Pseudomonas aeruginosa infections in Norway

An outbreak of *Pseudomonas aeruginosa* infection caused by contaminated Dent-O-Sept mouth swabs and invasive *Pseudomonas aeruginosa* infections in Norway 1992-2002

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Summary

Infections occurring as a result of stay in hospitals are costly for society and cause much suffering in the patients. A sizeable proportion of these hospital acquired infections are preventable. The hospital patient population is changing with more patients being susceptible to opportunistic infections. The *Pseudomonas* species is ranked among the top ten causes of bacteraemias in hospitals. Medical devices have often been reported to cause outbreaks in hospitals.

The overall aims of this thesis were to investigate a large outbreak of *Pseudomonas* infections and gain knowledge from it, to explore theories for causality and responsibility, and to describe the epidemiology and investigate risk factors for contracting invasive *Pseudomonas aeruginosa* infection.

The research originates from a large outbreak of *P. aeruginosa* infection discovered in 2002 which was caused by a contaminated medical device. From it we explore four areas: 1. the outbreak investigation; 2. the contamination of the medical device involved; 3. theories for causality of the outbreak; and 4. the epidemiology of invasive *Pseudomonas aeruginosa* infection.

Although the research in time moved from the specific *P. aeruginosa* outbreak to explore more general issues the thesis is organised the other way moving from the general to the specific as this gives a better introduction to the subject and is more pedagogical.

Paper I describes an outbreak investigation of *P. aeruginosa* infections, in particular how a nationwide, multicentre investigation was organised and conducted. The team-work and combination of epidemiological and microbiological methods were essential in finding the cause and stopping the outbreak. A total of 231 patients from 24 hospitals were identified with the outbreak strain of *Pseudomonas aeruginosa*; 71 of them died while hospitalised. Genotypically identical strains of the bacterium were isolated from patients, several batches of the Dent-O-Sept swab and from the production plant. We conclude that susceptible patient groups should use only documented quality-controlled, high-level disinfected products and items in the oropharynx.

Paper II describes the investigation of the swabs, the moisturising liquid and the production facility. A total of 76 swabs from 12 different batches produced over a period of 30 weeks were contaminated with the outbreak strain of *Pseudomonas aeruginosa*. Many swabs were

also contaminated with other microbes. More than 250 of 1565 examined swabs were contaminated with one or more microbial species. A system audit revealed serious breaches of production regulations. Biofilm formation in the wet part of the production is proposed as the most plausible explanation for the continuous contamination of the swabs. The legal requirements for microbiological purity of medical devices in Class 1 are not optimal.

Paper III explores the theories for causality of the outbreak of *Pseudomonas aeruginosa* infections. Applying various theories for causality and responsibility from different fields like science, philosophy and law on the actors and acts involved in the outbreak helped elucidating their roles and responsibilities, especially legal theories and counterfactual reasoning. We conclude that many factors contributed to causing the outbreak, but that contamination of a medical device in the production facility was the major necessary condition. The reuse of the medical device in hospitals contributed primarily to the size of the outbreak. In addition there were many errors in the chain from the production of the swabs, through purchasing and storage systems in the health care institutions to the use of the swabs and reporting of defective devices. The unintended error by its producer – and to a minor extent by the hospital practice – was mainly due to non-application of relevant knowledge and skills, and appears to constitute professional negligence. Due to factors outside the discourse of causality, no one was criminally charged for the outbreak.

Paper IV investigates the epidemiology of invasive *Pseudomonas aeruginosa* infection in Norway. Although *P. aeruginosa* usually do not cause infection in healthy persons, it frequently does in patients with certain underlying diseases, and in patients with disrupted barriers, especially in the ICU. Invasive *P. aeruginosa* infection is a rare disease with an incidence rate of 3.16 per 100 000 person-years at risk or 0.20 per 1 000 hospital stays, but very serious for those contracting it with a 30 day case fatality rate of 33%. Patients with malignant neoplasms of lymphoid and haematopoietic tissue and other diseases of blood and blood-forming organs have the highest risk of infection. Prudent antibiotic use is one possible explanation for much lower rates of infection in Norway compared with all other published studies.

Acknowledgements

This thesis is based on work carried out in 2002-2008 at the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health. The research all originates from a large outbreak of *Pseudomonas aeruginosa* infection discovered in 2002. I would like to thank the Institute – my working place since 1994 – for providing good working conditions, assisting me when necessary and encouraging me to finish.

First and foremost I would like to thank my supervisor and boss, Preben Aavitsland. Without him there would have been no thesis and scarcely any published articles. He guided the outbreak investigation, initiated the research protocol following up the outbreak investigation, and has encouraged and supported me throughout the process. Despite his hectic daily schedule he has always given me quick and thorough responses to my drafts and my many questions. I would also like to thank my contact supervisor at the University of Oslo, Per Nafstad, who came in later in the process. With fresh eyes and long experience he has quality assured the thesis and helped improving it greatly.

Outbreak investigation is team work. In this outbreak investigation more than 50 hospitals were involved, and many persons in each institution: local outbreak investigation teams, people from microbiological laboratories, infection control personnel, clinicians and administrators. We have collaborated closely with the Ministry of Health and Care, the Norwegian Board of Health Supervision, the Directorate of Health and other institutions, and in addition we have received input from patients and their next of kin and from the media. You have all been indispensable parts of this work. Many of you have been named as co-authors or thanked in the acknowledgements in the individual papers. I thank you all again.

I would especially like to thank Trond Jacobsen at St Olavs Hospital in Trondheim who was just as impatient and enthusiastic as I was. We worked well together and our collaboration exemplifies how genotechnological microbiology and epidemiology supplement each other synergistically in outbreak investigations. Bjørn Hofmann aroused my curiosity and renewed interest for philosophy of science and guided me through a jungle of concepts and terms in a new discipline. The crossing of outbreak investigation with causality and tort law has been fascinating. Thank you. Infection control nurses are the core of hospital infection control and prevention. Sissel Berg-Larsen at Feiringklinikken brought the attention to the mouth swab and Berit Bue at Stavanger University Hospital exemplifies the hard-working, helpful and inspiring infection control nurse. Thank you, both.

Of the many people involved in the outbreak investigation at the Norwegian Institute of Public Health I would especially like to thank my close colleague and friend Hanne-Merete Eriksen. She worked shoulder to shoulder with me during the outbreak investigation and has always been supportive, encouraging and a good critic. Of my colleagues who were not involved in my thesis I am especially indebted to my former boss, Professor Arve Lystad who introduced me to the world of epidemiology, taught me all the links in the chain of infection and raised me in a tradition of infection control and prevention which is broad-minded and interdisciplinary, and based on sound and sober judgements.

My parents, Bjørg and Halfdan, have always stimulated me to be curios and have encouraged me to ask questions and to try to find the answers in encyclopaedias, maps and other books. In the family the standard answer to any question has been the same for generations: Go, look it up!

Finally, I would like to thank the love of my life, my husband and best friend, Bjørn, who for periods has had to put up with not seeing me a lot, and who has caringly coaxed me to finish the thesis.

List of papers

This thesis is based on the following published papers. They will be cited by their roman numbers:

I Iversen BG, Jacobsen T, Eriksen HM, Bukholm G, Melby KK, Nygard K, Aavitsland P: **An outbreak of *Pseudomonas aeruginosa* infection caused by contaminated mouth swabs.** *Clin Infect Dis* 2007; **44:** 794-801.

II Iversen BG, Eriksen HM, Bo G, Hagestad K, Jacobsen T, Engeset E, Lassen J, Aavitsland P: ***Pseudomonas aeruginosa* contamination of mouth swabs during production causing a major outbreak.** *Ann Clin Microbiol Antimicrob* 2007; **6:** 3.

III Iversen BG, Hofmann BM, Aavitsland P. **Questions on causality and responsibility arising from an outbreak of *Pseudomonas aeruginosa* infections in Norway.** *Emerg Themes Epidemiol* 2008, **5:**22.

IV Iversen BG, Brantsæter AB, Aavitsland P. **Nationwide study of invasive *Pseudomonas aeruginosa* infection in Norway: Importance of underlying disease.** *J Infect* 2008; 57: 139-46.

List of abbreviations

AFLP – Amplified fragment length polymorphism
CE – Communauté Européenne
CFR – Case fatality rate
CI – Confidence interval
CSF – Cerebrospinal fluid
DNA – Deoxyribonucleic acid
EU – European Union
HAI – Hospital acquired infection
HELICS – Hospitals in Europe Link for Infection Control through Surveillance
HUS – Haemolytic uremic syndrome
ICD-10 – International Classification of Diseases, 10th Revision
ICU – Intensive care units
IPSE – Improving Patient Safety in Europe
MRSA – Methicillin resistant *Staphylococcus aureus*
MSIS – The Norwegian Notification System for Communicable Diseases
NIPH – The Norwegian Institute of Public Health
NNIS –National Nosocomial Infections Surveillance System (CDC, USA)
NOIS – The national surveillance system for hospital infections
OR – Odds ratio
PFGE – Pulsed-field gel electrophoresis
PIAH – The national point prevalence surveillance system for hospital infections and antibiotic use
PYAR – Person-years at risk
SPC – Statistical process control
USA – The United States of America
UTI – Urinary tract infection
VAP – Ventilator associated pneumonia

1. General introduction

1.1. Microbes and hosts

Infections have always been a serious threat to mankind, causing disease and death. Through much of historic times man has fought a battle against infectious diseases and its causes. Religious and traditional rules were created to prevent, treat and control the diseases and epidemics (1). Some were based on experience like cleanliness and hygienic measures; others were mere superstition (2), like phlebotomy to cure infections. When microbes were discovered as causes of infectious diseases, the search for cures were intensified and with the advent of antimicrobial therapy some voices in the medical community heralded the end of the era of infectious diseases. The emergence of antibiotic resistance and the increase in the number of debilitated persons with increased susceptibility for infections have curbed this optimism.

Microbes play a natural part in the interaction with humans. They colonise the skin, the outer part of certain orifices and are important for food digestion in the colon and distal ileum. Prudent use of antimicrobials and disinfectants are believed to be important to minimise the disturbance of equilibrium between the different microbes and between microbes and hosts (3).

Microbes are categorised in many ways, one is by pathogenicity. Some bacteria, like *Yersinia pestis* or *Vibrio cholera* usually cause disease in the human host and are called pathogenic whereas others like coagulase negative staphylococci under normal circumstances rarely cause disease and are called apathogenic. In between these two groups there is a continuum of pathogenicity of microbes that can cause disease under certain circumstances when one or more defence mechanisms fail, like disruption of barriers introducing bacteria into sterile body sites or the weakening of immunity during cancer treatment. This group of microbes is called opportunistic. *Pseudomonas aeruginosa* is an opportunistic bacterium (4-7). In addition, within each species of microbes there may be great variability. Among *E. coli* for example one may find apathogenic, opportunistic and pathogenic strains depending on presence of virulence factors.

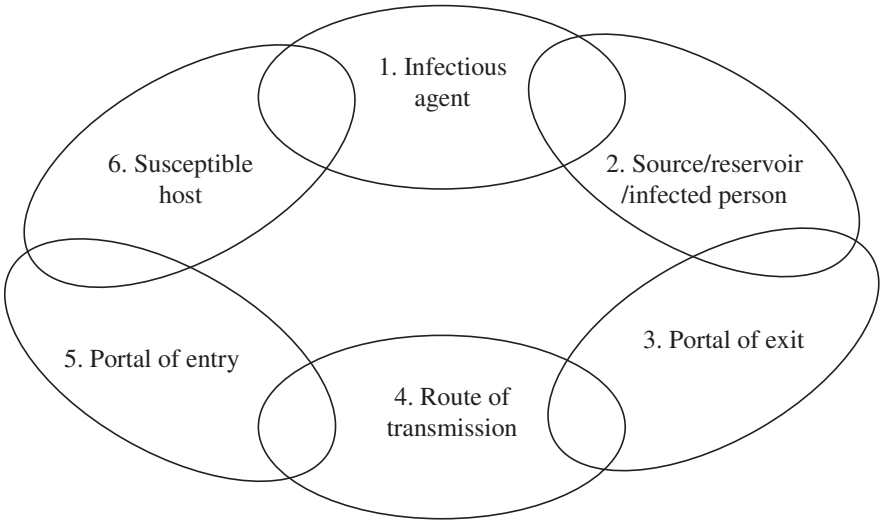
1.2. Communicable diseases

Epidemiology of communicable diseases differs from epidemiology of chronic diseases mainly in that the patient, the case, can become infectious and thus be the source of disease

for new cases. The epidemic potential for an infectious disease is mathematically described through the basic reproductive rate, called R_0. It is dependent on the risk of transmission per contact (β), the average number of contacts (κ) and the duration of infectivity for an infected person (D), mathematically described as: $R_0 = \beta \times \kappa \times D$ (8). In order for an infectious disease to expand and spread each infected person on average has to infect more than one new person, i.e. $R_0 > 1$. Some non-infectious diseases can be said to spread in populations through altered behaviour patterns. Diet and drinking trends may spread and cause epidemics of obesity and alcoholism. In addition, some infections are caused by microorganisms already present on the patient, the so called endogenic infections. However, in epidemiology communicable diseases and infectious diseases are usually seen as synonyms (9).

A conceptual model for communicable disease spread is the chain of infection (Figure 1) (1, 10). This model consists of six links which all have to be present for an infection to spread. If one link is broken, propagation ceases. Consequently the model is used in infection control and prevention to study where to intervene to prevent the spread of infections and to stop outbreaks.

Figure 1. *The chain of infection*



To break the chain if infection in the hospital setting one needs to analyse each link.

1. **Infectious agent:** All pathogenic microbes, most opportunistic and even some microbes generally considered to be apathogenic, may cause infections due to the susceptibility of many patients. A general reduction of all potentially pathogenic microbes would reduce the risk of infection.

2. **Source/reservoir/infected person:** There are three groups of reservoirs in hospitals, a) patients and personnel, b) the hospital environment, and c) medical devices, pharmaceuticals, food and water.

   2 a) patients and personnel: Infectious people can be treated and made non-infectious.
   2 b) the hospital environment: Contaminated surfaces can be cleaned and disinfected. Certain areas are more important to control like door knobs, hand rails and switches where many people touch frequently whereas ceilings and floors are less important from an infection control perspective. For moisture-prone bacteria wet areas like sinks, faucets, flasks, other containers and tubes need to be disinfected and controlled regularly. One needs to analyse where bacteria can multiply into numbers posing as a risk for patients and implement preventive measures. In specialised rooms like operating theatres even the air needs to be monitored for bacterial contamination.

   2c) medical devices, pharmaceuticals, food and water: Depending on type of use medical devices need to be clean, disinfected or sterilised and there needs to be monitoring systems in place to verify the microbial status of the products. In many hospital outbreaks where medical devices have been involved, deficiencies in the cleaning, disinfecting, and sterilising process of reusable equipment and in the control systems of these processes have been demonstrated. Food and drinking water is not sterile and may contain microbes that can cause infections in susceptible patient groups. Potentially harmful foods have to be removed for these selected groups.

3. **Portal of exit:** The main portals of exit of microbes from a patient are the mouth and airways, urethra, anus, damaged skin (where blood and pus can emerge), and intact skin. To prevent microbe containing body fluids, solid body parts or excretions from being transmitted, the mouth and nose can be covered with a surgical mask, cuts, bruises and abscesses covered with bandages, diapers used and intact skin can be covered to prevent the spread of exfoliations.

