The Importance of Bioaerosols in Hospital Infections and the Potential for Control using Germicidal Ultraviolet Irradiation.


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Abstract
In recent years a number of factors have stimulated an increased awareness of the presence of potentially pathogenic bioaerosols in indoor and outdoor environments and the detrimental health effects associated with them. The incidence of hospital acquired infection is a serious and widespread problem, with an estimated 1 in 10 patients acquiring an infection during a hospital stay. There are a whole range of potentially pathogenic microorganisms associated with nosocomial infections many of which are opportunistic pathogens which frequently cause respiratory infections particularly in immunocompromised patients. In order to determine the extent of the problem it may be necessary to carry out sampling in order to verify and quantify the presence of bioaerosols in the air. There are a large number of different air samplers available and the choice of sampler type will be highly dependent upon the location in which the sample is to be taken and the information required. In sensitive locations such as hospitals the primary consideration may be the need to cause as little disturbance to the patients as possible. Once an airborne route of infection has been identified as a transmission route there are several possible control strategies available including the use of germicidal ultraviolet disinfection. In the USA in particular ultraviolet disinfection systems are currently widely used in hospitals and other health care environments in order to protect patients and healthcare workers. Microorganisms are particularly vulnerable to UV light at wavelengths close to 254nm since this represents the maximum absorption wavelength of their DNA molecule. Photons of UV light strike a biological cell and the energy is absorbed by nucleic acids in the DNA molecule leading to the formation of pyrimidine dimers and other lethal photoproducts. The formation of pyrimidine dimers leads to changes to the double helix structure, cell mutation and ultimately to the death of the cell. Research carried out at the University of Leeds and elsewhere has shown that a whole range of potentially pathogenic bacterial species are susceptible to UV. It has also highlighted the importance of environmental factors such as relative humidity and the positive effect high levels of relative humidity can have on the survival of bacteria after UV exposure. Although history shows that the uptake of UV disinfection has progressed slowly the future looks extremely promising. With increasing concerns over rising numbers of TB outbreaks, new pathogens such as SARS and avian flu and also the increased risk of bioterrorism threats the market for new disinfection technologies is great. In addition to clinical applications, this technology would also be similarly applicable to waste management facilities for controlling potential pathogens such as Aspergillus fumigatus.

Key Words: Bioaerosols, airborne microorganisms, nosocomial infection, ultraviolet, UV, air disinfection, UV susceptibility

Introduction
In recent years a number of factors have stimulated an increased awareness of the presence of potentially pathogenic bioaerosols in indoor and outdoor environments and the detrimental health effects associated with them (Lin & Li, 2002). One of the main driving forces has been the re-emergence of tuberculosis as a major health concern mainly in the developing world but also more recently in the more developed countries of Europe. The situation has been compounded by the rise in the number of multi-drug resistant strains of Mycobacterium tuberculosis which has made antibiotic treatment of the disease increasingly problematic.

The importance of bioaerosols on hospital infections
Humans can fall prey to a whole range of viral and bacterial diseases such as measles and tuberculosis which are transmitted via the airborne route from one infectious individual to another susceptible victim. Infectious aerosols tend to be extremely small (< 5μm) and can therefore remain suspended and viable in the air stream over long periods of time. As a result the risk of airborne infection in high-risk locations (hospitals and clinics) and confined spaces (trains and aeroplanes) is extremely high.
Nosocomial infection (i.e. those acquired during a hospital stay) is a serious and widespread problem, with an estimated 1 in 10 patients acquiring an infection during a hospital stay (Mertens 1996). While many of these infections are associated with person-to-person contact, there is increasing evidence that some infections are transmitted by the airborne route. It has been calculated that the airborne route of transmission may account for as much as 10 - 20% of all endemic nosocomial infections (Brachman 1970). In England and Wales alone this is estimated to cost approximately £100 - 200 million per annum in England alone (National Audit Office, 2000).

