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ORIGINAL ARTICLE

Microbial aerosol generation during laboratory accidents and subsequent risk assessment

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Abstract

Aim: To quantify microbial aerosols generated by a series of laboratory accidents and to use these data in risk assessment.
Methods and Results: A series of laboratory accident scenarios have been devised and the microbial aerosol generated by them has been measured using a range of microbial air samplers. The accident scenarios generating the highest aerosol concentrations were, dropping a fungal plate, dropping a large bottle, centrifuge rotor leaks and a blocked syringe filter. Many of these accidents generated low particle size aerosols, which would be inhaled into the lungs of any exposed laboratory staff. Spray factors (SFs) have been calculated using the results of these experiments as an indicator of the potential for accidents to generate microbial aerosols. Model risk assessments have been described using the SF data.
Conclusions: Quantitative risk assessment of laboratory accidents can provide data that can aid the design of containment laboratories and the response to laboratory accidents.
Significance and Impact of the Study: A methodology has been described and supporting data provided to allow microbiological safety officers to carry out quantitative risk assessment of laboratory accidents.

Introduction

Laboratory acquired infections have been widely reported in the scientific literature (Pike 1979, Collins and Kennedy 1998). Many of these incidents have obvious causes such as needle-sticks injuries (Campbell *et al.* 2002) and contaminated hand to mouth or eye contact (Takata *et al.* 1991). These accidents can normally be prevented by appropriate training of laboratory staff and adherence to codes of laboratory practice. However, a microbial aerosol release within the laboratory can pose an infection risk, which may not be immediately recognized and may affect all users of the laboratory. Aerosols may be generated by using uncontained or malfunctioning high-energy equipment such as centrifuge and homogenisers. Aerosol generating accidents may happen such as dropping of glassware or plates, or by catastrophic equipment failures. These releases may lead to infection by inhalation of the aerosol. The equipment used in microbiological laboratories should be designed to prevent the release of these aerosols but accidents still can occur.

Publications by Kenny and Sabel (1968) and Ashcroft and Pomeroy (1983) have attempted to measure the aerosol generated during accidents. Kenny and Sabel studied small accidents within a safety cabinet, such as use of a blender and handling lyophilized cultures, using *Serattia marcescens* as a tracer. Ashcroft and Pomeroy studied fermenter malfunctions and even explosions using *Bacillus atrophaeus* spores as the tracer. Dimmick (1973) and Dimmick *et al.* (1974) also carried out this type of research and developed the concept of the spray factor (SF), which was a ratio of the aerosol output divided by the original suspension concentration (SC). The SF was used as an indication of the seriousness of an accident and as a component in risk assessment.

In this study a series of experiments have been designed to mimic potential accident scenarios, potential sources of release of aerosols and actual accidents. These

experiments mainly consist of equipment malfunctions or glassware smashes, which may be liable to cause large aerosol releases. Most of the experiments were carried out using the same concentration of an aqueous *B. atrophaeus* spore suspension and so are directly comparable. However, in two cases the concentration of the *B. atrophaeus* suspension was varied to investigate whether SC affected the SF developed by Dimmick (1973). The accidents have been monitored by a range of microbial air samplers in order to provide information on the number of microbes, number of microbe containing particles, and the particle size distribution of the aerosols produced by the accidents.

Materials and methods

Methodology

All the simulated accidents described in this section were carried out in a clean room (dimensions $3 \times 3 \times 2$ m^3) at the Health Protection Agency Centre for Emergency Preparedness and Response, Porton Down. The air sampling devices listed below were operated remotely to sample the microbial aerosol generated by the accidents.

Cyclone sampler
A large volume Cyclone sampler operating at 750 l min^{-1}, using a collection fluid consisting of phosphate buffer containing manucol and antifoam, as described by Decker *et al.* (1969) was used to sample the total number of micro-organisms in the aerosol. The sampler was operated for 10 min.

Casella slit sampler
A Casella slit sampler operating at a rate of 30 l min^{-1}, containing tryptose soya broth agar (TSBA) plates was operated for 10 min to count the number of microbially contaminated particles generated by the accident.

Andersen sampler
An Andersen six stage sampler containing six TSBA plates, operating at 28 l min^{-1} was operated for 5 min to measure the particle size distribution of the microbial aerosol (May 1964).