4. **Route of transmission:** There are mainly three transmission routes: a) direct and indirect contact transmission, b) droplet transmission and c) airborne transmission. Direct and indirect contact transmission is by far the most important mode of transmission and the hands of health care workers are in most hospital hygiene publications considered to be the most important vehicle for transport of infectious agents in the hospital. Isolation and quarantine or other ways of physical distancing to prevent the infectious from coming into contact with the non-infected are other methods for breaking the route of transmission. The measures instituted depend mainly on the mode of transmission for the particular infectious agent and on the severity of disease that it may cause.

5. **Portal of entry:** This is often the reverse of link 3 where all natural and artificial orifices and the intact skin and other outer surfaces can be a portal. By blocking the portal of entry using a surgical mask or respirator covering the mouth and nose, covering intact or cut skin, eyes etc. and depending on the mode of transmission, one can block the infectious agent from entering. In the hospital setting there are many more entry portals due to artificial openings stemming from surgery, catheters and other medical devices which have broken many of the natural defence barriers. That is why it is so important only to use quality-controlled equipment and to perform all critical procedures with the highest hygienic standards.

6. **Susceptible host:** In the hospital, many patients are especially susceptible for contracting infections. The main means of reducing their susceptibility is through immunisations where a vaccine is available and through improving their general conditions in order to better fight off any intruding microbe. In some instances like for certain surgical procedures short course antibiotic prophylaxis is given to reduce the risk of infection.

1.3. Hospital acquired infections

The risk of contracting an infection is much larger inside a hospital than outside. There are several reasons for this.

Firstly, people in hospitals are already ill. They are often bedridden and pacified making them more susceptible to infections of the skin and airways. Many patients have a reduced capacity to battle infections due to a weakened immune system. Trauma, surgical procedures and medical devices like catheters have disrupted natural defence barriers making it easier for microbes to gain access and cause infection.

Secondly, in hospitals there are many patients with infectious diseases and these may be infectious sources to other patients who concequently more easily may contract new

microbes. And the microbes are easily transferred from patient to patient through direct or indirect contact via health care workers, fixture or medical devices, or for some microbes via droplets or through the air.

Thirdly, the use of antimicrobials per population is much larger inside hospitals than in the general community (although the total consumption is much larger outside) (3). This antibiotic pressure drives a selection for more resistant strains of bacteria causing the bacterial flora in hospitals to be quite different from the one outside. All this makes the risk of contracting an infection much higher in hospitals than outside. It also makes it more difficult to treat due to antibiotic resistance and the susceptibility of the patients.

A hospital acquired infection (HAI) is usually defined as an infection that follows a stay in hospital, but that was not present or incubating at the time of admission to the hospital (11). For bacterial infections a standard incubation period of 48 hours is usually used meaning that infections occurring at least 48 hours after admission to the hospital are considered to be hospital acquired. Hospital acquired is synonymous to nosocomial which is the Greek word pertaining hospital. A wider term often used is health care associated infections which includes all infections that can be associated with hospitals, nursing homes or the outpatient setting in primary or specialist health care. Although an infection may be hospital acquired, this does not necessarily mean the patient acquired the microbe inside the hospital. A large proportion of HAIs results from microbes belonging to the patient's normal bacterial flora. Catheter-related urinary tract infections (UTIs) may be caused by *E. coli* from the patient's intestinal flora and a surgical site infection from the patient's normal skin flora.

1.4. Surveillance of infectious diseases

Public health surveillance is defined as the ongoing systematic collection, analysis, and interpretation of outcome-specific data essential to the planning, implementation and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know (12). The final link in the surveillance chain is the application of these data to prevention and control.

Surveillance of infectious diseases can be categorised in several ways (see Table 1 for categorisation 1a – 6b). In Norway the Norwegian Notification System for Communicable Diseases (in Norwegian abbreviated MSIS) consists of several subsystems. The system for the major part of notifiable diseases in MSIS is a cohort (category 1b) for the whole country (2b) receiving clinical and microbiological information (3a and b) on an individual basis (4b), is mostly passive (5b) but with some active follow-up of missing data (5a) and mainly manual

(6a) but with an aim to increase the electronic transfer, especially from medical microbiological laboratories (6b). Most developed countries have somewhat similar systems. In the period 1975-1991 detection of most bacteria from blood or cerebrospinal fluid (CSF) were individually reportable from medical microbiological laboratories to MSIS. *Pseudomonas aeruginosa* was among the bacteria to be notified but MSIS did not publish tables on a bacterial genus level, only based on the site of infection where the microbes were detected from.

For HAI, Norway has had a tradition since 1979 of repeated national point prevalence studies (in Norwegian abbreviated PIAH). On a given day and time all patients occupying a bed in somatic wards in hospitals are counted (denominator) as are those with one of the four most frequent HAIs: UTIs, lower respiratory tract infections, surgical site infections and blood stream infections (numerator). The infections are not specified by causative microbial agent, so the number of *P. aeruginosa* infections cannot be specified. Numerators and denominators are summed up by ward and hospital and aggregated data sent to the Norwegian Institute of Public Health (NIPH). From 2002 all hospitals and nursing homes have been asked to submit data to NIPH twice-yearly. From 2004 they have had the opportunity to enter the date electronically via Internet. (According to the list this surveillance system is: 1a, 2b, 3a, 4a, 5a, 6b.)

In 2005 NIPH implemented a national surveillance system for hospital infections (in Norwegian abbreviated NOIS). In this system, surgical site infections following certain surgical procedures will be subject to surveillance during a given 3-month period each year. The system is in accordance with the European hospital surveillance network (Hospitals in Europe Link for Infection Control through Surveillance (HELICS) / Improving Patient Safety in Europe (IPSE)) which in turn is based on the National Nosocomial Infections Surveillance System (NNIS) from The United States of America (USA). Participation in NOIS is mandatory for all hospitals. (According to the list this surveillance system is: 1b, 2b, 3a, 4b, 5a, 6b.) In the NOIS system the causative microbial agent is not specified.

Table 1. *Categorisation of systems for surveillance of infectious diseases, with three Norwegian systems as examples.*

Category	Description	MSIS	PIAH	NOIS
1. By study type	a. Repeated cross-sectional study (prevalence study or survey)		X	
	b. Cohort study (incidence study)	X		X
2. By selection of reporters	a. Sentinel reporting			
	b. Regional or total coverage	X	X	X
3. By source of information	a. Clinical information	X	X	X
	b. Microbiological detection and information	X		
	c. Serological marker of infection			
	d. Surrogate markers of infection (Hospital economical reimbursements, mortality statistics, work force absenteeism etc.)			
4. By type of data	a. Aggregated data		X	
	b. Individual data	X		X
5. By type of data collection	a. Active surveillance	(X)	X	X
	b. Passive surveillance	X		
6. By mode of data transfer	a. Manual, paper-based system	X		
	b. Electronic, automated system	(X)	X	X

In some Norwegian hospitals with on-site microbiological laboratories infection control personnel get regular reports of all detections of certain indicator bacteria. For some they even can get alerted after single findings. There is no national standard for which bacteria to cover or how to report. By selecting *P. aeruginosa* as an indicator bacterium the hospitals can measure the occurrence of detection of this microbe.

Nationally and internationally there is increased emphasis on patient safety and quality assurance systems. Hospitals are increasingly required to measure and report on risks for hazards occurring in hospitals and to set up plans for minimising these risks. Surveillance systems form a basis for these efforts and increasing resources are spent on developing, improving and implementing systems for surveillance of infectious diseases in hospitals.

In Norway, NOIS is currently covering surveillance of surgical site infections following a few surgical procedures. In addition to include more surgical procedures new modules are being developed in other high risk areas for acquiring infections in hospitals as in intensive care units (ICU). Modules are also being developed to more detailed measure the consumption of

antimicrobial drugs in hospitals in order to detect overuse and misuse, and consequently to be able to suggest alterations and improvements. Development of better systems for on-site microbiological laboratory surveillance will also be a priority in the coming years. Improved and better accessible databases will ease this development. In addition surveillance systems measuring the incidence of infections and of antibiotic use in nursing homes are being piloted.

To date, only results from national prevalence studies have been published in scientific papers (13-21). Results from NOIS have only been published on NIPH's Internet pages but several papers will be submitted for publication in the near future.

1.5. Outbreak investigations

Outbreak investigations in hospitals and elsewhere follow the same general structure and regardless of causative agent. An outbreak can be defined in several ways, the simplest being "an event involving more cases than usual". A more elaborate definition is: An event involving more cases than expected of a certain disease within a given time and area. Another definition is two or more cases of the same disease and with a presumed common source (22).

Outbreaks in hospitals are either common source outbreaks or caused by local spread via patients, personnel, equipment or environment, or to a lesser degree, through air. One can also find mixed-pattern outbreaks where a common source introduces the microbe which then in turn spreads locally.

1. Common source
    a. Medical devices, medicine or other remedy produced locally or procured
    b. Food, drinks or water produced locally or procured
    c. A fixed, maintained source in the hospital (contaminated sink, faucet, ice machine, flower pots and vases, ventilator system or other)
    d. An environmental condition that enables microbiological growth, e.g. moist walls or ceilings where fungus or bacteria can grow
    e. A chronic carrier among the personnel (e.g. a chronic MRSA (Methicillin resistant *Staphylococcus aureus*) nasal carrier or a surgeon with a chronic blood-borne viral infection transmitting to some of her patients during surgery)
2. Local spread
    a. Contact transmission from person to person (patients and personnel), either directly or indirectly via the environment

b. Droplet transmission from person to person being in close proximity to each other (usually less than 1 meter)
c. Airborne transmission where microbes can travel longer distances through air (i.e. more than 1 meter)

Outbreaks caused by *P. aeruginosa* can either be common source or through local spread. As the bacterium has affinity for water, moist products, moist environment, or moist areas of the human body are usually the reservoir for the bacterium. The spread is usually through contact, but droplet transmission can also occur, especially from droplet generating procedures.

There are two main ways of detecting outbreaks

1. Indicator based surveillance: Ongoing routine surveillance systems detect an increased number or cases under surveillance or unusual patterns in the data.
2. Event based surveillance: Outbreaks are detected through unstructured reporting systems like media, international alerts, outbreak reporting, and unusual events reporting from the health services.

It is crucial to have systems in place to detect outbreaks as early as possible. Better and more elaborate surveillance systems for events, diseases and microbes will improve our ability for early detection. However, the more sensitive a system is the more "noise" is also detected. And no matter how elaborate an indicator based surveillance system is in detecting outbreaks, we always will have to appreciate the unease or sixth sense of health care personnel as a valuable, additional alert system.

In outbreak investigations there is a range of tasks to undertake, preferably in a logical, chronological order, although many of the tasks needs to be performed in parallel or repeated several times.

The following major tasks for outbreak investigations can be listed (12, 22):

- Prepare and plan
    - Have a general, structured plan ready.
    - Know your potential collaborators, their legal position and skills.
    - Maintain your skill through training.
- Detect and verify

    - Have a system in place to receive and assess warnings and notifications about possible outbreaks in order to determine whether further investigations are necessary.
- Alert and inform stakeholders
    - To mitigate the extent and consequences of an outbreak it is important to notify all relevant stakeholders. Norwegian laws and regulations give detailed instructions on whom to inform and when. For example all suspected and verified outbreaks in health care institutions are to be reported immediately to the chief medical officer in the county and to NIPH. (23, 24)
- Make a case definition, identify and verify cases
    - Keep in mind that the case definition can change over time as the knowledge of the outbreak increases. For example one may in the beginning of an outbreak use a syndromic diagnosis to be replaced by an etiologic diagnosis later.
- Describe the outbreak in terms of time, place and person
    - Use basic, epidemiological tools. Describe also who are at risk of becoming ill.
- Generate hypotheses
    - Base your hypotheses on all available information at the time
- Test hypotheses
    - Once hypotheses are generated they are to be tested against the information gathered. The main tools are:
        1. Epidemiological studies
        2. Microbiological sampling
        3. Performing environmental investigations and assessments
    - Based on the preliminary findings from the hypothesis testing, decide on whether to plan for a more systematic study.
- Implement control and preventive measures
    - If the outbreak is sufficiently serious it may be necessary to implement measures on limited knowledge
- Communicate findings
    - Keep detailed minutes of all actions from the very start
    - Prepare a written report

- Keep the mass media informed. Coordinate the main messages with the other stakeholders. In the past years increasing time is spent on keeping the mass media informed. When the outbreak involves children, deaths, differences of opinion among investigators or stakeholders, errors made or political issues, the media attention can be particularly intense.

In outbreak investigations the lack of time is in conflict with the need to be precise, systematic and deliberate. This urgency is the main difference between outbreak investigations and prospectively planned epidemiological studies. The ideal epidemiological study is prospective, well planned, with unambiguous definitions and clear and profound hypotheses to be tested. This would be ideal for outbreak investigations as well but is not feasible most of the time, and one need to make compromises. For some outbreaks the number of cases is few and the statistical power may be low. For outbreaks with serious outcomes like death and debilitating disease the need for a quick result may force the investigators to compromise on the accuracy of the protocol. As a consequence all results from an outbreak investigation need to be interpreted with caution. When the media pressure is high and the public outcry to come up with an explanation is loud, it is tempting to conclude prematurely and too confidently.

Another contrast with planned epidemiological studies is that the hypotheses, definitions and the protocol may change over time. At the start of an outbreak investigation there is little knowledge so the investigation needs to begin broadly. As knowledge is gained, hypotheses can be more specific, definitions narrower and the protocol more structured. In addition it may be necessary to implement control measures before the investigation is complete which can make it more difficult to come up with clear results. However, whereas the aim of many planned epidemiological studies is to detect small differences between various exposed groups, the main aim of an outbreak investigation is detect the reasons for the outbreak in order to prevent further cases.

1.6. Medical devices

Prior to 1995 unsterile medical devices were poorly regulated in Norway. Products like mouth swabs were only regulated through general regulations on product control. In 1995 the Act on medical devices and its regulation were introduced (24, 25). The purpose of the Act and its regulation is to prevent harmful effects, mishaps and accidents and to ensure that medical

devices is tested and used in a professional and ethically justifiable way (24). When in doubt the Ministry of health and care defines whether a product is to be called medical device.

Through the European Economic Area Agreement Norway abides by much of the legislation within the European Union (EU), including European Council Directive 93/42/EEC concerning medical devices (26). Norwegian jurisdiction on medical devices today is to a large extent, direct translations of EU council directives.

A medical device is defined in the Council Directive as:

> "'medical device' means any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application intended by the manufacturer to be used for human beings for the purpose of:
>
> - diagnosis, prevention, monitoring, treatment or alleviation of disease,
> - diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap,
> - investigation, replacement or modification of the anatomy or of a physiological process,
> - control of conception,
>
> and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means; " (26)

The Directive classifies medical devices into four classes, I, IIa, IIb and III. The classification rules are based on the vulnerability of the human body taking account of the potential risks associated with the technical design and manufacture of the devices. Annex IX of the Directive gives detailed rules for classification of medical devices into these four classes.