Although it is recognised that in some diseases such as tuberculosis (TB), measles and pulmonary aspergillosis the airborne route is of primary importance, the contribution made by this route towards the overall burden of nosocomial infection is unclear. As a result there is a great deal of uncertainty regarding the role played by aerosolised microorganisms, with the result that for many years the importance of airborne microflora in hospitals has been largely ignored. Very little work has been carried out in this field since the early 1960s (Greene et al. 1960; 1962a; 1962b) and as a result, the epidemiology associated with airborne infection remains poorly understood. Nevertheless, there is increasing evidence to implicate airborne transmission in nosocomial outbreaks of *Staphylococcus aureus* (including methicillin-resistant strains - MRSA) (Rutula et al. 1983; Farrington et al. 1990), *Pseudomonas* spp. (Grieble et al. 1974), and *Acinetobacter* spp. (Allen and Green 1987; Bernards et al. 1998), as well as norovirus outbreaks.

In order for airborne transmission to take place the microorganisms of interest must enter the air stream which can happen through a variety of routes. The microorganisms can be dispersed violently into the air as respiratory droplets/droplet nuclei when an infected person coughs or sneezes (Figure 1). They can also be released on skin squames which are shed continuously at a rate estimated to be approximately $3 \times 10^8$ skin squamae per person per day (Rhame 1998). Other activities such as bed making can release large quantities of microorganisms into the air and Greene *et al.* (1960) reported that the number of bacteria in the air could exceed 6000 cfu/m$^3$ during bed making. Overall it is thought that most airborne microorganisms found in hospital buildings originate from the staff, patients and visitors within the building rather than entering from outside.

Although microorganisms associated with skin squamae are important in terms of their potential risk of causing infection this risk tends to be rather localised. Their relatively large size means that they will quickly fall out of the air and therefore their range is relatively
small. More important are those microorganisms contained within droplet nuclei which were first described by Wells in the mid 1930’s as the primary mode of airborne infection. Droplet nuclei are formed by the evaporation of droplets produced when an infected person coughs or sneezes. An infected person coughing or sneezing will produce 1000’s of droplet nuclei many of which may contain pathogenic microorganisms.

During sneezing droplets are expelled into the atmosphere at velocities as high as 100m/s in some cases and the droplets produced tend to be approximately 10μm in diameter. The larger of the droplets will fall to the ground very quickly and the smaller ones will evaporate and rapidly decrease in size and become droplet nuclei. Droplet nuclei are typically 1-5μm in diameter, settle slowly and can remain suspended in the air for long periods. These droplet nuclei are so small that they bypass the innate host defence mechanisms of the human upper respiratory tract and are deposited in the alveoli in the lungs.

Because droplet nuclei can remain suspended in air for several hours (a 2μm droplet in a calm room will take 4.2 hours to fall 2 metres), they can travel over long distances and thus can be distributed widely throughout buildings. The chain of infection is therefore very much influenced by the ventilation conditions that are experienced in any particular setting. In terms of infection control it is believed that airborne transport of microorganisms represents a weak link in the infection transmission route and one where control measures may have the greatest chance of breaking the infection cycle.

Potential microorganisms involved
There are a whole range of potentially pathogenic microorganisms associated with nosocomial infections. Although some are serious human pathogens many others are simply opportunistic pathogens which to a healthy human being would present very little risk but which can frequently cause serious respiratory infections in immunocompromised patients. It is beyond the scope of this paper to discuss all the potential pathogens associated with nosocomial infection however some of the more important are discussed below. The list includes some of the more recognised airborne infections together with others which are less well know but which have been included because they are of particular interest in the context of the research currently being carried out at the University of Leeds which will be discussed in more detail in a later section.

Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis* and is a classical example of an infection which can be transmitted via the airborne route. Although it is not normally considered a nosocomial pathogen, its transmission within hospitals is well recognised. Recently in the UK several outbreaks of multi-drug resistant tuberculosis have highlighted the ever-present potential for transmission within the hospital environment (Breathnach *et al*, 1998). The emergence of multi-drug resistant strains of *M. tuberculosis* has increased the concern regarding this disease.

