification.

The type of bacteria (Gram-Positive or Gram-Negative) governs the type and formulation of the agar to be used in air sampling.

Culturing of Gram-Positive bacteria during air sampling reflects considerations about exclusion of Gram-Negative bacteria. The American Society for Microbiology (ASM) recommends Phenyl Ethyl Alcohol (PEA) agar and Mannitol Salt agar (MSA) for the culture of Gram-Positive bacteria [46] [47]. Phenyl Ethyl Alcohol inhibits growth of Gram-Negative bacteria while permitting growth of Gram-Positive bacteria [46]. Mannitol and other additives enable separation of pathogenic *Staphyloccus* bacteria from non-pathogenic *Staphyloccus* bacteria. Salt was toxic to most microorganisms except *Staphyloccus* [47].

Culturing of Gram-Negative bacteria follows similar considerations. MacConkey agar (MAC) is a selective and differentiating agar that supports growth only by Gram-Negative bacteria [48]. It can further differentiate the Gram-Negative organisms based on their metabolism of lactose [49]. *Klebsiella* is a lactose fermenter. MAC contains a pH indicator that turns pink under acidic conditions. Therefore, lactose-fermenting-Gram-Negative bacteria will form pink colonies, while non-lactose fermenters will form off-white opaque colonies. Even within lactose-fermenters, species will show a varying rate of growth. The rate of growth is also a way to further differentiate organisms in the MAC medium.

MAC on agar plates was used successfully in air sampling using an impactor device as described above [50]. Sampling occurred at a rate of 100 L/min for 10 minutes using 100 mm plates. MAC was included to minimize the growth of background non-target microorganisms (predicted to be mostly fungi or Gram-Positive bacteria) while still allowing the growth of *B. thailandensis* or *K. pneumoniae*. Plates were incubated aerobically at 35˚C for 24 h. Analysis of colonies on the plates occurred by instrumental means.

6. Discussion

This review has examined the involvement of the Gram-Negative bacteria, *Klebsiella pneumoniae* in a life-threatening pneumonia. While well known in medicine and healthcare because of sectoral initiatives to educate and protect these personnel, *Klebsiella pneumoniae*, its occurrence and properties are virtually unknown in other domains, starting with occupational settings, visits to foreign countries due to business and tourism and large-scale sporting and entertainment events.

Information concerning critical properties of *Klebsiella pneumoniae*and their relationship with occupational settings is scattered throughout the literature in medical and academic microbiology. Information summarized from several sectors indicates that *Klebsiella pneumoniae* is a facultative anaerobic, opportunistic pathogen, strains of which have developed resistance to various antibiotics.

As with other bacteria, *Klebsiella pneumoniae* is ubiquitous in the environment. As a facultative anaerobe, *Klebsiella pneumoniae* lives in both aerobic and anaerobic environments and experiences metabolic advantage in the aerobic environment. As an opportunistic pathogen, *Klebsiella pneumoniae* potentiates pneumonia caused by other bacteria in patients having immune system stressed by various factors. In the absence of a vaccine that targets *Klebsiella pneumoniae*, reliance is made on prevention of pneumonia by vaccination against other pneumonia-causing bacteria and antibiotics that kill the microorganisms that cause pneumonia.

Prevention of potentiation of pneumonia by opportunistic pneumonia-causing bacteria is an important strategy in vaccination against *Streptococcus pneumoniae*. Prevention of infection by *Streptococcus pneumoniae* suppresses potentiation by opportunistic pathogens by preventing undue stress on the immune system. Vaccines against pneumonia-causing bacteria vary in breadth of protection. Some have limited lifetime.

Various strains of *Klebsiella pneumoniae* have developed varying levels of antibiotic resistance. Antibiotic resistance of some strains is sufficient to render untreatable pneumonia involving participation by *Klebsiella pneumoniae*.

Given the ubiquity of *Klebsiella pneumoniae* and other species of bacteria that share the same properties, an important component of protection is to identify occupations and activities outside of healthcare most at risk to suffer the consequences of bacterial attack in the lung. The risk of bacterial attack in the lung is widely recognized in healthcare and receives considerable attention from medical professionals for this reason. Outside of healthcare, the risk is little appreciated.

The first group includes workers exposed to large quantities of bacteria in air in performance of their jobs. In particular, these workers have potential exposure to aerosols emitted from wastewater and possibly from manure. *Klebsiella pneumoniae* is just as possible in aerosols emitted aerobic waters as from anaerobic waters.

The second group includes workers engaged in animal husbandry where exposure to emissions from manure is a daily occurrence.

The third group includes other workers exposed to emissions from water sources containing an aerobic layer above an anaerobic layer especially where disturbance is possible and likely to occur.

The fourth group comprises persons who travel to cities where sanitation is substandard compared to modern practices. In particular these situations especially include combined sewer systems that collect surface water and sewage. During storm events overload of the sewer system can lead to discharge of the raw sewage/stormwater mixture into rivers, lakes and ocean areas not normally subjected to such burdens. This situation can affect any city whose infrastructure contains a combined sewer system, regardless of the level of sanitation practice.

The fifth group comprises persons who work in locations frequented by large numbers of people some of whom are exhaling aerosols containing infectious bacteria. These locations include passenger terminals in airports and train stations, venues that house large-scale entertainment and sporting events, confined spaces such as the cabins in aircraft and other mass transit equipment, among other possibilities.