In order to mark a medical device with CE (Communauté Européenne) the Council Directive states that the producer must produce a declaration of conformity. The declaration of conformity is the procedure whereby the manufacturer ensures and declares that the products concerned meet the provisions of the Directive which apply to them. The list of provisions is quite detailed.

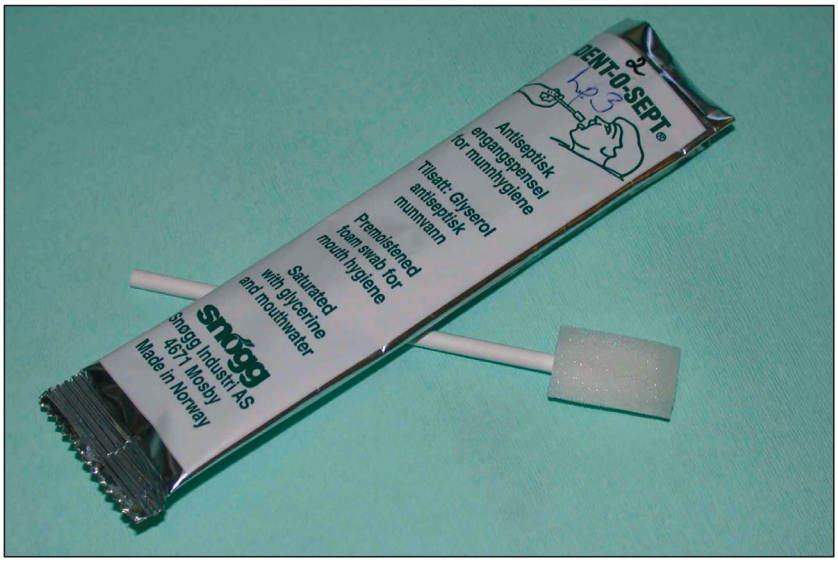
For non-invasive medical devices in Class I, there are no demands for sterility. The devices must, when used, "not compromise the clinical condition or the safety of patients". "The devices and manufacturing processes must be designed in such a way as to eliminate or

reduce as far as possible the risk of infection to the patient, user and third parties." Beyond this, the directive does not specify the microbial quality of the product.

In comparison, pharmaceutical preparations for use in the respiratory tract are according to the European Pharmacopoeia classified in a Category 2 where – in addition to other microbiological requirements – the absence of *Pseudomonas aeruginosa* needs to be documented (27).

In the aftermath of the outbreak of *Pseudomonas aeruginosa* described in this thesis there were public discussions on how to classify the Dent-O-Sept swab (Figure 2). Was it a medical device or a cosmetic product? The Act (28) and Regulation on cosmetics is rather general and defines cosmetics and body care products as: "Any product intended for use on the bodily surface (like skin, head hair and other hair growth, nails, lips and external genitals) or on the teeth and the mucosa of the oral cavity, in order to exclusively or mainly cleanse, scent or change their appearance or influence bodily odours or protect them or maintain them in good condition." (29). The Regulation also states that the producer shall produce and have available a dossier which describes "The physico-chemical and microbiological specifications for the raw materials and the finished product and the purity and microbiological control criteria of the cosmetic product". This is in accordance with EU legislation (30). Although the legislation is rather general the European Commission has several scientific committees to provide more detailed guidelines and opinions. The DG Health and Consumer Protection's Scientific Committee on Consumer Products has issued Notes of guidance for the testing of cosmetic ingredients and their safety evaluation, currently in its 6th revision. These "Notes of Guidance" should not be seen as a checklist, but have been compiled to provide assistance in the complex process of the testing and safety evaluation of cosmetic ingredients. In its chapter 6-4: Guidelines on microbiological quality of the finished cosmetic product it is – among other requirements – specifically stated that *Pseudomonas aeruginosa* must not be detectable in cosmetic products (31). In addition it recommends challenge testing with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* to test the efficacy of the preservation. However, this challenge testing does not take into account the altered microbiological properties of biofilm formation as described in chapter 1.8 of the thesis. It is also worth noting that the requirements for microbiological purity and product documentations are stricter for cosmetics and for comparable pharmaceutical products than for medical devices, class I. The producer of the Dent-O-Sept swab as well as the national health authorities agreed on classifying the Dent-O-Sept swab as a medical device, class I and not a cosmetic product.

***Figure 2**. The Dent-O-Sept swab, a non-invasive medical device in Class I.*



1.6.1. Use of medical devices in hospitals and its control

The industry of medical devices is big business. According to the European commission when it launched the revision to the Medical Device Directives in 2005, there are some 10 000 types of medical devices from some 7 000 business entities in Europe employing upwards of 350 000 Europeans in the EU. The products range from simple bandages and spectacles, through life maintaining implantable devices, equipment to screen and diagnose disease and health conditions, to the most sophisticated diagnostic imaging and minimal invasive surgery equipment (32).

There are no national statistics on the use of medical equipment in Norway. A network organisation supporting suppliers to the health sector (Leverandørforeningen for helsesektoren) has indicated that the total sales of equipment to the hospitals amounts to 8-10 billion NOK annually where approximately half the sum concerns medical devices.

Infection control personnel in hospitals have indicated increasing complexity of highly advanced, technical machinery, including ventilators. Many of them are difficult to clean and disinfect and some are not possible to check whether cleaning and disinfection have been effective. The personell also claim an increasing use of single-use equipment all over the

western world. Many of these devices are expensive and for some years there has been an international debate on reprocessing of expensive single-use devices for reuse.

According to national regulations all hospitals must have written guidelines on the purchase and control of medical devices (33). It is recommended that infection control personnel take part in all purchases of medical devices when relevant for infection control. The purchasing process is not always performed according to guidelines in most hospitals.

The producer, seller, owner, and user of medical devices are all obliged to report errors of medical devices according to previous and current legislation (25). Depending on type of equipment and type of error (product error, electrical error, error concerning radiation, error in use of product) there are different reporting systems. The efficiency and completeness of the reporting systems have been questioned (34). For years there has been ongoing work to improve the error reporting systems from the health care services.

1.6.2. Contamination of medical devices

Medical devices may be contaminated in each of the steps from ingredients and building materials, trough production, packing and transport to storage, use and reuse of the final product (Table 2).

Table 2. *Critical areas in the production and use of medical devices; problems and possible solutions.*

Area	Problem	Solutions
Ingredients, building materials	Any of the components used to make up the product or which are used in the production process can contain microbes. When introduced into the production process the microbes can establish and remain without new introductions.	Risk assessment and microbiological quality control of all identified components used in the production. When the final product is sterilised, this is less relevant.
Production facilities	The facilities can be contaminated from any of the components, from personnel or other environmental factors during the production	Risk assessment and microbiological quality control of all identified critical points. When the final product is not sterilised, microbiological quality control of the final product is warranted.

Area	Problem	Solutions
Packing	Packing material and environmental conditions (e.g. moisture) during the packing process may contaminate the outer surfaces.	Risk assessment and microbiological quality control of the final, packed product.
Transport and storage	Packaging and products can be damaged and contaminated during transport and storage.	Control of product and packing upon arrival and before use. Control of any product declarations and expiry dates.
Use	Devices can be contaminated during use, and whether it was sterile or not at commencement, bacterial growth can occur.	Risk assessment of critical points. Implementation of preventive measures to reduce risk. Follow user guidelines.
Reprocessing of reusable devices	Errors can occur in any of the steps of cleaning, disinfection and sterilisation of reusable devices. Many devices can be difficult to reprocess due to ruffled surfaces, small lumina, unreachable inner areas etc.	Follow detailed general and specific guidelines. Microbiological quality control after the finished process. Surveillance and tracking systems for the use of the devices.

1.6.3. Outbreaks caused by medical devices

A range of microbes can cause outbreaks in hospitals. When an association with medical devices or environmental reservoirs is described, often the bacteria in question are Gram-negative water-prone bacteria (7, 35-73). *Pseudomonas aeruginosa* is often the causative agent of hospital outbreaks (35-73); other common bacterial sources are *Serratia marcescens* (65, 74-78), *Acinetobacter baumannii* and other *Acinetobacter* spp. (79-83), other species of *Pseudomonas* (84-87), *Stenotrophomonas maltophilia* (87, 88), *Burkholderia cepacia* (89-92), *Klebsialla pneumonia* (93) and enterococci (94).

Medical devices can either introduce the causative bacterium of the outbreak or also maintain the outbreak due to wrongful use of the product or faulty cleaning and disinfection procedures between patients (35-37, 41, 43, 46, 54, 57, 64-67, 72, 78, 86, 87, 89, 95-98). In other

outbreaks personnel and environmental reservoirs are important (38, 45, 51, 59, 60, 62, 99). Cross-colonisation and cross-contamination within hospitals has been documented and can maintain outbreaks for longer periods (47, 52, 56, 59, 61, 63, 71, 74, 81-83, 93, 94, 100-102). Liquid or moist pharmaceuticals can cause or maintain outbreaks (37, 85, 89, 90, 103) as can moist cosmetics and water (35, 42, 67, 70, 84, 92, 99, 103, 104). Even transplanted organs can transmit bacteria like *P. aeruginosa* and cause an outbreak (58).

Many outbreaks are linked to ICUs and ventilator treatment (35, 38, 42, 45, 47, 50, 53, 78-80, 88, 96, 99). *Pseudomonas aeruginosa* is the most common gram-negative bacteria causing ventilator associated pneumonia (VAP) (105). Oropharyngeal colonisation is important for the development of VAP (106) and oral care may prevent pneumonia (107). For many of the outbreaks reported no definite source introducing the bacteria into the hospital has been verified. Nonetheless, the outbreaks have been brought to an end following the introduction or enforcement of strict infection control routines regarding personnel behaviour, standard precautions, cleaning, disinfection and sterilisation of medical devices, usage of only sterile or high-level disinfected moist products and thorough disinfection of all moist environmental surfaces (36-38, 41, 49-51, 53, 54, 59, 61-63, 65, 66, 70, 71, 74, 77-79, 82, 84, 86, 89-93, 95, 96, 98, 99).

1.7. *Pseudomonas aeruginosa*

The name *Pseudomonas* meaning “false single units” was given to this group of bacteria when detected in the late 19th century in water. *Aeruginosa* means “copper rust” and denotes the blue-green pigment seen in laboratory cultures.

1.7.1. Microbiology

Pseudomonas aeruginosa is a gram-negative, non-spore-forming, rod-shaped bacterium with one polar flagellum. It is almost exclusively aerobic, have minimal nutritional requirements and can utilise carbon from a variety of sources. In the laboratory it is easily identifiable (4-6).

P. aeruginosa produces several virulence factors. Polysaccharides and lipopolysaccharides serve as a barrier between the cell wall and the external environment and form a basis for the biofilm that the bacteria can produce. The bacteria also produce pigments that can act as virulence factors, and different exotoxins and proteases (5). In addition, *P. aeruginosa* produces several signal molecules, important for biofilm formation (108-112).

Pseudomonas is naturally detected in a variety of environments like soil, water, plants and animals, including humans. The bacterium has a predilection for moist environments.

Consequently, in humans it is usually detected in moist areas like the ear, perineum and axilla. Likewise it is detected in moist areas of the hospital, like sinks, taps, mops, water containers, humid medical devices, medicines, food, and in any non-sterilised water.

1.7.2. Epidemiology and clinical infection

P. aeruginosa is often characterised as an opportunistic bacterium which denotes that it rarely causes infection in healthy humans but may do so following disruption of physical barriers and in patients with certain underlying illnesses. Outside the hospital setting, skin infections, especially after skin burns and external otitis in frequent swimmers are the most common clinical manifestations (4, 5, 7).

In hospitals the clinical *P. aeruginosa* infection often reflects the patient's underlying diseases. In addition to bacteraemia and endocarditis, infection of the urinary tract, respiratory tract, central nervous system, ear, eye, bone, joints and skin are most often reported (4, 5, 7, 113, 114). Immunocompromised patients are vulnerable for infections in most body sites. Burns and disruption of the skin barrier can cause severe infections of the skin. Burn wound infections progressing to septicaemia are in ¾ of patients caused by *P. aeruginosa* and have a case fatality rate (CFR) of more than 50% (5). In patients receiving mechanical ventilation, other patients in ICUs and other patients with manipulation of airways *Pseudomonas* pneumonia is common. Patients with cystic fibrosis have an especially high risk for chronic colonisation and infection of the airways. Biofilm formation is an important factor in disease persistence for this patient group (115). *Pseudomonas* septicaemia and UTIs are also common clinical manifestations of *P. aeruginosa* infections.

Pseudomonas species is ranked among the top ten causes of bacteraemias in hospitals (116-121). In-hospital crude case-fatality from invasive disease is high, ranging from 18% to 61% (113, 122-132). In a Norwegian single-hospital study of bacteraemia in patients with malignant blood diseases, *P. aeruginosa* ranks number five in frequency as causative agent and these patients had a CFR of 21% within 30 days after bacteraemia diagnosis (133). *Pseudomonas* species other than Pseudomonas aeruginosa infrequently cause infection (6).

1.8. Biofilm formation

Bacteria exhibit two distinct modes of behaviour, a planktonic mode with free floating single bacteria and as a biofilm where the bacteria appear as structured communities (108, 111, 134). Most of our knowledge about bacteria stem from studies on planktonic bacteria. Studies in

recent years indicate that biofilm is an important – if not the most important – mode that bacteria appear in.

P. aeruginosa is well known to form biofilms (111, 134, 135). Biofilms are structured, specialised communities of adherent microorganisms encased in a complex extrapolymeric substance matrix (134) which can form on any surface although some surfaces are known to retard adherence (111). When a biofilm is formed and reaches a critical mass the quorum sensing molecules excreted alter many of the functions of the bacteria, including slowing its metabolism and increasing the production of a glycocalyx matrix (108, 112). These and other factors reduces the bacteria's susceptibility to antibiotics and disinfectants (111, 135). It has been shown that *P. aeruginosa* can reappear after biofilms on polyvinylchloride pipes have been exposed to a variety of disinfectants for seven days (136). To eradicate the viable bacteria in a biofilm, heat is preferred. Alternatively mechanical removal or the use of oxidative biocides to slowly dissolve the biofilm matrix (135) are suggested. Once a biofilm has formed and matured it can spread to new locations either through single cell dispersal or the shedding of clumps of biofilm (111, 112, 134).

1.9. Molecular typing methods

In epidemiology and outbreak investigation support from microbiological investigations often is essential in order to delimit an outbreak and determine who is a case and who is not. It is not sufficient to be able to identify *Pseudomonas* spp. or *Salmonella* enteritidis in patients to determine whether they are part of the same outbreak. More specific methods are needed. This is similar to criminal investigations by the police where different so-called fingerprint methods are used to link persons to locations.

In classical microbiology phenotypical and serotypical methods are used to differentiate between bacteria of the same species. Most of these methods are technologically simple and the results easy to compare between laboratories, but the methods are time consuming due to much manual work. Bacteria can further be differentiated on the presence of toxins and virulence factors and on antibiotic resistance patterns (5, 6).

The genome of bacteria has certain areas that are conserved within a species and certain areas that show different degrees of variability. The degree of variability varies considerably between bacterial species. To be able to distinguish between bacteria one needs to identify areas of the bacterial genome that are sufficiently variable to be able to discriminate between

clones, but not too variable so all bacteria appears different. The different molecular typing methods are developed to fit the specific properties of the different bacteria.