Pulmonary aspergillosis is caused by the inhalation of the spores of *Aspergillus* sp which are widespread in the outdoor environment. The spores are thought to enter hospital buildings through ventilation ducts with inadequate filtration. High concentrations of *Aspergillus* sp. spores are commonly associated with building work with can liberate spores into the atmosphere. Immunocompromised individuals are particularly vulnerable to infection from *Aspergillus* sp and mortality rates are significant, especially in bone marrow transplant patients.
Although *Acinetobacter* sp are ubiquitous in the environment they are associated with a wide range of infections particularly in immunocompromised individuals. Infections are becoming more difficult to treat as multi-drug resistant strains emerge. There is increasing evidence both from the work carried out at the University of Leeds and elsewhere (Allen and Green, 1987, Bernards et al., 1998) that *Acinetobacter* sp are transmitted via the airborne route. *Acinetobacter* sp. have been isolated from the air in the intensive care unit at a Leeds hospital (Beggs et al. 2002)

*Staphylococcus aureus* or more importantly the methicillin resistant strain (MRSA) is a major problem in hospitals throughout the world. Although most outbreaks are associated with the a contact route for infection, the airborne route is thought to play an important role in particular locations such as intensive care wards and burns units.

There are a whole range of bacteria which are of concern regarding those individuals which are immunocompromised and in particular those with cystic fibrosis. *Pseudomonas aeruginosa* is an opportunistic pathogen and is a common nosocomial pathogen which is generally associated with catheter-related urinary tract infections, infected ulcers, burns and eye infections. It is commonly associated with infections almost exclusively limited to those with a compromised lower respiratory tract or immune system. Colonisation of cystic fibrosis patients by mucoid *P. aeruginosa* is common and difficult if not impossible to treat. It is notorious for its resistance to antibiotics and it is therefore a particularly dangerous and dreaded pathogen. It accounts for approximately 16% of nosocomial pneumonia and 12% of nosocomial urinary-tract infections.

*Burkholderia cepacia* is a significant cause of morbidity and mortality in patients suffering from cystic fibrosis and studies have shown that it can be found in approximately 5% of those with cystic fibrosis. Pulmonary colonisation reduces survival of patients by as much as 50% and approximately one third to a half of the patients succumb to "cepacia syndrome", a rapidly fatal necrotising pneumonia. *Stenotrophomonas maltophilia* was originally considered to be a harmless commensal but is now emerging as an important nosocomial pathogen in the immunocompromised, in cancer patients, in transplant recipients and patients undergoing peritoneal dialysis. It was first isolated from the respiratory tract of cystic fibrosis patients in 1975 and since then the number of cystic fibrosis centres reporting isolation of *S. maltophilia* from patients has continued to rise. As with *B. cepacia* it is estimated to be found in approximately 5% of those with cystic fibrosis. *Serratia marcescens* was originally thought to be benign but more recently found to be pathogenic to some individuals. It is now believed to be a nosocomial pathogen responsible for 4% of hospital acquired pneumonias and multi-drug resistant forms are common and in addition to this some strains will develop resistance during therapy.

**Methods for monitoring bioaerosols**

Numerous types of sampling devices are available for the collection of bioaerosols from both indoor and outdoor environments. Each device will have its own set of advantages and disadvantages and will be more or less applicable to a particular environment. Generally the collection of bioaerosol particles is based on the same principles as those for non-biological aerosols. However, ensuring the survival or biological activity of the bioaerosol particles during and after collection is an important concern. Furthermore sample handling and storage, as well as the analysis of the collected aerosol are considerably different from general particle sampling (Nevalainen et al., 2001).
The purpose of bioaerosol sampling is most often to verify and quantify the presence of bioaerosols in the air and in most cases no single sampling method can collect, identify and quantify all of the bioaerosol components existing in a particular environment. One important factor to consider is that different bioaerosol types will be more or less difficult to sample. For example, bacterial and fungal spores tend to be hardy and are resistant to environmental stresses whereas vegetative cells are easily damaged and their viability may be compromised by both environmental and more importantly sampling stresses (Cox, 1987). This is particularly important when considering the hospital environment where one is mainly concerned with gram negative vegetative bacterial species. These are thought to be the most sensitive to environmental stress and may suffer damage during the sampling process which will affect their viability and ultimately their detection.