Recent trends in vaccination-avoidance are creating a large pool of people who potentially lack protection against bacteria that cause pneumonia because of stresses on their immune systems involved in fighting colds, influenza, covid, measles, and other infectious diseases for which vaccines are readily available.

An issue of considerable concern exposed during this investigation involved inconsistencies in the thoroughness of inclusion information provided in various sources. This investigation identified sources where *Klebsiella pneumoniae* was identified as being present during a study and other sources investigating the same situation where *Klebsiella pneumoniae* was not identified as being present.

Another area of inconsistency was the source of exposure to *Klebsiella pneumoniae* that potentiated pneumonia caused by other bacteria. This review has documented numerous investigations performed by other authors that demonstrated that *Klebsiella pneumoniae* is present in air especially where handling of wastewater occurs in open-topped chambers.

A report prepared by the U.S. Centers for Disease Control and Prevention (CDC) [51] takes the opposite view and states that "*Klebsiella* does not spread through the air". The reality is that in order to suffer infection by *K. pneumoniae*, a person must be exposed to the bacteria. In other words, *K. pneumoniae* must enter the respiratory tract to cause pneumonia, or the blood to cause a bloodstream infection [24]. The report produced by the CDC [51] offers neither context nor support for the position reported on their website. Still further, the ubiquitous nature of the distribution of *Klebsiella* worldwide begs the question about how an immobile bacterium can migrate to so many places. The CDC website listed in Google searches makes no attempt to provide context. Hence, readers lacking detailed training in this area will apply whatever context is relevant to them.

The uncertainty created by these conflicting views demands resolution. The inconsistencies are manifested through inclusion versus omission in listing *Klebsiella* in various subjects reported here. Some articles mention *Klebsiella* in various topics whereas others do not. Hence, careful reading with cross-checking between articles cited in various places in this article provides the means to confirm the likelihood of involvement by *Klebsiella* when it is not listed. Consistency in reporting information concerning *Klebsiella pneumoniae* in the literature is absolutely critical in order to produce properly informed responses.

Given the ubiquity of *Klebsiella pneumoniae* and the demonstrated presence in the air, the question then becomes, why some persons in demonstrated good health are affected and others are not? The answer to this question appears to be related to a factor that is easily overlooked in the remainder of the discussion. This is related to how the stress on the immune system of an otherwise healthy person can develop. While stress on the immune system leading to a compromised response is a theme throughout this discussion about *Klebsiella pneumonia* and by extension, other bacteria sharing similar properties, interpersonal factors remain to be considered.

In the example provided in the beginning of this review, about 5 days passed from the time of exposure to the initial coughing episode; 10 days from the exposure to admission to the first treatment facility; about 17 days to admission to the hospital; and about 26 days to the start of treatment with the antibiotic needed to kill the infection by *Klebsiella pneumoniae*. During this period the immune system of the patient was engaged in fighting the pneumonia caused by other bacteria assisted by antibiotics used in that type of treatment. It would seem that the delay in detecting and determining the presence of *Klebsiella pneumoniae* in the lungs provided the time needed for development of the massive infection by *Klebsiella pneumoniae*.

In retrospect, this explanation makes sense to explain what happened and provides a cautionary example for others. The delay occurred despite efforts for the patient made in good faith by medical professionals, including one having extensive experience in this area. There is a natural inclination when otherwise healthy people suffer respiratory infection to delay medical intervention because of confidence in the ability of the body to combat the invader based on previous success and lack of recognition by lay people about the tipping point at which time medical intervention is necessary. In addition, even for those people for whom a family doctor is available, delay in obtaining an appointment is inevitable. People for whom a doctor is not available must depend on publicly available resources including emergency rooms in hospitals and primary care facilities, as mentioned in the example in a previous section.

In addition, the episode of exposure is not always recognizable as it was in the example. Hence, the delay in receiving treatment could easily equal or exceed that described here.

Given the realities presented above, the scenario of being completely unprotected against onset of pneumonia and potentiation of the infection by *Klebsiella pneumoniae* is entirely feasible. Overcoming this situation depends on the knowledge level of the patient and initiative to obtain vaccination against pneumonia-causing bacteria either privately or through an employer; and ultimately, the knowledge and skill of the doctors involved in the case, and the ability to kill the bacteria causing the infection with the relevant antibiotic(s).

The ability to obtain positive outcomes starting with prevention of pneumonia depends on education of people who might be affected by this situation starting with information concerning the availability and limitations of existing vaccines and the duration of protection. This also requires further education of medical doctors and the general public.

7. Conclusion

The episode documented in this article serves as the introduction to a discussion concerning the vulnerability of people to infection by pneumonia-causing bacteria and potentiation of this vulnerability by facultative anerobic, opportunistic bacteria, the most important of which is *Klebsiella pneumoniae*. Resistance of *Klebsiella pneumoniae* to many antibiotics compounds this problem and is not generally recognized or when recognized, not appreciated to the level of seriousness that is necessary to protect vulnerable populations outside those already served by healthcare professionals. Vulnerable populations include workers and business and pleasure travellers potentially exposed to pneumonia-causing bacteria. A consistent and coherent explanation for this situation becomes the foundation for strategies and vaccination to expand vaccination in order to eliminate to the extent possible deaths due to *Klebsiella pneumoniae.*

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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