There is a whole array of molecular typing methods used for bacteria. All are based on the same principle, which is to identify and extract specific areas of the bacterial genome, amplify them and display them in ways to be able to compare the different bacterial samples.

For *Pseudomonas aeruginosa* mainly two molecular typing methods were used in the period when the Dent-O-Sept outbreak occurred. One is called pulsed-field gel electrophoresis (PFGE) where the bacterial genome is amplified, cut and spread in an agar by an electric field creating bands of different sizes in a specific pattern (137). By visually comparing, one can determine the degree of similarity between isolates. Due to uncontrollable differences in the processing between laboratories, it is not advisable to compare results between different laboratories. Another disadvantage of the method is that it is labour-intensive.

The other method commonly used is amplified fragment length polymorphism (AFLP). AFLP uses restriction enzymes to cut genomic DNA (deoxyribonucleic acid), followed by ligation of complementary double stranded adaptors to the ends of the restriction fragments. A subset of the restriction fragments are then amplified using two primers complementary to the adaptor and restriction site fragments. The fragments are visualised on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies (38, 101, 138, 139) .

In investigations of hospital outbreaks different molecular typing techniques are commonly used to distinguish cases from non-cases. The first challenge is to identify the best typing method for the microbe in question. Another is to define the genotypical criteria for including and excluding a bacterial isolate among the cases. There may be some variability among the bacteria at the onset of the outbreak and the bacteria may change during the course of the outbreak due to random mutations, antibiotic pressure, transfers of plasmids between bacteria etc. Most of the outbreaks referred to in chapter 1.6.3 have used one or more molecular typing methods to define the cases belonging to the outbreak and to link the cases to sources among medical devices, other equipment, the environment and personnel.

1.10. Causality

In complex situations many factors influence each other and it is rare to find simple cause-effect episodes like “the person died (effect) because he was shot through the heart (cause)”.

There is a definite association between smoking and lung cancer, but not a one-to-one relationship. Not all smokers contract lung cancer and not all with lung cancer have smoked.

The philosophical basis of the dominant approach for testing theories in medicine is the hypothetico-deductive model as described by for example David Hume and Karl Popper. According to this model it is impossible to achieve absolute proof for a scientific hypothesis; tests performed can only corroborate or falsify the hypothesis. Consequently one can never prove causality between factors and an outcome, only strengthen or weaken a proposed association. In this tradition, Sir Austin Bradford Hill listed nine viewpoints from which to study the association of two variables in order to claim causation (140).

Classic epidemiology has been mainly backward looking, seeking an explanation to an event. In much of the 19th century there was a profound debate on what caused many of the major diseases of the time, being it miasmata (stench or bad air) or contagions (141). For a disease like cholera, John Snow, the father of epidemiology, was in favour of the theory of a contagion which he called ”morbid matter” (142). Late in the 19th century, a prominent German microbiologist, Robert Koch, formulated a set of postulates that needed to be fulfilled in order to claim that a micro-organism caused a specific disease (143, 144). According to his postulates we need both necessary and the sufficient conditions to claim causal relationship between a microbe and a disease.

1.10.1. Counterfactual theories

A century later MacMahon stated that there are two ways of classifying ill persons, either by *manifestational criteria* (grouping ill persons according to symptoms or clinical signs, e.g. common cold, schizophrenia or meningitis) or by *causal criteria* (grouping ill persons with respect to a specified experience believed to be a cause of their illness, e.g. lead poisoning or meningococcal disease) (145). To have a *Pseudomonas aeruginosa* infection implies by name and definition causality of the bacterium.

The central question in counterfactual theories of causation is “What would have happened if not event c had happened?” And the answer is: “If not event c had occurred, then the event e would not have occurred” (146). Counterfactual reasoning can be used both in deterministic and probabilistic models. In daily life and in medicine counterfactual reasoning is extensively used. “If the needle hadn’t been contaminated, the patient would not have acquired hepatitis.” “If you hadn’t been exposed to asbestos, you would not have contracted mesothelioma.” “If the producer had adhered to the regulations, the outbreak would not have occurred.” Many of

the epidemiological study designs have counterfactual thinking embodied (147). In cohort studies we compare exposed and unexposed individuals for a certain risk factor. The unexposed group can be viewed as "what if this exposure did not occur". When calculating the attributable risk fraction, also called the etiological fraction, we assume that all association between the exposure studied and the outcome is causal, and in addition imply that if not the exposed group had been exposed, the rate of outcome among them would have been at the same level as among the unexposed.

1.10.2. Determinism and probabilism

Determinism is based on the idea that that every event is necessitated by antecedent events and conditions together with the laws of nature (148). According to causal determinism the causal relationships are invariant: Every time a certain configuration of conditions occurs, the outcome will be the same. We may have causal determinism even if the situation is complex and the outcome is hard to predict. Probabilistic causality on the other hand claims that the causal relationship is probabilistic, and not invariant. That is, the outcome (effect) may vary according to probability distribution. Probabilistic theories of causation state that causes raise the probabilities of their effects (149).

In epidemiology, probabilistic approaches are most often used in the conceptual thinking of a relationship and in the statistical testing of the strength of association (149). Here, Hill's set of nine viewpoints to explain the association between two variables are commonly used (140). Only the one of temporal sequence of association is essential. This list of "Guidelines for causation" is more in tune with modern epidemiological science as they emphasise the strength of association rather than pure mechanical determinism. However, many have criticised Hill's list and in recent years there has been a resurge in the debate about causality (150-154). Often in communicable disease epidemiology, including outbreak investigation, it is useful to apply Hill's nine viewpoints to assess association. However, with the advent of modern microbiological methods where one can detect genotypically identical strains of a bacterium in different locations and thereby more or less confirm the association, the other viewpoints play a lesser role.

2. Background and outline of thesis

2.1. Background about the outbreak

In late February 2002, the NIPH was alerted to a possible increase in the number of *Pseudomonas* infections in the clinical wards of Norwegian hospitals, especially in ICUs (Table 3). Infection control personnel in different hospitals had vague impressions of seeing more *Pseudomonas* infections than normal. On 8 March 2002, investigators at St. Olavs Hospital in Trondheim, Norway, discovered genotypically identical strains of P. aeruginosa in patient samples from two hospitals in different regions, and 10 days later, they discovered a genotypically identical strain from a third hospital in yet another region. We launched a national outbreak investigation. In retrospect we have created a timeline or log over the main events:

Table 3. *Time-line of the main events in the Dent-O-Sept case.*

Time	Event
1977 or 1978	Production of the Dent-O-Sept swab started
1995	New regulations on medical devices made legal for Norway. In order to CE mark a medical device, the producer needs to make a declaration of conformity.
1999	External evaluation of the production of the Dent-O-Sept swabs after complaints about discoloured swabs. The producer complied with some, but not all of the recommendations.
10.04.2000	The producer was certified by an independent body according to the standard NS-EN ISO 9002, 1994.
20.11.2000	The first patient with the *P. aeruginosa* later to be indetified as the outbreak strain was tested.
12.04.2001	The first patient with *P. aeruginosa* later to be identified as the outbreak strain in blood culture was tested.
17.09.2001	The first swab to be detected contaminated with the outbreak strain was produced this week.
01.11.2001	A rapid increase in new cases with the outbreak strain started.
Nov. 2001	Some clinicians in hospitals started to question whether they were seeing an increase in *Pseudomonas* infections, especially in ICUs.

Time	Event
27.02.2002	Telephone from a doctor at the hospital in Stavanger to NIPH where she told us about a perceived increase in *Pseudomonas* infections and requested us to enquire other hospitals about it.
28.02.2002	E-mail from NIPH to all regional centres in hospital infection control and prevention where we ask whether they have noticed an increase. Quick preliminary answers from most regions.
08.03.2002	St. Olavs Hospital in Trondheim detected genotypically identical strains of *P. aeruginosa* in hospitalised patients from Stavanger and Trondheim.
12.03.2002	General alert of possible outbreak in MSIS-rapport, a newsletter from Norwegian Institute of Public Health.
18.03.2002	The outbreak strain detected in a third hospital, Ullevål in Oslo.
21.03.2002	The outbreak strain detected in a fourth hospital, Ahus, outside Oslo.
21.03.2002	Trawling questionnaire sent to all hospitals with patients with the outbreak strain.
02.04.2002	Telephone from the infection control nurse at Feiringklinikken. They had sent a discoloured Dent-O-Sept mouth swab for microbiological analysis and *Pseudomonas* sp. was detected. The lab had discarded the culture.
03.04.2002	E-mail sent to all involved hospitals to check the Dent-O-Sept swabs.
08.04.2002	At 5.18 PM, mail from St. Olavs Hospital: A genotypically identical strain of *Pseudomonas aeruginosa* was detected in Dent-O-Sept swab produced week 47 in 2001.
09.04.2002	All relevant parties were notified. A press conference was held. Large media attention. Production of the Dent-O-Sept swab ceased permanently.
10.04.2002	The Directorate for Health and Social Affairs asked orally NIPH to perform a mapping of all aspects of the outbreak.
12..04.2002	The Directorate for Health and Social Affairs organised a system audit of the manufacturer. The police started an investigation of the producer.

Time	Event
25.04.2002	The minister of Health gave an orientation on the outbreak of *Pseudomonas* infections in the parliament.
14.06.2002	The Norwegian Board of Health asked NIPH formally and specifically to perform a mapping of the total extent of the outbreak.
28.06.2002	NIPH sent formal letters to all medical microbiological laboratories about the outbreak mapping.
02.09.2002	The police had finished the investigation of the producer and decided not to press charges and closed the case.
01.10.2002	Internal review report by the Ministry of health on the roles and responsibilities of the central health administration in the fields of medical devices, discrepancy report systems and infection control
09.12.2002	The Directorate for Health and Social Affairs appealed the police's decision not to press charges. The time limit for this kind of appeals is three weeks; hence the Attorney-General could not reopen the case.
21.08.2003	Preliminary report from NIPH on the outbreak investigations of patients.
17.01.2004	Final reports from NIPH, The Directorate for Health and Social Affairs and The Norwegian Board of Health.
18.02.2004	Status report from The Norwegian System for Compensation to Patients on the monetary claims and their assessment.
11.10.2005	Out-of-court settlement where the producer agrees to pay The Norwegian System for Compensation to Patients 1.2 million NOK without accepting any responsibility for the outbreak.
19.06.2006	Court settlement between the producer and one large hospital (Ullevål) where the hospital received a compensation of 3.3 million NOK for additional costs incurred.

2.2. Setting

Norway had a population of 4.5 million people in 2001-2002 and approximately 65 (mainly public) hospitals organised in 5 public regional health trusts, each of which had a centre for hospital infection control. The 22 medical microbiological laboratories in the country provided general bacteriological culturing services. There was no national surveillance system

for *P. aeruginosa* infection. There were around 1000 health care institutions for the elderly. Through the European Economic Area Agreement Norway abides by much of the legislation within the EU, including European Council Directive 93/42/EEC concerning medical devices (26).

The main data for this study was collected in 2002-2003 during and after an outbreak of *P. aeruginosa* infection in hospitals.

Most of the research for this study took place at the NIPH during and after the outbreak investigation. NIPH is a governmental non-regulatory institute mandated to conduct surveillance of infectious diseases (epidemiologically and microbiologically) and advice the health services and the public on prevention and control of infectious diseases. As with other outbreak investigations NIPH collaborated extensively with all affected parties: infection control teams and administration in the hospitals, the regional health trusts' centres for hospital infection control, the medical microbiological laboratories, the local Food Safety Authority, some nursing homes and municipal medical officers, some private persons, the national and county Board of Health, the Directorate of Health and Social Affairs and the Department of Health.

2.3. Outline of the thesis

In Chapter 1 I give a general introduction, high-lighting some of the special features of HAIs and outbreak investigation in a hospital setting, medical devices, the bacterium under study and also give a general introduction to the discourse on causality in epidemiology which became important in the public debate of the outbreak.

In Chapter 3 I list the aims of the study and in Chapter 4 I present the data sources and methods used. The main results are presented in Chapter 5. And in Chapter 6 the findings are discussed and strengths, weaknesses and limitations of the methodology used are put under scrutiny. Finally, in Chapter 7 I reiterate the main conclusions, suggest further studies and propose what actions that should be taken from the lessons learned.

3. Aims of the thesis

All parts of this thesis are in some ways related to a large outbreak of *Pseudomonas aeruginosa* infection in Norwegian hospitals that occurred in 2001-2002.

The overall aims were to investigate a large outbreak of *Pseudomonas* infections and gain knowledge from it, to explore theories for causality and responsibility, and to describe the epidemiology and investigate risk factors for contracting invasive *Pseudomonas aeruginosa* infection.

When an infectious disease outbreak of this size occurs in hospitals large resources are spent on the outbreak investigation. In addition, experience is gained and knowledge is acquired in many related areas. The organisational structure and information channels are tested, as are the guidelines and regulations, the behaviours and routines, and the ability of all parties involved to respond to the emergency occurring.

3.1. Investigating an outbreak of *Pseudomonas aeruginosa* infections

When an outbreak occurs the overriding goal is to stop the outbreak to minimise the damage. The specific aims were to

- describe the outbreak,
- identify risk factors for contracting the disease among the patients,
- identify the causes of the outbreak, and
- make recommendations for the prevention of future outbreaks.

3.2. Investigating contamination of the medical device

When the outbreak strain of *P. aeruginosa* was detected in a medical device, it was concluded that this device was the vehicle introducing the bacterium into the hospitals. All aspects of this device, its production and use, was explored and assessed. The specific aims of this part of the investigation were to

- examine how *Pseudomonas aeruginosa* contaminated the product,
- assess the extent of the contamination by *P. aeruginosa* and other microbes, and
- identify critical points in the production process that made the contamination possible.

3.3. Exploring theories for causality of an outbreak of *Pseudomonas aeruginosa* infections

The outbreak received heavy mass media attention at times. Many patients had fallen ill, many died while hospitalised. In the public debate differences of opinion among stakeholders

were visible, politicians took part (an orientation was given in the parliament), large economical resources and even work places were at stake.

With all these actors and acts and different agendas, it was difficult to see who did what and which role and responsibility each participant had. The literature on causality is plentiful and diverse. The specific aims were to

- examine theories of causality from different fields like science, philosophy and law,
- apply the theories on the various participants in the outbreak to examine their role, and
- discuss the responsibility and fallibility for two of the main actors.

3.4. Investigating the epidemiology of invasive *Pseudomonas aeruginosa* infection

Pseudomonas aeruginosa can cause serious disease in susceptible patients even when there is no outbreak. *Pseudomonas* species is ranked among the top ten causes of bacteraemia in hospitals (116-121). In-hospital crude case-fatality from invasive disease is high, ranging from 18% to 61% (113, 122-132). There is extensive knowledge on *P. aeruginosa* infections in general and especially from tertiary care hospitals. But little is known about the epidemiology of invasive *P. aeruginosa* infection in humans in unselected hospitals, and even less from Norway as these infections are not covered by any national surveillance systems. The specific aims of this part of the study were to

- describe the epidemiology of invasive *P. aeruginosa* infection in Norway,
- identify patient groups at increased risk of disease and of death from *P. aeruginosa* infection, and
- estimate national incidence rates and mortality rates of *P. aeruginosa* infections by groups of underlying diseases.