According to Nevalainen et al (2001) the overall sampling efficacy of a bioaerosol sampler can be divided into three components:

- The ability to extract particles from the ambient environment without bias regarding particle size, shape or aerodynamic behaviour
- The ability to remove the particles from the air stream and deposit them into or onto the collection medium
- The ability to remove biological particles without altering their viability or biological activity and to provide the proper conditions for the organisms to form colonies or otherwise.

Bioaerosol sampling involves the separation of the particle trajectory from the air streamline trajectory through the application of different physical forces. Samplers such as the Andersen sampler and the SAS sampler use inertial separation and impaction onto solid media. Inertial impaction is the most commonly used mechanism of particle removal in bioaerosol samplers and variations in the inlet nozzle dimensions and airflow paths can determine the cut-off size of particles a sampler will collect. In the 6-stage Andersen sampler (Plate 1) this principle is used to provide size distribution data for bioaerosols in the collected air stream. The Andersen 6-stage sampler has been used in many studies and can give valuable information regarding the size distribution of bioaerosols. The SAS samplers are one-stage multiple hole impactors (Plate 2) which allow portability and consecutive sampling. Both the Andersen and SAS samplers require statistical adjustment for multiple impaction.

Plate 1. Six stage Andersen sampler  
Plate 2. SAS Single stage sampler
Particle separation through centrifugal force is used by the Burkard cyclone sampler (Plate 3) which can be used with or without a collection liquid. The Burkard centrifugal sampler is portable and inconspicuous and therefore has the advantage in that it will cause minimal disturbance to a room's occupants. Cyclone samplers can be used to collect dry particles such as dust into an empty collection chamber or biological particles which are dispersed into a collection liquid. In terms of advantages and disadvantages they tend to be the same as the liquid impingers.

![Plate 3. Burkard Cyclone sampler](image)

Liquid impingement as used in the AGI-30 (Plate 4) mainly uses inertial forces to collect particles but also uses diffusion within the bubbles to enhance particle collection. As their name suggests they collect bioaerosols into a collecting fluid which can then be plated out for microbial counts, examined microscopically for manual identification and counting or subjected to immunochemical tests. The advantage of such a device is that a single sample can be used to enumerate a range of different organisms. However on the other hand they may be inefficient at collecting hydrophobic particles such as fungal spores, the liquid can evaporate over time and it is also necessary to sample over long periods since the sample flow rate must be relatively low (Cage et al., 1996).

Choice of sampler type will be highly dependent upon the location in which the sample is to be taken and the information required. In sensitive locations such as hospitals the primary consideration may be the need to cause as little disturbance to the patients as possible. This may exclude the use of a 6-stage Andersen sampler or even the liquid impinger, both of which require an external air pump which requires a power supply and may be quite noisy. In this case it may be more appropriate to use the Burkard or SAS samplers, both of which have their own battery powered pump and are very quite when being operated.
If sampling is being undertaken for a limited number (1 or 2) known species of bioaerosols it would be simple to use the SAS or Andersen sampler as these can be loaded with agar plates containing media selective for each organism. These can then be incubated back in the laboratory and the whole process is then very simple. If information is required regarding the size distribution of the bioaerosols then the 6-stage Andersen sampler must be used. On the other hand if the purpose of the sampling is to determine the presence and even quantity of a whole range of bioaerosols it would then be a better option to use either the Burkard sampler collecting into a liquid or the liquid impinger (AGI-30). The advantage being that a single sample can be used to evaluate a whole range of different species by plating out the collection liquid onto a wide range of different selective media. Using the Andersen sampler or the SAS sampler would mean taking large numbers of separate sample for each of the species being investigated.