The tracer suspension used, unless otherwise stated, was a 2×10^9 spores ml^{-1} suspension of *B. atrophaeus* (NCTC 10073). TSBA plates were used for microbiological analysis in all cases. The collection fluid from the Cyclone sampler was inoculated in duplicate ($0{\cdot}1 \times 2$ ml) onto the surface of TSBA plates. All plates were incubated aerobically at 37°C (±2) for between 18 and 24 h before being counted. In some of the experiments, sodium fluorescein was added to the spore solution. After the accident the room could be illuminated with a UV lamp and the fluorescent component would allow the extent of any splashes to be seen. In some experiments settle plates were laid out in the room to assess the extent of spread and magnitude of splashing.

All experiments were carried out with the room ventilation system turned off. The ventilation system was operated to clear aerosols after sampling to allow safe operator entry to the room and to return aerosol concentrations (ACs) to background levels.

Individual experiments

Thirteen experiments were designed to simulate various types of laboratory accident that could generate microbial aerosols.

Experiment 1: dropping a flask on the floor
A 250 ml flask containing 50 ml of the spore suspension was dropped from a height of 0·75 m on to the floor of the room.

Experiment 2: dropping a large volume Thompson bottle
Thompson bottles are large elongated bottles with flat sides, which are used for the growth of micro-organisms and are incubated on their sides. A bottle containing 300 ml of the spore suspension was placed in an upright position before being toppled from 0·75 m.

Experiment 3: a 15 ml spill from a height of 900 mm
A universal bottle containing 15 ml of the standard spore suspension was slowly spilled from a height of 0·9 m to simulate the effect of a spill on a bench running on to the laboratory floor.

Experiment 4: dropping of three 50 ml bottles, each containing 15 ml of culture
Three bottles in a rack were dropped 1 m to the floor.

Experiment 5: aerosol produced from blocked peristaltic pump
A peristaltic pump was primed from a 50 ml reservoir of the spore suspension. The outlet tubing was then blocked and both the pump and samplers were operated. The increase in back pressure caused a connector in the outlet tubing to become detached and the suspension was sprayed at the wall of the room.

Experiment 6: solution forced through syringe filter
A 10 ml syringe was filled with 10 ml of the spore suspension. A partially blocked 0·2 μm filter cassette was attached to the syringe. The samplers were started as the syringe was forcefully pressed; the pressure forced the fil-

ter off the syringe and the suspension was sprayed in all directions.

Experiment 7: dropping heavily loaded fungal plates
Four fungal plates with extensive growth of penicillium and heavy surface growth of spores were dropped onto the laboratory floor.

Experiment 8: centrifuge spill without rotor seal in place
An outdated Sorvall GSA rotor had its 'O' ring seal removed and had 10 ml of a 5×10^9 spore suspension gently pipetted into the rotor chamber. The rotor was accelerated to 4000 rev min^{-1} in a RC5B centrifuge, braked and the centrifuge lid opened while the air samplers were operated.

Experiment 9: unsealed bucket on a swing-out centrifuge rotor
A set of sealed rectangular centrifuge buckets with screw down lids was tested to find out if they generated microbial aerosols. It was found that these buckets were contained when the bucket seal was in place and applied with silicone grease supplied by the manufacturer. However, aerosols were generated when the seal was not in place. In experiment 9a the bucket contained two overfilled Falcon centrifuge tubes containing a 9×10^9 spore ml^{-1} suspension and in experiment 9b a 50 ml spill of the same suspension was 'rolled' around inside the bucket so that some of the fluid would rest on the inside walls of the lid before centrifugation.

Experiment 10: dropping bacterially loaded plates
Four plates contained 3-day-old colonies *of B. atrophaeus*, which were slightly dry, were dropped onto the laboratory floor.

Experiment 11: smashed flask in an orbital shaker
A 1 l flask containing 200 ml of a 9×10^8 spore ml^{-1} suspension was placed on the rack of a Gallenkamp chest-shaking incubator in a totally unsecured position. The shaker was then operated at 100 rev min^{-1} and the samplers were switched on. The flask smashed almost immediately and the shaker lid was opened 30 s after the broken glass pieces has settled.

Experiment 12: spill of 18 ml with different spore concentrations
A spill of 18 ml of *B. atrophaeus* spore suspension was created by spilling the contents of a universal container from about 0·8 m. This is a similar scenario to experiment 4, which consisted of a 15 ml spill. The tests were carried out with a $9{\cdot}1 \times 10^5$ (a), $9{\cdot}1 \times 10^6$ (b), $9{\cdot}1 \times 10^7$ (c) and 9·1 x 10^8 (d) spore ml^{-1} suspension.