4. Materials and methods

4.1. Investigating an outbreak of *Pseudomonas aeruginosa* infections

Following the alert of a possible increase, the NIPH immediately launched a classical outbreak investigation as outlined in Chapter 2.4: Infection control personnel in hospitals were alerted, preliminary epidemiological and microbiological investigations were performed, environmental sampling were performed, preliminary, and later definite case definitions were made. When the outbreak strain of *P. aeruginosa* was found in a product, systematic sampling of all available batches of the product was carried out. The production plant was inspected and sampled.

The Ministry of Health and its subordinate institutions, the Norwegian Board of Health and the Directorate for Health and Social Affairs, requested the detection of all patients involved in the outbreak. A systematic protocol was developed and approved. The outbreak strain of *P. aeruginosa* was identified. All available clinical, bacterial isolates were genotyped and compared with the outbreak strain. For each patient with the outbreak strain or with another strain of *P. aeruginosa* isolated from blood or CSF samples, the patient's physician were asked to fill in a detailed questionnaire.

We conducted a case-control study to investigate risk factors for having the outbreak strain of *P. aeruginosa* as compared with other strains of *P. aeruginosa*. We did not look for risk factors for having *P. aeruginosa* infection. Data obtained in the descriptive epidemiological investigation was used. To be able to pick comparable controls the source population was defined as only those with invasive *P. aeruginosa* infection. Case patients were persons with the outbreak strain isolated from blood or CSF samples during the period October 2001–December 2002, and control subjects were all the persons included in the study with genotyped strains of *P. aeruginosa* other than the outbreak strain isolated from blood or CSF samples during the same period.

To investigate risk factors for a fatal outcome during the stay in the institution for patients with an invasive *Pseudomonas* infection, we used a cohort design including all of the patients in the case-control study. The same variables as in the case-control study were included as possible risk factors for death, in addition to having the outbreak strain.

To evaluate whether the *Pseudomonas* infection had contributed to the death of individual patients, Bjørn G. Iversen and Preben Aavitsland meticulously assessed all of the available information for each of the dead patients. Among the information assessed was the course of

events for each patient, dates for hospital admission, diagnosis, transfers and death, underlying illnesses, clinical and microbiological information and the clinicians' assessment of a relationship between *P. aeruginosa* infection and death. Due to a large degree of variability between how the different clinicians assessed the relationship, we did not follow their assessments in all instances.

4.2. Investigating contamination of the medical device

Detailed environmental investigations were carried out for the product, the production facility and for the moist ingredients of the product.

When the outbreak strain of *P. aeruginosa* was found in a product, systematic sampling of all available batches of the product was carried out. Up to 10 items of each available batch of the product were asked to be examined. We asked the laboratories to identify and deep freeze monocultures of all findings of certain microbes whereas others were only to be noted and reported.

The Directorate for Health and Social Affairs organised a system audit of the manufacturer on 12 – 15 April 2002 by studying documents, interviewing selected personnel and inspecting the premises, including microbiological sampling which were cultured at the municipal Food Control Authority. On request from the producer, the laboratory at the municipal Food Control Authority performed environmental sampling in addition to what had been performed during the system audit.

Microbiological analysis (155, 156) were performed on each of the ingredients for the moisturising liquid used in the product (except tap water). The total viable aerobic count and specific detection of *P. aeruginosa* were tested in each of the liquids. Then the moisturising liquid undiluted and in 1:10 dilution were tested for their effect on the outbreak strain and a reference strain of P. aeruginosa (ATCC 9027 -MicroBioLogics) (157).

4.3. Exploring theories for causality of an outbreak of *Pseudomonas aeruginosa* infections

An analytical approach in the tradition of philosophy of science, and not using a strict epidemiological methodology, was used to discuss causality in the outbreak. Firstly, theories of causality from different disciplines were introduced. Then the *P. aeruginosa* outbreak was used as a case and the different theories were applied. The different theories of causality were put under scrutiny and the roles of the many actors involved were elucidated. Two actors are

especially central in this outbreak and their roles were further discussed to examine their responsibility and fallibility in the outbreak.

4.4. Investigating the epidemiology of invasive *Pseudomonas aeruginosa* infection

We used all information on patients with *P. aeruginosa* or *Pseudomonas* not identified at the species level (*Pseudomonas* spp.) isolated from blood or CSF during the period 1992-2002 collected during the outbreak investigation. We described the whole cohort and we analysed in detail the patients from the recent years (1999-2002), about whom we had collected much more information. Denominator data was collected from a variety of public sources. Population statistics and the number of beds in municipal nursing homes were downloaded from Statistics Norway. The number of stays and the number of days of hospitalisation in somatic hospitals by region, age and discharge diagnoses were supplied by The Norwegian Patient Register. Several disease categories that have been shown to be associated with increased risk of invasive *P. aeruginosa* infection were selected and grouped according to ICD-10 (International Classification of Diseases, 10th Revision) (158).

Descriptive and analytical epidemiological methods were used, and incidence proportions of infection and death for various groups were calculated.

4.5. Data management and statistical analysis

In the data collection, extensive efforts were made to ensure completeness and quality checks of data. Participating microbiological laboratories were given lists of all information they had sent NIPH to check for completeness of records and to fill in missing values and to correct improbable values. Hospitals were contacted by mail and phone to remind them of missing clinical forms and to check missing or improbable values and unreadable text.

Data were checked manually and electronically by listings, cross tabulations and by calculating time between admission, diagnosis, discharge and death. Some patients had been admitted to more than one hospital, and double entries were checked and removed. All patients were checked with an updated National Population Registry to search for deaths.

Paper I

All data were entered into an Epi Info software database, version 6.04d (Centers for Disease Control and Prevention), and analysed data using Epi Info (Centers for Disease Control and Prevention) and Stata 8 (Stata) statistical software. For the case-control study, odds ratios (ORs), 95% confidence intervals (CIs), and *P* values were calculated. In the multivariable

logistic regression analysis, all risk factors were initially included in the model, and the ones with the highest *P* values were removed one by one, until only variables with *P* values <0.05 remained. The variables that remained in the model were assessed and statistically tested for effect modification. In the cohort study examining risk factors for death, a similar binary regression approach was used.

Paper IV

We entered all patient data in an Epi Info version 6.04d database and analysed them in Epi Info, Excel, Episheet and Stata 8 and 9 statistical software. Incidence proportions, incidence rates and a comparison of these (risk ratios and rate ratios of different kinds) with 95% CIs were calculated in Episheet and Stata. To identify risk factors for dying among the cases we performed stepwise multivariable binomial regression analyses in Stata.

4.6. Laboratory analysis

Primary culturing of product and patient samples was performed at local laboratories. Culturing of samples of the product was performed at local laboratories according to our instruction: "Brush the swab against both a lactose and blood agar dish in a rotating manner so all sides of the foam tip touches the agar. It is not necessary to place the swab in a growth broth". The isolates were identified by standard procedures in use by the laboratories.

Culturing of samples from the system audit and the additional investigation of the production site were performed at the laboratory of the municipal Food Control Authority. The qualitative analysis of the samples was performed by direct seeding (except for dry Dent-O-Sept swabs) and seeding after enrichment overnight in a heart infusion broth on Kings Agar B and on blood agar. The quantitative analysis was performed by direct seeding of 0.1 mL undiluted or – if heavy contamination was expected – diluted liquid on Kings Agar B and for some samples also on blood agar. The plates were incubated at 37°C overnight before reading.

All available isolates of *P. aeruginosa* from patients, product and from the system audit (but not from the additional investigations of the production site) were sent to at least one of five reference laboratories for genotyping and comparison with the outbreak strain *(P. aeruginosa* found in the product batch 47.2001 on 8 April 2002). The reference laboratories reported whether the isolate belonged to the outbreak strain or not. Four of the reference laboratories used a protocol developed at St. Olavs Hospital for a PFGE method. The criteria of Tenover

et al. (137) were used to interpret identical (no band differences) or closely related (≥3 band differences) isolates. The fifth laboratory used an AFLP method. The method for genotyping by AFLP is slightly modified from a method described elsewhere (38). Isolates that displayed ≥85% similarity were considered to be closely genetically related and to belong to the same clone.

The AFLP and PFGE protocols were compared and found to be equal in detecting and discriminating the outbreak strain. If an isolate was not typeable by PFGE because of excessive activity of endogenous endonucleases, it was genotyped with AFLP.

4.7. Ethics

All public health work has to balance between the rights of the individual and the benefits for society. This is particularly evident in prevention and control of communicable diseases. A person with a communicable disease can have his freedoms restricted in order to protect the society at large. For example, a person with open pulmonary tuberculosis needs to be confined to an isolation room as long as he is contagious to prevent further spread, preferably voluntarily, if necessary by force. The Norwegian Communicable disease control act is build up around these principles (23). There was no need to isolate any patients with *P. aeruginosa* by force.

One regulation to this act is on the surveillance of communicable diseases and immediate notification of serious events and outbreaks. The immediate outbreak notifications shall not contain person identifiable information. When conducting an outbreak investigation it sometimes is necessary to obtain confidential information quickly in order to control the outbreak and minimise the harm and where seeking approval may seriously delay the investigation. This is acceptable and in accordance with consequentialistical ethical thinking. The National Committee for Research Ethics in Norway concludes that prior approval is not needed as long as the objective is to control the outbreak (159). Then it is also in accordance with deontological ethics.

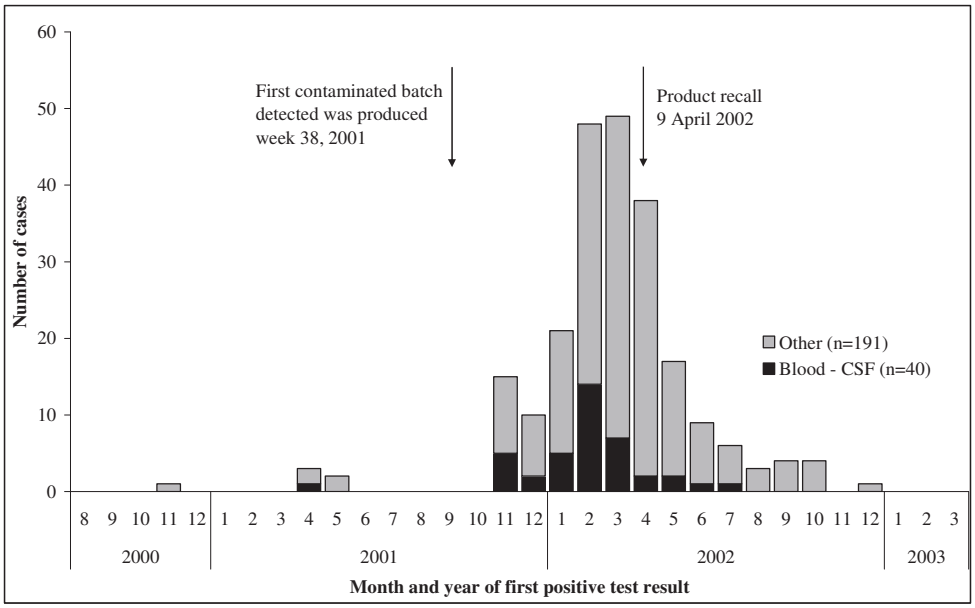
When the outbreak investigation moves beyond the need to control the outbreak, further approval is needed, hence the NIPH was authorised by the Norwegian Board of Health to perform the study. And the Data Inspectorate gave permission to create a database to store the information.

5. Synopsis of the results of the study

5.1. Investigating an outbreak of *Pseudomonas aeruginosa* infections

The outbreak strain of P. aeruginosa was isolated from 231 patients from November 2000 through December 2002, with a peak incidence during February–March 2002 (Figure 3). The patients with the outbreak strain were hospitalised at 24 different hospitals in all public regional health trusts (range, 1– 39 patients per hospital), whereas 5 lived in other institutions, and 3 were not hospitalised when they received a diagnosis. The median age was 65 years and 61% were men. Of the 231 patients 39 had positive blood culture results. Clinically 42 had sepsis, and 87 had pneumonia, whereas 70 patients were only colonised. Altogether, 156 patients were admitted to an ICU during their hospital stay, and 128 received mechanical ventilation within the previous 3 weeks before receiving a diagnosis of *Pseudomonas* infection.

Figure 3. *Epidemic curve of the outbreak showing the number of patients cases with the outbreak strain of Pseudomonas aeruginosa isolated from either blood or CSF sample or other sites, by month and year of the first positive culture result.*



Seventy-one patients (31%) died while institutionalised; all of the patients who died had severe underlying disease. An assessment of whether the *Pseudomonas* infection contributed to the patient's death concluded that it was probable for 34 patients, improbable for 21,

uncertain for 13, and impossible to evaluate because of a lack of information for 3. A total of 132 patients (69%) with the outbreak strain had definitely or probably used the Dent-O-Sept swab, whereas 58 (31%) had not or probably had not used it.

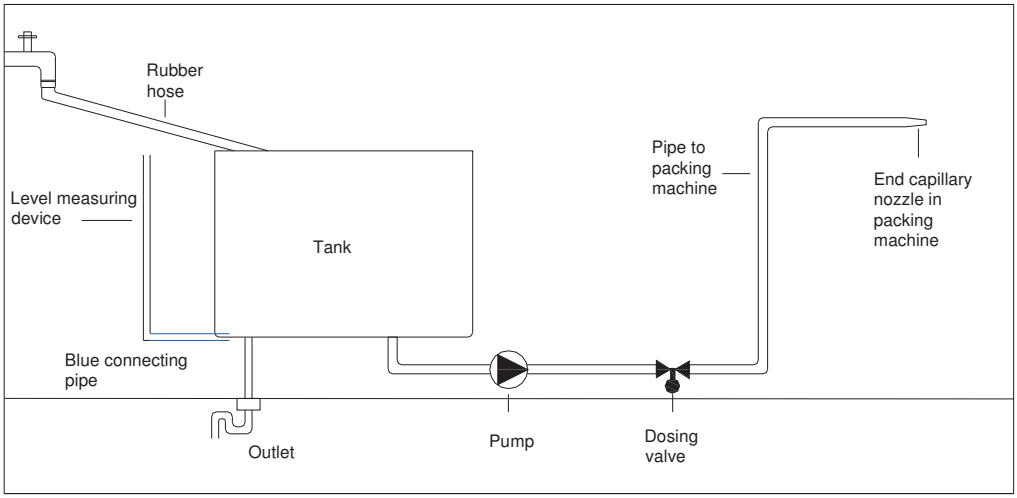
Among 39 case patients and 159 control subjects, use of the moist mouth swab (adjusted OR 5.3; 95% CI 2.0–13.6) and receipt of mechanical ventilation (adjusted OR 6.4; 95% CI 2.3–17.2) were associated with infection due to the outbreak strain.

5.2. Investigating contamination of the medical device

NIPH received information about stored batches of the Dent-O-Sept swab from 59 general hospitals, four other health care services and 20 private persons. A total of 1565 swabs were examined from 149 different batches. Although we asked for up to 10 swabs of each batch to be examined, an average of 18 swabs per available batch were examined for the years 2001 and 2002, ranging from one to 37 swabs per batch. The outbreak strain of *P. aeruginosa* was detected in 76 swabs from 12 different batches of the Dent-O-Sept swab produced from week 38 in 2001 to week 15 in 2002 when production ceased. All genotyped strains of *P. aeruginosa* were identical to the outbreak strain. In total, more than 250 swabs were found to contain one or more species of microorganisms, mainly gram-positive bacteria which were predominantly discovered in the earlier batches. Gram-negative rods including *Acinetobacter baumanii* were isolated in swabs produced in 1999 and 2001.