**Control of nosocomial infections using UV germicidal irradiation**

Ultraviolet germicidal irradiation is now a recognised method of inactivating a wide variety of biological agents (Rice & Ewell, 2001) and in particular airborne microorganisms. Recent increases in the incidence of airborne diseases such as tuberculosis have focussed attention upon the use of this technology and in the USA ultraviolet disinfection systems are currently widely used in hospitals and other healthcare environments in order to protect patients and healthcare workers (Dumyahn & First, 1999). It has previously been noted in the literature that the efficacy of UV irradiation is a function of many different locational and operational factors including UV intensity, exposure time, lamp placement, air movement patterns and the relative humidity of the air (Lin & Li, 2002; Peccia et al, 2001; Ko et al, 2000).

Microorganisms are particularly vulnerable to UV light at wavelengths close to 254nm since this represents the maximum absorption wavelength of their DNA molecule. Photons of UV light strike a biological cell and the energy is absorbed by nucleic acids in the DNA molecule leading to the formation of pyrimidine dimers and other lethal photoproducts (Beggs 2002). The formation of pyrimidine dimers leads to changes to the double helix structure, cell mutation and ultimately to the death of the cell (Figure 2).
The germicidal properties of UV light have been known about for many years; in the pre-antibiotic era UV lamps were used extensively in tuberculosis (TB) wards to control the spread of infection (Lidwell 1994), but with the development of anti-bacterial drugs and, in some countries the introduction of vaccination against TB, UV air disinfection fell out of favour. However, in recent years, with the global rise in TB (Miller et al. 1996, Tancock 1998), there has been renewed interest in its use as an infection control measure and several research programmes have been initiated in this field.

UVGI can be applied as either duct irradiation in which UV devices are mounted within the ducts supplying air to a particular location or upper room UVGI in high risk settings. Upper room UVGI as the name suggests involves the installation of an open field UV device such as that shown in Plate 5 to create a UV zone within the upper portion of a room. Since exposure to UV light is particularly harmful, such devices are fitted with a number of louvers to prevent the UV light from penetrating the lower room. The goal of upper room UVGI is to inactivate the infectious airborne microorganism in the upper room and supply the lower room with disinfected upper room air. Upper-room installations rely on natural convection currents, rather than fans, to carry airborne microorganisms through the UV field. By using the convection currents that occur naturally in rooms it is possible to disinfect very large volumes of air relatively quickly (Miller, et al. 1999).
The effect of UV is ultimately related to the dose received since this will determine the number of photoproducts produced and the number of microorganisms inactivated. Another factor to be considered is the microorganisms concerned as the degree of damage caused will vary between different microbial species. The susceptibility of any given microorganism to UV damage is quantified by its UV susceptibility constant (Z, the higher the Z value the more susceptible the organism), which is the gradient of the line produced when the natural logarithm of the survival fraction is plotted against UV dose. Survival fraction is calculated as below where \( N_{\text{UV}} \) is the average number of organisms surviving after UV exposure and \( N_0 \) is the average number of organisms before UV exposure.

\[
\text{SF} = \frac{N_{\text{UV}}}{N_0}
\]