Experiment 13: centrifuge accident with different spore concentrations
A GSA rotor was overfilled with 10 ml of spore suspension as in experiment 8. The centrifuge was accelerated up to 4700 rev min^{-1} within a minute, braked and the lid opened while the samplers were operated for a 10 min period. This experiment was carried out with the four different suspensions used in experiment 12.

Calculation of SF

The results of many of the laboratory experiments are summarized in the table below and allowed the calculation of the SF. The SF used in this paper is defined as

$$SF = \frac{AC(CFU\ m^{-3})}{SC(CFU\ ml^{-1})}. \quad (1)$$

This formula is adapted from Dimmick (1973) whose original SF has a time component (i.e. it used a per minute aerosol output). However, many laboratory accidents are completely instantaneous making the use of a time factor problematic. The use of the spray factor in this paper allows a quick determination of the AC to be carried out.

Results

The accidents that generated the highest levels of microbial aerosol were those generated by the dropping of fungal agar plates, the centrifuge rotor accident, the large bottle drop and the syringe filter blockage (Table 1). All these accidents were also associated with a low particle aerosol. The Casella results showed that these small particle aerosols remained at a fairly constant concentration over the sampling period. Settle plates and fluorescein contamination showed that in the first two experiments contamination spread over a 2 m distance.

The results in Table 2 and Figs 1 and 2 shows that there is a reasonably direct relationship between suspension and AC. The relationship between the Casella concentration and the SC is not as direct in experiment 12. This is because the Cyclone measures the number of airborne spores, which appears to be directly proportional with the SC. The Casella measures the number of airborne particles containing micro-organisms. As the concentration of spores in the suspension increases the average number of micro-organisms per particle increases, and so affects the relationship between the Casella AC and spore suspension. The relationship is more direct in experiment 13 as the aerosol produced is of a lower particle size and therefore the aerosol particles will be mainly monodispersed.

Table 1 Results of experiments 1–11

	Accident	Casella (CFU m^{-3})	Andersen (CFU m^{-3})	Cyclone (CFU m^{-3})
1	Dropping a flask	173	643	$1{\cdot}03 \times 10^3$
2	Dropping a Thompson bottle	$2{\cdot}48 \times 10^3$	$3{\cdot}48 \times 10^3$ *	$1{\cdot}37 \times 10^4$
3	15 ml spill	387	493†	$2{\cdot}07 \times 10^3$
4	Dropping three bottles	588	$1{\cdot}06 \times 10^3$ *	$3{\cdot}98 \times 10^3$
5	Peristaltic pump	634	886	$5{\cdot}18 \times 10^3$
6	Syringe filter	$3{\cdot}70 \times 10^3$	$3{\cdot}43 \times 10^3$†	$1{\cdot}77 \times 10^4$
7	Fungal plate	$>3{\cdot}3 \times 10^3$	$1{\cdot}34 \times 10^5$ *	$>1{\cdot}56 \times 10^5$
8	Centrifuge spill	$>3{\cdot}3 \times 10^3$	$1{\cdot}71 \times 10^4$†	$2{\cdot}30 \times 10^4$
9a	Centrifuge bucket	150	64	142
9b	Centrifuge bucket	$3{\cdot}00 \times 10^3$	$1{\cdot}10 \times 10^3$ *	$1{\cdot}50 \times 10^3$
10	Bacterial plate	26·7	3·6	8·2
11	Orbital shaker	$1{\cdot}15 \times 10^3$	818*	871

*Over 50% of particles less than 2·2 μm.
†Over 90% less than 2·2 μm.
‡50% less than 1·5 μm.

Table 2 Results of experiments 12 and 13

		(CFU m^{-3})			
Experiment	Sampler	Susp. A ($9{\cdot}1 \times 10^5$ ml^{-1})	Susp. B ($9{\cdot}1 \times 10^6$ ml^{-1})	Susp. C ($9{\cdot}1 \times 10^7$ ml^{-1})	Susp. D ($9{\cdot}1 \times 10^8$ ml^{-1})
12	Andersen	nd	3·57	nd	1071·4
	Cyclone	nd	20·1	211·5	896·1
	Casella	6·7	56·7	296·7	1083·3
13	Andersen	17·9	53·6	nd	3442·9*
	Cyclone	14·2	196·9	388·2	3972·2
	Casella	16·7	153·3	nd	3160·0

nd, below detection limit (7 CFU m^{-3} for the Andersen sampler and 1 CFU m^{-3} for the Cyclone sampler), *82% <1·5 μm.