During the system audit and the additional investigations of the production facilities samples for microbiological examinations were taken from several places along the production line. The outbreak strain of *P. aeruginosa* was detected from the end capillary nozzle in the packing machine (Figure 4). In the additional investigation *P. aeruginosa* (which were not genotyped) were cultured from the blue connecting pipe, the level measuring device and a rubber hose.

Figure 4. *Schematic figure showing the wet part of the production of the Dent-O-Sept swab.*



The system audit concluded that the production deviated from the existing regulations in several areas:

- The production process, including the recipe for Dent- O-Sept, did not ensure that the product had the qualities and properties stated by the producer nor that the risk of contamination was avoided or reduced to a minimum.
- Neither the boxes nor wraps of the Dent-O-Sept gave the user the necessary information. The CE (Communauté Européenne) marking was unjust because the producer's declaration of conformity with the regulations, including the risk analysis, was poorly based and documented. The technical documentation did not give a third party a basis for assessing whether the device was in accordance with the demands of the regulations.
- The producer did not comply with the obligation to report defects and deficiencies in medical devices to national health officials and had not adequately followed up errors in the production demonstrated in an external review in 1999.

No bacteria were detected in any of the ingredients for the moisturising liquid. When the outbreak strain of P. aeruginosa was added to the Dent-O-Sept solution and to the two concentrations of the disinfectant we observed a 6 log reduction in 15 minutes and for the 1:10 diluted Dent-O-Sept solution a 6 log reduction after 3-6 hours. For the reference strain (ATCC 9027) there was a 6 log reduction in 15 minutes for all four liquids.

5.3. Exploring theories for causality of an outbreak of *Pseudomonas aeruginosa* infections

The *P. aeruginosa* outbreak was used as a case and various theories of causality from different disciplines (epidemiology, other sciences, philosophy and law) were applied to discuss the roles and responsibilities of some of the parties involved. Mackie's concept of INUS conditions, Hill's nine viewpoints to study association for claiming causation, deterministic and probabilistic ways of reasoning, all shed light on the issues of causality in this outbreak. Moreover, applying legal theories of causation (counterfactual reasoning and the "but-for" test and the NESS test) proved especially useful, but the case also illustrated the weaknesses of the various theories of causation.

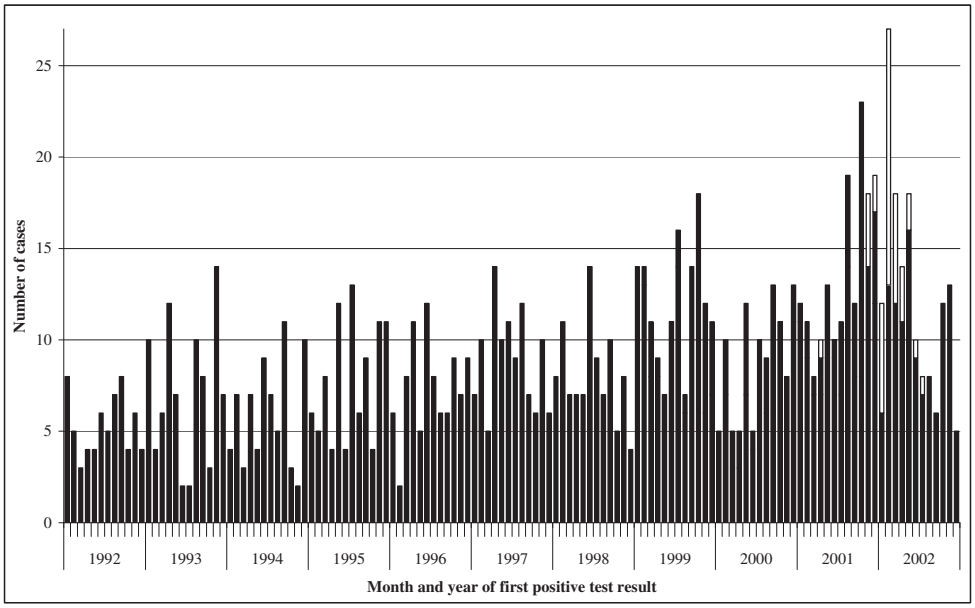
We concluded that many factors contributed to causing the outbreak, but that contamination of a medical device in the production facility was the major necessary condition. The reuse of the medical device in hospitals primarily contributed to the size of the outbreak. The unintended error by its producer – and to a minor extent by the hospital practice – was mainly due to non-application of relevant knowledge and skills, and appears to constitute professional negligence. Due to criminal procedure laws and other factors outside the discourse of causality, no one was criminally charged for the outbreak

5.4. Investigating the epidemiology of invasive *Pseudomonas aeruginosa* infection

In the 11 year period 1992-2002, 1174 patients with invasive disease were identified, of which 1079 (92%) had isolates of *P. aeruginosa* and 95 had *Pseudomonas* not identified at the species level (*Pseudomonas* spp.), resulting in an overall incidence rate of 2.43 per 100 000 person-years at risk (pyar) (Figure 5). The median age of the patients was 72 years and 67% were male.

For the period 1999-2002, 567 incident cases (representing 565 patients) were identified, corresponding to an incidence rate of 3.16 per 100 000 pyar (95% CI, 2.90-3.43). In hospitals the incidence rate was 3.33 per 100 000 person-days (95% CI, 3.06-3.62), or 0.20 per 1000 hospital stays (95% CI, 0.18-0.21).

Figure 5. *The monthly number of cases of invasive Pseudomonas aeruginosa infection in Norway 1992-2002. Forty cases (white bars) belonged to an outbreak caused by a contaminated mouth swab.*



The rate of infection was much higher in males, 5.1 per 100 000 person-days in hospital as compared with 1.9 in females (incidence rate ratio [IRR], 2.6; 95% CI, 2.2-3.1). For hospital-acquired cases the rates were 2.9 and 1.0 per 100 000, respectively (IRR, 3.0; 95% CI, 2.3-3.8).

A total of 55% of the cases were hospital-acquired and an additional 10% were living in or being hospitalised from a nursing home. The remaining 34% were community-acquired and 1% was unknown. For hospital-acquired infection the rate was 671 per 100 000 person-years as compared with 1.13 for community-acquired infection, and 37 in nursing homes.

Of the patients with invasive *P. aeruginosa* infection, 14% received mechanical ventilation within a period of 3 weeks before the sample positive for *P. aeruginosa* was taken, and 30% were admitted to an ICU during their stay in the hospital.

The highest risk for invasive *P. aeruginosa* disease was found in patients with malignant neoplasms of lymphoid and hematopoietic tissue (ICD-10 categories C81–C96) (risk per 1000 hospital stays 1.9; 95% CI 1.5-2.3) and other diseases of blood and blood-forming organs (D70–D77) (2.2; 95% CI 1.2-3.7). The CFR was 35%. The highest CFR was seen among

patients with transplanted organs (Z94) (67%) and patients with certain diseases of the respiratory system (J10–J22; J40–J96) (52%).

In the multivariable regression analysis, the following variables were independently associated with an increased risk of dying (CFR) while hospitalised among the cases: Having one or more underlying risk diagnoses (risk ratio [RR], 2.52; 95% CI, 1.50-4.25), admission to an ICU at some time during the stay (RR 1.55; 95% CI, 1.26-1.91), being 60 years or older (RR, 1.53; 95% CI, 1.14-2.06), and having received immunosuppressive treatment within the past three weeks before the sample with *P. aeruginosa* was taken (RR, 1.39; 95% CI, 1.13-1.73). Having a *Pseudomonas* UTI as the most serious clinical *Pseudomonas* diagnosis was highly protective (RR 0.09; 95% CI, 0.01-0.63).

6. Discussion

The outbreak of *Pseudomonas aeruginosa* infection in 2001-2002 was a major wake-up call for many groups in the society, including hospital managers, hospital infection control personnel, national health service administration, politicians in the parliament and elsewhere and producers of medical devices. Prior to the outbreak few would have believed that a seemingly inconspicuous mouth swab which had been produced for decades, could have caused serious disease in so many patients.

The outbreak reminds us of the change in the patient population in hospitals over the last decades with an increasingly larger part being severely ill and susceptible for an increasing number of opportunistic bacteria. Consequently infection control becomes increasingly important in hospitals.

Questions of causality, responsibility and blame have always been a part of the history of infections. During and after the outbreak investigation, questions of causality, responsibility and liability were raised: Who and what caused the outbreak, who were responsible for the extent of the outbreak, could the damages have been mitigated by acting sooner or differently, should anyone be punished?

The outbreak prompted action in many areas. All actors involved analysed their situation, wrote statements and reviews, a national action plan against HAIs were published; regulations and guidelines for infection control and prevention in hospitals were revised (3, 25, 33, 34, 160-166).

6.1. An outbreak of *Pseudomonas aeruginosa* infections

The *Pseudomonas aeruginosa* outbreak in Norway 2001-2002 is possibly the largest published outbreak of its kind to date with 231 confirmed cases, 161 of whom had a clinical infection. A total of 71 (31%) of the patients died while in an institution. All of those who died had severe underlying illnesses such as terminal cancers, multiple traumas, or severe respiratory or vascular disease. The cause of death was multifactoral for all these patients. To decide whether the *P. aeruginosa* infection contributed to the patients' deaths can only be based on a best judgement as one has to assess all the different factors and estimate the impact of each factor. In an assessment based on all available information of whether the *Pseudomonas* infection contributed to the patient's death, we concluded that it was probable for 34 of the 71 patients, improbable for 21, uncertain for 13, and impossible to evaluate

because of a lack of information for 3. In conclusion at least 161 patients had a clinical infection and 34 died prematurely as a consequence of the outbreak.

The outbreak received much media attention, especially when the link to the Dent-O-Sept swab was documented. Afterwards the attention gradually subsided. Later Norway has experienced other large national outbreaks. In 2006 there was an outbreak with *E. coli* infection causing haemolytic uremic syndrome (HUS) (167). The outbreak – where 10 of 17 cases had HUS as a clinical manifestation and one child died – received much more intense media coverage than the Dent-O-Sept outbreak. In 2005, a legionellosis outbreak in the county of Østfold with 56 confirmed cases and 10 deaths received less media attention than the HUS outbreak but more than the Dent-O-Sept outbreak from an subjective point of view at NIPH (168). There may be several reasons for this. One is a behaviour change of the media being increasingly aggressive and when an event catches on, journalists try to outdo each other in digging up minute details and blowing them out of proportions. Increasingly frequent the media coverage of outbreaks escalates to a level involving the national government and the parliament. The other main reason can be that whereas the Dent-O-Sept and legionellosis outbreaks to a large extent involved elderly and already ill people, the HUS outbreak mainly struck children.

But for the small outbreaks or when the source is obvious, outbreak investigations are both resource and time consuming. When investigating a large, multicentre outbreak like the Dent-O-Sept outbreak it is important to create a flexible central team and a robust network reaching all involved parties. With many participants one of the challenges is to let all voices be heard but at the same time make all pull in the same direction once a matter has been discussed and a decision reached. Not to loose sight when drowned in details, it can be wise to have one or a few “opponents” to evaluate the progress of the outbreak investigation at some distance in order to suggest corrections if needed.

During the *P. aeruginosa* outbreak most of the communication between the central outbreak investigation team at NIPH and the local teams in the hospitals and the laboratories were by e-mail. This was the first large outbreak investigation at NIPH were e-mail was the main mode of communication, and it proved to be quick and very resource efficient. On the day when the source of the outbreak was verified the head investigator sent and received a total of 33 e-mails which was an immense amount in 2002 (but not today). The active use of postings on the NIPH website on the Internet was still in its beginning and would have been used more extensively today. The cooperation between all parties but a few exceptions was exemplary.

Only one local hospital outbreak team worked counter-actively in periods by creating an alternative trawling questionnaire to the one the others had agreed on and sending it to some of the other hospitals, requesting extra information on questionnaires to neighbouring hospitals in addition to what the central investigation team had collected and thereby placing extra burdens on them. At the height of the outbreak investigation we participated in several meetings with the top administration, including the managing director, of this hospital.

Most hospital outbreaks are spread locally via persons, local products or the environment. When three and steadily more hospitals were involved the attention was directed towards a moist product mainly used in the ICU. However which product was difficult to elucidate as there is a huge array of moist devices and pharmaceuticals used in the ICU. A systematic trawling and testing of products had started when an infection control nurse tipped us about discoloured Dent-O-Sept swabs that had yielded *Pseudomonas* sp. but where the bacterial culture had been discarded before it was examined further.

Similar to criminal investigations, genetic fingerprinting has become an important means of connecting cases to sources and causes. We were able to detect genotypically identical bacteria from patients, the product and the production facility thereby beyond doubt confirming the source of the outbreak. An increasing number of outbreaks are investigated with the help of microbiological genotyping (45, 60, 66, 88, 169). In addition, these methods are often used in the case definitions to separate the patients with the outbreak strain from other patients with infections with other strains of the same microbe. Genotyping of the many isolates of *P. aeruginosa* has been an expensive but indispensable part of this outbreak investigation.

6.2. Contamination of the medical device

Medical devices may be contaminated in each of the steps from ingredients and building materials, trough production, packing and transport to storage, use and reuse of the final product.

Some, but not all of the Dent-O-Sept swabs were contaminated. How the *P. aeruginosa* bacteria came into the production equipment was never ascertained. One plausible hypothesis is that it entered via the municipal drinking water. Drinking water is not – and is not required to be – sterile. Moisture-prone bacteria like *P. aeruginosa* will naturally occur in municipal drinking water. The misconception of the purity of drinking water is also seen in the health

care setting were tap water is used in areas where only high-level disinfected or sterile water should be used.

In the production facilities the cleaning and disinfection routines of the production equipment did not eradicate the bacteria. And there was no quality control system in place checking the microbiological quality of the final product. This was one of the recommendations given during an external evaluation and not followed up (162).

However, other actors in addition to the producer were not abiding by regulations and guidelines. Many health care institutions found their logistic systems for purchase, storage and use of the swab to be deficient. Examples of reported deficiencies were: no centralised procedure for purchases, infection control personnel not taking part in the purchasing process and boxes of old batches were found in remote storage places. Many also lacked adequate reporting systems for faulty medical devices.

Anecdotal information from several hospitals described that, after use, nurses would store the swab in a glass of tap water on the night stand and later reuse it for the same patient. The extent of this practice is unknown but where it occurred, the bacterial load these patients were exposed to may have increased exponentially as indicated in a report (170). The risk of becoming colonised or infected increases with the dose of bacteria the patient is exposed to.