A number of researchers have carried out experiments in order to determine the UV susceptibilities of a whole range of microorganisms and some of these are presented in Table 1 although the list is by no means comprehensive. All the data presented in this table has been determined for microorganisms in the airborne state and it is generally considered that microorganisms are more susceptible to UV irradiation when airborne than when on solid media or in a broth.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Z value (m²/J)</th>
<th>Medium</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis (spores)</td>
<td>0.0190</td>
<td>Air</td>
<td>Rentschler 1941</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>0.0510</td>
<td>Air</td>
<td>Sharp 1938</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.5721</td>
<td>Air</td>
<td>Sharp 1940</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0.0950</td>
<td>Air</td>
<td>Rentschler 1941</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0.1050</td>
<td>Air</td>
<td>Sharp 1938</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0.2140</td>
<td>Air</td>
<td>Riley, et al. 1976</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0.4449</td>
<td>Air</td>
<td>Sharp 1940</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>0.0120</td>
<td>Air</td>
<td>Rentschler 1941</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>0.4721</td>
<td>Air</td>
<td>Riley, et al. 1976</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.3476</td>
<td>Air</td>
<td>Sharp 1940</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.9602</td>
<td>Air</td>
<td>Luckiesh 1946</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.3759</td>
<td>Air</td>
<td>Sharp 1940</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>0.0546</td>
<td>Air</td>
<td>Jensen 1964</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>0.1528</td>
<td>Air</td>
<td>Jensen 1964</td>
</tr>
<tr>
<td>Aspergillus (Eurotium) amstelodami</td>
<td>0.00344</td>
<td>Air</td>
<td>Luckiesh 1946</td>
</tr>
</tbody>
</table>

The data presented in Table 1 shows the great variability in the UV susceptibilities of different microorganisms, for example it can be seen that the bacterium Eschericia coli is approximately a hundred times more susceptible to UV irradiation than the fungus Aspergillus (Eurotium) amstelodami. What also becomes apparent is the variability between different researchers in the UV susceptibility data presented for the same organism. For example, susceptibility values for Serratia marcescens range from 0.095 to 0.445 m²/J which may be due to the use of different strains of the bacterium and also the experimental techniques used (Beggs, 2003).

Research work carried out at the University of Leeds has looked at the effect of ultraviolet disinfection upon a whole range of airborne bacterial pathogens. The results have shown that all are susceptible to the effects of UV but the magnitude of the effect is extremely species
dependent as highlighted in the table above. Research has also shown the importance of
environmental factors such as relative humidity and the positive effect high levels of relative
humidity can have on the survival of bacteria after UV exposure. The research that has been
carried out at the University of Leeds and elsewhere can only serve to benefit the ultraviolet
disinfection industry as a whole. Only through research like this will manufacturers be able to
develop better products which are more efficient and safer to use. The development of
guidelines as to correct installation strategies through the use of computer modelling looking
at the number of devices required in a particular situation and the best locations for them is
also progressing.

The full scale use of UV disinfection in a range of clinical and non-clinical environments has
been investigated (Beggs et al, 2000, Nardell et al, 1986 and 1991). Although history shows
that the uptake of UV disinfection has progressed slowly the future looks extremely
promising. With increasing concerns over rising numbers of TB outbreaks, new pathogens
such as SARS and avian flu and also the increased risk of bioterrorism threats the market for
new disinfection technologies is great. In addition to clinical applications, this technology
would also be similarly applicable to waste management facilities for controlling potential
pathogens such as Aspergillus fumigatus.

Aerobiology research at the University of Leeds
The Aerobiology Research Group at the University of Leeds is a multidisciplinary team with
research interests in amongst other things the control of hospital acquired infection through
the use of novel technologies such as ultraviolet radiation air disinfection and negative air
ionisation. The group is perhaps unique in that it is made up of experts in the fields of applied
microbiology, medical microbiology and computer modelling together with building services
and epidemiology. This gives the group a broad understanding of all the issues associated
with the research area and allows the subject to be approached from a whole host of different
angles.

At present the group are involved with a research project looking at the impact of negative air
ionisation on bioaerosols. The work is being carried out at laboratory scale using a range of
specially designed test apparatus and also within a large bioaerosol test chamber, one of only
a handful in the world. The research into the negative air ionisation has been undertaken after
successful trials using negative air ionisers in an intensive care unit in a UK hospital (Beggs et
al, 2002). The research is focussing upon the mechanisms by which the negative ions interact
with the bioaerosols and the possible physical or biological impacts.

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