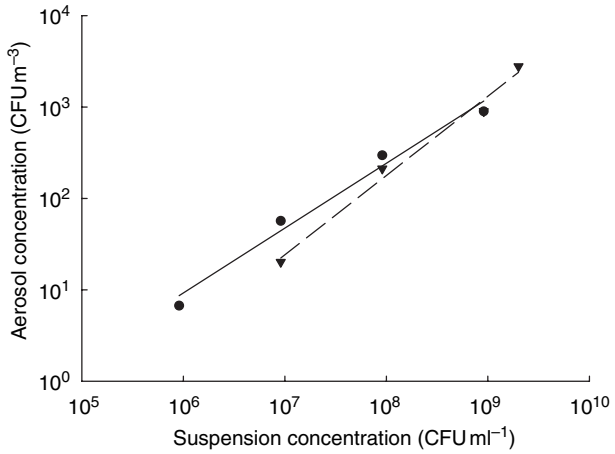

Figure 1 Aerosol formed during the spilling of spore suspensions (●, Casella: $y = x^{0{\cdot}71}/1950$, $r^2 = 0{\cdot}980$; ▼, Cyclone: $Y = x^{0{\cdot}87}/49000$, $r^2 = 0{\cdot}986$).

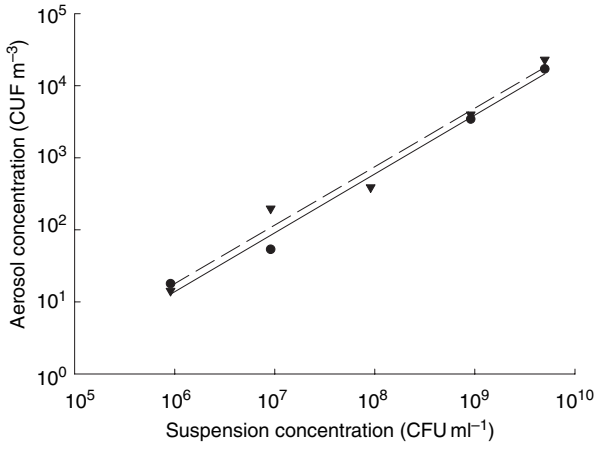

Figure 2 Aerosol generated from centrifuge accident (●, Andersen: $y = x^{0{\cdot}82}/5828$, $r^2 = 0{\cdot}989$; ▼, Cyclone: $y = x^{0{\cdot}81}/4169$, $r^2 = 0{\cdot}975$).

Spray factor

The SFs obtained using a selection of the accident data were calculated as shown in the methodology and are shown in Table 3.

When a SF is calculated from Kenny and Sabel's (1968) data for the dropping of flask containing 200 ml of bacterial suspension (7·99) the value is similar to that found in this study from the Thompson bottle drop experiment (6·85).

Table 3 Spray factors obtained from accidents

Type of accident	SF ($\times 10^6$) ml m^{-3}
Smashed flask (50 ml)	0·52
Smashed Thompson bottle (300 ml)	6·85
Spill of 15 ml	1·04
Three bottle drop	1·99
Blocked peristaltic pump	2·59
Blocked syringe filter	8·85
Centrifuge rotor spill	4·60
Centrifuge bucket spill	0·17
Shaking Incubator	1·28

SF, Spray factor.

The SF is intended for use in the prediction of aerosol production due to an accidental release of microbial aerosol in the laboratory. This should give an indication of the level of containment required for different laboratory procedures or exposure in the case of an accident. By reversing eqn (1) the AC can theoretically be calculated from the SF and SC as follows:

$$AC = (SF \times SC). \quad (2)$$

However, if this equation is to be applied, the relationship between the AC and the SC needs to hold over a range of concentrations, and so the SF value needs to be stable. When SFs are calculated from the Cyclone results from experiment 12, the range of SFs were found to be $1{\cdot}09–2{\cdot}21 \times 10^6$.