When a microbe is introduced into a hospital setting for example via a medical device, it may contaminate or colonise patients, personnel, the environment or other medical equipment. Approximately 1/3 of the cases in the *P. aeruginosa* outbreak had probably or definitely not used the swab. And in addition to having used the swab, receipt of mechanical ventilation was an independent risk factor for harbouring the outbreak strain of *P. aeruginosa* compared with having another strain of the same bacterium. We present two possible explanations for this:

1. Injury to the tracheal epithelium may favour adherence of *Pseudomonas*, and *P. aeruginosa* is commonly found in ventilator associated pneumonia (4). When the outbreak strain repeatedly was introduced from the swabs, environment or persons it could easily lead to colonisation or infection in susceptible, ventilated patients.
2. The ventilators themselves posed as a risk implying that the cleaning and disinfection of the ventilators between patients did not sufficiently eradicate the bacteria once it had been introduced. The problem of adequately disinfecting all parts of the ventilator or other medical equipment is well known from other outbreaks (64, 66, 80, 98).

Typical areas prone to *P. aeruginosa* contamination are sinks, faucets, flasks, other containers and tubes; in short, any moist area. When the bacterium first is introduced it needs hardly any nutrition to survive or even to multiply. Many containers with ordinary tap water are left to stand for too long periods without checking, cleaning and disinfection. Health care institutions need to perform risk assessments of all moist environments and to institute guidelines for microbiological quality control of these environments and guidelines for when only to use sterile water.

6.3. Microbial control of moist products

Where there is water there are microbes if not the water is completely sterile. And if bacteria are present they will multiply. A bacterium like *P. aeruginosa* has affinity for moist environments and has minimal nutritional requirements (4, 7). There are several ways of controlling microbial growth in moist products. The first way is to sterilise the product with heat, gas or radiation. For products that are exposed to the environment, microbes will eventually enter the product and some sort of preservative is essential. In cosmetics and pharmaceuticals a range of preservatives have been used to control growth, of which parabens and benzoic acid and its chemical derivatives are common. In foodstuffs salt, sugar, acids, alcohols and gaseous environments are also commonly used. Most disinfectants intended for use as technical disinfection are too toxic to add to these moist products. There are few moist medical devices on the market that does not need to be sterile.

All preservatives and other additives to these kinds of moist products are strictly regulated by law and categorised. For example, all approved food additives in the EU are listed in the Codex alimentarius and given an E-number. All preservatives are E200-E299, and sodium benzoate, for example is E211.

Medical devices: In Norway laws and regulations of medical devices (24, 25) are for all practical purposes identical with those of the EU, including European Council Directive 93/42/EEC concerning medical devices (26). For non-invasive medical devices in Class I, there are no demands for sterility. The devices must, when used, “not compromise the clinical condition or the safety of patients”. “The devices and manufacturing processes must be designed in such a way as to eliminate or reduce as far as possible the risk of infection to the patient, user and third parties.” Beyond this, the directive does not specify the microbial quality of the product, e.g. the absence of *P. aeruginosa*. And without any assistance from a third party the producer can draw a declaration of conformity. The responsibility for control

of medical devices is with the Directorate of Health and Social Affairs (now called the Directorate of Health).

Pharmaceutical preparations: All medicinal products are strictly regulated and needs to be approved by the Norwegian Medicines Agency or a counterpart in another country in the EU in order to receive marketing authorisation (171, 172). According to the European Pharmacopoeia, pharmaceutical preparations for use in the respiratory tract are classified in a Category 2 where – in addition to other microbiological requirements – the absence of *Pseudomonas aeruginosa* needs to be documented (27).

Cosmetics: As for the products mentioned above, cosmetics and body care products are regulated through an Act and a regulation (28, 29). As for medical devices the legislation is almost identical with that of the EU (30). The Regulation states that the producer shall produce and have available a dossier which describes: "The physico-chemical and microbiological specifications for the raw materials and the finished product and the purity and microbiological control criteria of the cosmetic product". The appendices to the regulation give examples of groups of cosmetics and body care products and list which preservatives can be used. The "Notes of guidance" giving detailed recommendations to the Council Directive states that *Pseudomonas aeruginosa* must not be detectable in cosmetic products (31). The Norwegian Food Safety Authority is responsible for the control of cosmetics.

There seems to be less specific demands for microbiological purity for non-invasive medical devices in Class I compared with other moist products. In their final report the Directorate of Health and Social Affairs stated the need to discuss whether non-sterile, moist medical devices should be reclassified to a higher class than Class I (164, 165). The issue was thoroughly debated at meeting for the authorities on medical devices from the Nordic countries in February 2004. The other Nordic authorities were quite reluctant to reclassify these products. Their main argument was that if the manufacturer had adhered to the current regulations and classifications the outbreak would not have occurred.

The area of medical devices has not been prioritised for resources in the central health administration. This is one of the clear conclusions the Ministry of health drew in an internal review after the outbreak (34). Compared with the other Scandinavian countries, Finland and Great Britain, Norway had spent a lot less resources on the administration and control of medical devices. The resources allocated had mainly been used on controlling medical

devices of higher classes and controlling the technical control organisations. Only on direct enquiries the Board of Health (and later the Directorate of Health and Social Affairs) had followed up on Class I medical devices.

6.4. What preservatives were used in the Dent-O-Sept moisturising liquid?

Two of the major questions after the outbreak were: What preservatives in the moisturising liquid suppressed bacterial growth and how could the bacteria survive in the production facilities and in the individual wrapped swabs?

One batch of moisturising liquid consisted of: Tap water (147 litres), 96% ethanol (3 litres), Glycerol (16 litres) and Vademecum, a commercially available mouth rinse (6 litres). The main ingredients of the mouth rinse are ethanol (44%) and sodium benzoate (5.25%) in addition to water. Of the three major possible preservatives the final concentration in the Dent-O-Sept moisturising liquid was calculated to be 2.3% ethanol; 9.3% glycerol and 0.18% sodium benzoate (173). The producer had used ethanol as an antimicrobial agent, glycerol as a moisturiser and Vademecum for taste and comfort.

It is believed that ethanol and glycerol in such low concentrations have little documentable bacteriostatic effect. Sodium benzoate on the other hand, has antimicrobial effect in concentrations starting from 0.01%. However, the effect is largely dependant on the acidity of the solution, being at its maximum at pH 2.5-4.0 and weak above pH 4.5. In an expert report made for the Directorate of Health and Social Services the acidity measured in a limited number of swabs was approximately pH 7.0 (173). We remade a small batch of the moisturising liquid and measured a pH of 6.8 (174). At this pH level sodium benzoate has minimal antibacterial effect.

In a controlled test two different strains of planktonic *P. aeruginosa* were added to the moisturising liquid. The bacteria were rapidly inactivated even in a 1:10 dilution of the liquid. So far it has not been elucidated what ingredients in the moisturising liquid that have the rapid bactericidal effect on *P. aeruginosa*. Nonetheless, the moisturising liquid inactivated planktonic bacteria even at a pH level of 6.8. Still the outbreak strain of *P. aeruginosa* survived in the production plant and in a number of wrapped swabs.

Several hypotheses have been put forward to explain this apparent discrepancy. Our main hypothesis is biofilm formation. *P. aeruginosa* is well known to form biofilms (111, 134, 135). Bacteria in biofilms have an increased ability to withstand antibiotics and disinfectants

(111, 135). We have shown that the cleaning and disinfection process in the production line probably did not reach all areas in the tank and piping system. A mature biofilm may spread to new locations through single cell dispersal or the shedding of clumps of biofilm (111, 112, 134). Such clumps still have biofilm properties and may well have survived on a wrapped swab surrounded by a liquid with antimicrobial effect.

An alternative hypothesis has been launched in the popular and medical press (175-179). This suggests that the pH may have risen in the liquid inside the wraps due to the chemical influence either from the glue used to attach the foam rubber heads to the stick or other materials inside the wrap. According to this hypothesis an increase in the pH may have lowered the antimicrobial effect of sodium benzoate. It is an interesting hypothesis. However it has one major flaw: The pH of the moisturising liquid and in different approximate remakes of it has been measured to be in the range of 6.4-7.7, and never below 6.4 (174, 178). In this range sodium benzoate has minimal preservative effect and pH alterations within this range do not alter sodium benzoate's bactericidal ability.

In conclusion, we still do not know which ingredients in the Dent-O-Sept liquid that are exerting its rapid bacteriostatic effect. It cannot be singly ethanol, glycerol or sodium benzoate in the concentrations and pH range of the mixed liquid. Whether they may have the tested bacteriostatic effect on planktonic *P. aeruginosa* by working in consort or if there are other unknown preservatives in the Vademecum mouth wash has not been tested. Out of pure curiosity it would be interesting to find the answer. However, the production of Dent-O-Sept swabs ceased in 2002 and any new producer of moist swabs would need to produce a declaration of conformity for their product where the issue is addressed.

6.5. Medical devices as a source of infection

The quantity of published hospital outbreaks is almost innumerable. A quick Internet search on PubMed searching for "hospital" and "outbreak" yielded 9384 articles. In the winter season epidemics of norovirus infections can be rampant as can influenza epidemics and outbreaks with the spread of Methicillin resistant *Staphylococcus aureus*.

Medical devices and other equipment, solutions and liquids are responsible for a considerable number of outbreaks. Often they are related to moisture in one way or another.

Pseudomonas aeruginosa is the dominant causative agent for outbreaks in hospital among the gram-negative opportunistic bacteria (35-37, 39, 41-44, 46, 49, 50, 53-55, 57-59, 64-67, 70, 72, 84, 85, 99, 104, 180-183). Most are caused by medical devices directly or indirectly but

some are related to liquids like flush devices, hydrotherapy, bath toys or even bottled drinking water to name a few (35, 37, 39, 42, 67, 70, 85, 99, 104, 181, 182). Outbreaks have even been caused by lens prosthesis implants (44) and after a multiple organ transplantation (58).

Of the other microbes related to medical devices and other equipment (74-76, 78-80, 86-90, 92, 95-98, 103, 105) many are like *P. aeruginosa* gram-negative bacteria like *Serratia marcescens* (65, 74-78), *Acinetobacter baumannii* and other *Acinetobacter* spp. (79, 80), other species of *Pseudomonas* (84-87), *Stenotrophomonas maltophilia* (87, 88), and *Burkholderia cepacia* (89, 90, 92).

All outbreaks are unique in one or more ways regarding causative agent, mode of transmission, duration, extensiveness, size, consequences, liability, etc. In most of these aspects the Dent-O-Sept outbreak is not unique, encompassing *P. aeruginosa*, a medical device, moisture, hospital setting, ICU, and a mixture of direct transmission from the medical device and indirect via the hospital environment. However, the Dent-O-Sept outbreak is possibly the largest *Pseudomonas* outbreak ever to be published regarding the number affected (231 patients, 161 with infection, 70 colonised), the number of deaths (71 while hospitalised, and where the *Pseudomonas* infection probably contributed to the death for 34 of them) and the number of health care institutions involved (24 hospitals).

6.6. Claiming causality

In the scientific laboratory all variables are known, and the scientist can change one factor at the time and measure its effect on the outcome variables. Modern epidemiology is complex; a range of factors contribute to an outcome, many of which are unknown. And in outbreak investigations, the investigator cannot control the exposure variables because they have already occurred. In addition, there is a time constraint.

The basic concept of causality is a fundamental and integral part of daily language. However, when trying to define the term in philosophy (146, 148, 149), science (140, 144, 145, 147, 152-154, 184-186) or law (187-191), it becomes utterly difficult and complex to a degree that some even discourages the use of the term. Instead euphemisms like ‘associated with’, ‘linked to’, ‘related to’, and ‘due to’ are used signalling causation but to a somewhat weaker degree.

Tradition on causal theories differs between the different scientific disciplines. Applying the various theories on the major actors and events in the outbreak shed light from different angles and proved to be helpful in the analysis. Especially using counterfactual reasoning which is frequently used in tort law simplified the complex picture. Counterfactual theories

have been used in epidemiology where the term "the counterfactual ideal" has been coined to describe the perfect unexposed experience (192). However, it would be helpful to develop the counterfactual reasoning from tort law further in epidemiology. When used on the Dent-O-Sept outbreak the main actors responsible were easier to identify. Our analysis concluded clearly that the major necessary condition causing the outbreak was the contamination of the swabs in the production facility. Without this contamination, the Dent-O-Sept outbreak would not have happened. Many other factors contributed to the outbreak and the size of it, the reuse of the single use swabs in the hospitals being the most important.

Moral responsibility is related to legal responsibility. As mentioned earlier, outbreak investigations have similarities with police investigations. In large outbreaks, especially where people are injured or die, the police regularly start an investigation. With a clear causal association between the contaminated swabs and *Pseudomonas* infection and death among patients, it would have been interesting to see whether the conclusion would have been the same in a criminal court case.

6.7. Invasive *Pseudomonas aeruginosa* infection

For the period 1999-2002, we found a rate of invasive *P. aeruginosa* infection of 0.20 per 1 000 hospital stays. Studies from other countries have indicated much higher rates, between 0.94 and 1.8 per 1000 hospital stays (114, 120, 123-125, 127, 128, 130) in tertiary referral hospitals and university hospitals and 0.43 and 0.59 per 1000 hospital stays in community hospitals (118, 119). The low incidence in Norway compared with studies from other countries may partly be explained by study design. Our study was nationwide and population-based and included all somatic hospital stays in Norway. Certain hospital departments such as dermatology and gynaecology and obstetrics, and community or specialty hospitals with less than 5000 discharges per year, are known to have low rates of *P. aeruginosa* invasive infection so these departments may have contributed to the low reported overall rates. However, no Norwegian hospital had a higher rate than 0.42 per 1000 hospital stays.

To our knowledge only one other population based study on invasive *P. aeruginosa* infections has been published (193). This study based on the population of a county in Minnesota, USA in 1999-2006, has higher incidence rates than our findings with 10.8 patients per 100 000 pyar in men and 3.7 in women compared with 4.3 and 2.0 respectively per 100 000 pyar in our study.

We suggest that one explanation for the low rates was the prudent use of antibiotics in hospitals in Norway with low overall antibiotic consumption and low use of broad spectrum antibiotics. The use of narrow-spectrum drugs is encouraged (194-196). Indiscriminate use of antibiotics, especially broad-spectrum antibiotics, is known to be associated with development of resistance and selection of resistant bacteria, such as *P. aeruginosa* (4-7, 197).

The use of empiric broad spectrum antibiotics such as most 3rd generation cephalosporins, which may give *Pseudomonas* an advantage, are generally discouraged. For empiric treatment of septicaemia where broad spectrum coverage is necessary, penicillin plus an aminoglycoside is the recommended standard treatment in most hospital departments. In general, aminoglycosides are active against *Pseudomonas* and have a high threshold for development of resistance (tobramycin considered most active), thereby avoiding selection pressure favouring these bacteria (198).

Several risk factors for invasive *P. aeruginosa* infection and death have previously been identified (4, 5, 7, 113, 123, 126, 127, 129, 130, 199). Using complete national discharge statistics, we were able to calculate absolute rates of infection among patients with various underlying diseases. Our study confirms that *P. aeruginosa* infection is a major risk to patients with cancer, immunodeficiency, renal failure and certain other diseases.

Invasive *P. aeruginosa* infection is a serious disease with a high CFR, 35% in our study. Most patients who died from invasive *P. aeruginosa* infection died within a short time of being diagnosed. The patient groups with the following underlying diseases had the highest mortality risk per 1000 discharges: Malignant neoplasms of lymphoid and haematopoietic tissue, other diseases of blood and blood-forming organs, organ transplantation and renal failure. Among the patients with invasive *P. aeruginosa* infection the following factors were independently associated with an increased CFR: Having an underlying risk diagnoses associated with *P. aeruginosa* infection; admission to an ICU; old age and immuno-suppressive treatment prior to infection. A clinical *P. aeruginosa* diagnosis only of UTI was protective, whereas meningitis, pneumonia and septicaemia increased the risk although not significantly. Other studies list similar risk factors (123, 126, 127, 130). As demonstrated by others (124), bacteraemic pneumonia had a high CFR.