The relationship between the AC and SC given by linear regression for the Cyclone experiment 12 (Correlation coefficient $r^2 = 0{\cdot}986$) is

$$AC = (SC)^{0.89}/(4{\cdot}9 \times 10^4). \quad (3)$$

Therefore as a rough estimate of the AC for this accident scenario, the SC can be multiplied by 3×10^{-6} [the 'SF' (m^3 ml^{-1})] to give the AC in the SC range between 10^6 and 10^{10} (SF = $1{\cdot}98–4{\cdot}46 \times 10^{-6}$ taken from the linear regression formula).

The same analysis was carried out for the results of experiment 13. The range of SFs for the Cyclone results was $4{\cdot}4–15{\cdot}6 \times 10^6$. The relationship between the AC and SC given by linear regression for the Cyclone (Correlation coefficient $r^2 = 0{\cdot}975$) is

$$AC = (SC)^{0.81}/(4{\cdot}3 \times 10^3). \quad (4)$$

For a rough estimate of the AC for this accident scenario, the SC can be multiplied by 9×10^{-6} [the 'SF' (m^3 ml^{-1})] to give the AC in the SC range between 10^6 and 10^{10} CFU ml^{-1} (SF = $2{\cdot}93–16{\cdot}8 \times 10^{-6}$ for the Cyclone, taken from the linear regression formula).

Discussion

Laboratory accidents are a rare occurrence in well-run microbiology laboratories. Nevertheless, these events will require prompt and practical evidence-based responses to be taken. The use of the SF in simple risk assessment models can be used to assess the seriousness of accidents with aerosol transmitted pathogens, the correct medical response and the time required before the laboratory can be entered safely. The use of the assessment can best be explained by use of an example.

Accident

A flask is dropped containing 50 ml of a 5×10^9 ml^{-1} suspension of Agent X. A technician who dropped the flask remains in the laboratory for 10 min to clear the mess while a colleague leaves immediately (30 s) to raise the alarm and get assistance.

Assumptions

The exposed personnel breathe at a rate of 15 l min^{-1}. The aerosol does not significantly deposit during the time period and all aerosolized micro-organisms are deposited in the lung. The laboratory has an air change rate of 12 air changes per hour.

Calculations

SF for this accident is $5{\cdot}3 \times 10^{-7}$ and

$$\begin{aligned} AC(CFUm^{-3}) &= SC \times SF \\ &= 5 \times 10^9 \times 5{\cdot}3 \times 10^{-7} \\ &= 2650\ m^{-3} \\ &= 2{\cdot}65\ l^{-1}. \end{aligned}$$

$$\text{Dose}\,(30\,\text{s}) = 7{\cdot}5 \times 2{\cdot}65 = 19{\cdot}9\ \text{CFU}$$

$$\text{Dose}\,(10\,\text{min}) = 150 \times 2{\cdot}65 = 398\ \text{CFU}$$

It can be seen that one of the technicians received a dose of 398 CFU and the other received a dose of 19·9 CFU. Now depending on the infectious (and lethal) dose of the agent concerned, decisions can be made on the requirement for antibiotic and other treatments. This calculation can be carried out for any of the procedures previously mentioned and any concentration of agent. Data on other accidents can be taken from Table 3 or from Kenny and Sabel or Dimmick.

The figures obtained above can also be used to work out how long it will take to disperse the aerosol within the laboratory. The original 2650 m^{-3} concentration will

be reduced by sedimentation and aerosol decay but the main factor will be the laboratory air change rate.

In the laboratory in which the accident occurred the air change rate was 12 h^{-1}. Using figures taken from ACDP (2002), in 12 min the AC should be 265 m^{-3}, in 23 min it will be 26·5 and in 35 min it will be 2·65 m^{-3}. Again depending on the agent used, decisions can be made as to when the laboratory can be safely re-entered in order to deal with the spill (wearing appropriate PPE including respiratory protection with acceptable protection factor) and decontaminate the laboratory probably using appropriate procedures such as gaseous disinfection. Similar calculations of aerosol decay rate can be carried out for all procedures using the SF data in Table 3.

A study has been carried out to measure the microbial aerosol produced by a range of microbiological accidents. These data have been analysed using a form of 'SF' originally proposed by Dimmick. The relationship between SF and SC has been investigated for two experiments and has been found to be a useful simplification of the aerosolization process during accidents. A scheme for using the SF in microbiological risk assessment has also been demonstrated.

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