6.8. Methodological weaknesses and limitations

An outbreak investigation is getting the best possible results in a chaotic world with limited resources and under heavy time pressure. Once the source of the outbreak is detected and

removed, the time constraint is relieved and a more thorough collection of data can be performed. However, if it takes too long the interest of the collaborators may subside and the quality of data may weaken. For the Dent-O-Sept outbreak the source was detected 8 April 2002, the scientific protocol following the outbreak investigation was finalised in late June 2002 and the last information entered into the crude database in August 2003.

There are always four possible explanations to a finding: 1. it can be true; 2. random error; 3. bias; or 4. confounding. In addition, there is always a possibility that the study design is flawed or inadequate to answer the hypothesis raised, or that inferences drawn from the results are invalid.

6.8.1. Random error

Random error is the portion of variation in a measurement that has no apparent connection to any other measurement or variable, generally regarded as due to chance (9). It is the variability in the data that we cannot readily explain after the systematic error is eliminated. A confidence interval is used to indicate the amount of random error in the estimation (192). By convention most results in epidemiology are given with a 95% confidence interval. This means that if the study were repeated many times and in an identical manner, the confidence interval should include the correct measure 95% of the time, on the condition that there are not other errors. For all effect ratios presented in our studies the point estimate with a 95 % confidence interval were given to account for possible random error.

6.8.2. Bias

Bias is defined in several ways. The textbook definition is all deviations of results or inferences from the truths (9). This will include random error, confounding, effect modification, flawed design, prejudices and wrong interpretations. In practical epidemiology the term bias is usually restricted to two forms of bias, selection bias and information bias.

Selection bias is mainly a problem that affects case-control studies where it gives rise to non-comparability between cases and controls when cases are not representative of the population that produced the cases. Selection bias can also occur in cohort studies when completeness of follow-up or case ascertainment differs between exposure categories.

Information bias is a flaw in measuring exposure or outcome data. There are two types of misclassification: 1. Non-differential misclassification where the probability of exposure being misclassified is the same regardless of outcome status. This type of misclassification will principally weaken the measured effect. 2. Differential misclassification where the

probability of exposure being misclassified depends on the outcome status and vice versa. Differential misclassification can either weaken or strengthen a measured effect. There are two main types of differential misclassifications: 1. Recall bias which is typical in case-control studies where cases and controls remember exposures differently. 2. Observer bias where those collecting data classifies data differently for different groups under study.

Case-control-study

This study in Paper I to identify causes of infection with the outbreak strain was restricted to severe disease, i.e. where the bacterium had been isolated from blood or CSF. The advantage was that the case definition was clearer and we need not speculate whether for example a skin infection was merely a colonisation. However, by only selecting patients with the most severe disease, the analysis loses generalisability. That said, from a clinical and epidemiological perspective, it is more important to look for risk factors among the most severely ill patients.

The aim of the study was to to identify the causes for infection with the outbreak strain as compared to being infected with other strains of *P. aeruginosa*. The aim was not to identify risk factors for *P. aeruginosa* infection by *any* strain. This aim had consequences for the choice of control group, namely patients with non-outbreak strain *P. aeruginosa* identified in blood or CSF. We reasoned that these patients had the same risk factors as cases for getting a severe *P. aeruginosa* infection, for instance immunosupression. Thus, we avoided the cumbersome task of controlling for the unspecific concept "underlying diasease". In epidemiological terms, we defined the source population as patients with *P. aeruginosa* isolated from blood or CSF. If, on the other hand, we had defined the source population as all hospitalised patients and sampled controls among them, the analysis would have been biased towards severity of disease and not of having the outbreak strain. It would have been hard to differentitate the risk factors of invasive *P. aeruginosa* infection in general from risk factors for outbreak strain infection. Had controls been selected among patients admitted to the ICU, the analysis could have been biased the other way as only 2/3 of the case patients had been admitted to the ICU during their stay.

Choosing as cases patients infected with one certain strain or subtype of a microbe and as controls patients with other strains or subtypes has been named a case-case study (200). This design can be useful for communicable diseases where only a potentially non-representative fraction of the infected patients are identified and thus eligible for being cases. This is the situation for salmonellosis for example where most patients do not seek medical care. To control for this selection process of those identified with the disease, controls are chosen

among those identified with other subtypes of the same microbe and who concequently have gone through the same selection process. In essence the case-control study in Paper I is a case-case study where cases and controls differ only regarding the genotype of *P. aeruginosa*. However, our purpose was not to control for the selection process of the cases. In the hospital setting blood cultures are taken from almost all patients were invasive disease is suspected and the detection rate is high. Hence, few patients with invasive *P. aeruginosa* infection will go undetected. Our choice of the case-case variant of the case-control study was instead guided by the aim of the study, i.e. to identify the cause of the outbreak as efficiently as possible.

Microbiology

The main challenge in outbreak investigations is that of bias. It is crucial to detect all cases and to gather correct information on all of them which can be said to be a form of selection bias. The clear assignment from the Ministry and the politicians was to detect everyone who was colonised or infected with the outbreak strain of *Pseudomonas aeruginosa*. All stored cultures of *Pseudomonas* were genotyped. Laboratories had mainly stored cultures of *P. aeruginosa* from blood and CSF and in the retrospective part of the study we may have missed cases with the outbreak strain from other body sites. In the prospective part of the study we believe that a great majority of the cases were detected. For the whole period we do not know how many with the outbreak strain that were not sampled, especially outside hospitals. In conclusion the real number of affected patients was probably much higher. However, there is good reason to believe that most of the missing cases have only been colonised or had less serious infections. Consequently one can claim a selection bias towards more serious cases, but none of the studies intended to give a numerical distribution of the clinical presentation of *Pseudomonas* infection.

For the study of invasive *Pseudomonas aeruginosa* infection the same dataset for the cases was used. As the laboratories keep records and store samples from blood and CSF we believe few cases were missed. Some samples were not available for genotyping which could have influenced the comparison between the outbreak strain and the others.

Five laboratories genotyped the bacteria to identify the outbreak strain, four using the same PFGE typing method, the fifth AFLP. The methods had been compared in their ability to detect the outbreak strain and no discrepancies in the results were detected.

Environmental sampling

All initial environmental sampling from the production facilities and from the swabs were done by direct seeding on a lactose and blood agar dish. Without prior enrichment in a growth broth we may have some false negative results. This was indicated when the laboratory at the municipal Food Control Authority some weeks after the system audit performed repeated environmental sampling of the production facilities and used an enrichment broth. Then they detected the *Pseudomonas* bacterium in several places where the system audit had not (170). Whether to use an enrichment broth or not is a trade off between spending resources and increasing the sensitivity somewhat. More than 1500 swabs were examined in addition to patients and environmental samples.

Clinical information

For all included patients the clinicians were obliged to fill in a one page questionnaire; and we received it for all but two patients. Although great efforts were made to ensure that the outbreak investigation was given highest priority, the quality of the returned questionnaires varied. Extensive efforts were made to ensure completeness and quality of the data, including contacting the clinicians and linkage with the National Population Registry to search for deaths among patients. Consequently, we believe most of the collected patient data is accurate, but some information may have been missed, especially regarding some of the subordinate discharge diagnoses. However, all but 3 of the 567 patients had at least one main underlying disease recorded other than the *Pseudomonas* infection. There were few patients in several of the risk diagnosis groups (as listed in Paper IV, table 3). Missing information in either of these groups could have influenced the incidence and mortality rates and the case fatality, and the results need to be interpreted with caution. However, all numerators and denominators are given in the table, making it possible for the reader to judge the results.

Most of the variables are factual (e.g. whether the patient had been admitted to the ICU during the hospital stay) and are not much influenced by observation bias. However, one variable especially was vulnerable for subjective assessment: If the patient died, “May the patient’s death be related to the detection of *Pseudomonas* infection?”. To control for that two of the paper’s authors meticulously assessed all available information for each of the dead patients, including the clinician's assessment, underlying illnesses, dates of onset, diagnosis and death, and other clinical and microbiological information. There was varying degree of missing values for the variables, most for the question asking whether the patient had used the Dent-O-Sept swab during the stay. This variable was also the one most subjected to observation

bias as most clinicians knew whether the patient had harboured the outbreak strain or not. However, for 1/3 of the patient with the outbreak strain the clinician wrote that that patient had not or probably not used the swab.

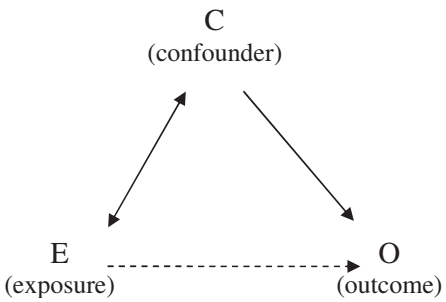
Denominator data

Denominator data in Paper IV were all collected from quality controlled national databases like Statistics Norway and The Norwegian Patient Register. There is always a challenge in combining data from different datasets as the variable definitions may vary. For the dataset in Paper IV it was most crucial for the definition of underlying disease. From the national register we picked the main and up to seven subordinate discharge diagnoses whereas on the clinician's forms there was free space to record as many as they wanted. No more than 10 underlying diagnoses were detected and recorded.

6.8.3. Confounding

The word confounding is derived from Latin *confundere* meaning to mix together (9). A confounder (C) is a variable that is associated with the exposure variable (E) and has an effect on the outcome (O) and is not an intermediate factor in the causal pathway between the exposure and outcome variables (figure 6). For example a measured association between coffee drinking and pancreatic cancer may be due to a higher proportion of coffee drinkers among smokers than among non-smokers. A reanalysis stratified by smoking status may show no association between coffee drinking and cancer in either group of smoking status. Then smoking status is confounding the first measured association. However, not all associated variables are confounders (201).

Figure 6. *Association between exposure, outcome and confounder*



Confounding can be controlled for in several ways: **1. Restriction** of the population or cases studied; **2. Matching** cases and controls on certain variables; **3. Randomisation** of study subjects in experimental-type studies; **4. Stratification** of the study population on the presumed confounding variables; and **5. Multivariable modelling**, a powerful technique to control for several possible confounders. The first three methods are study design strategies and the last two are analysis strategies.

Most of the results from the outbreak investigation are descriptive with no association between variables posed and no statistical analysis performed. As described above, all effect ratios are presented with a point estimate and a 95 % confidence interval to account for possible random error.

In Paper I we showed a clear association between the use of the Dent-O-Sept swab and having the outbreak strain of *Pseudomonas aeruginosa*. When controlling for known possible confounders the strength of association dropped from OR=7.9 to OR=5.3. There may also be other, unknown confounders that we did not control for. In addition, use of the Dent-O-Sept swab was the variable with the highest number of missing values in this case-control analysis (33 of 198 missing). If differential, it may introduce observation bias.

However, the association between having the outbreak strain and the Dent-O-Sept swab was also established in other ways. Genotypically identical strains of the bacterium were detected in patients and in Dent-O-Sept swabs (and in the production plant) and thereby unequivocally establishing how the outbreak strain was brought into the health care services. In complex settings there are usually more than one factor contributing to an outcome. In this outbreak approximately 1/3 of the cases had not used the swab. Consequently, indirect transmission in the hospital setting via health care workers, medical devices or the environment appears to be an important mode of transmission in this outbreak, although not tested specifically in the outbreak investigation.

In two papers we analysed risk factors for dying among patients with invasive *Pseudomonas aeruginosa* infection. In Paper I several factors were identified in the univariable analysis but only having used the Dent-O-Sept swab remained independently associated in the multivariable regression analysis. Logically it does not make sense that using a mouth swab would increase the risk of dying among patients with invasive *P. aeruginosa* infection. Consequently we interpreted the results as residual confounding where having used the swab served as a marker for severe underlying disease.

In Paper IV including a larger number of patients over a longer time interval (567 patients as compared with 198 in Paper I) and several new variables, more variables were found to be independently associated with an increased risk of dying in the multivariable regression analysis. This time, swab use was not independently associated with dying. One explanation for the difference can be controlling for the residual confounding by adding new clinical variables on underlying disease and clinical manifestation of the *P. aeruginosa* infection; another is larger inaccuracies in the recording of Dent-O-Sept swab use outside of the outbreak period.

In Paper IV, except for the analysis of risk factors for dying, only univariable analyses were performed because denominator data were aggregate data. Thus, we were unable to do individual level analyses of relative risks for infection, nor controlling for potential confounders by multivariable regression analysis. As age and gender are the two major possible confounders that influence the incidence risks, we may not easily compare the groups of underlying diseases. As explained above for the CFR we were able to control for confounders by binomial multivariable regression.

6.8.4. Effect modification

Effect modification – sometimes called effect-measure modification – refers to the situation in which a measure of effect changes over values of some other variable (192). Effect modification can be tested statistically by looking for interaction using statistical software programmes. However, eyeballing and thorough assessment is the best way to judge whether there is effect modification. In Paper I we assessed whether there was effect modification with the two variables "use of swab" and "receipt of mechanical ventilation" regarding the outcome of having the outbreak strain of *P. aeruginosa*. One may hypothesise that the presence or absence of swab use in mechanically ventilated patients influenced the variable "receipt of mechanical ventilation" as a risk factor for having the outbreak strain. We did not find clear indications for effect modification. However, one should bear in mind that the figures are small (Paper I, Table 3).

6.8.5. Analysis of causality

Paper III on causality is of a different genre than the other three. Rather than being quantitative and analysing figures it is qualitative and analyses and debates concepts and theories. Consequently there are no figures with confidence intervals but a discourse aiming at

elucidating the concept of causality through a real life example. In its nature the paper is more subjective.

7. Main conclusions and further studies

7.1. Main conclusions

In the outbreak with *Pseudomonas aeruginosa* in Norway, we detected a total of 231 patients with genotypically identical strains of the bacterium from 24 hospitals in 2000-2002. Seventy-one of the patients died while hospitalised, and for 34 the *Pseudomonas* infection probably contributed to the patients' deaths. The same outbreak strain was isolated from 76 mouth swabs called Dent-O-Sept from 12 different batches produced from September 2001 to April 2002 and from the production facility. In total more than 250 of 1565 examined swabs were contaminated with one or more microbial species.

In a complex situation like an outbreak with many acts, actors and factors playing larger or smaller parts, applying various theories for causality and responsibility from different fields like science, philosophy and law – especially legal theories and counterfactual reasoning – helped elucidating their roles and responsibilities. Many factors contributed to causing the outbreak, but contamination of a medical device in the production facility was the major necessary condition. The reuse of the medical device in hospitals primarily contributed to the size of the outbreak. In addition there were many errors and flaws in the chain from the production of the swabs, through purchasing and storage systems in the health care institutions to the use of the swabs and reporting of defective devices. The unintended error by its producer – and to a minor extent by the hospital practice – was mainly due to non-application of relevant knowledge and skills, and appears to constitute professional negligence.

This outbreak is possibly the largest published *P. aeruginosa* outbreak to date. Although *P. aeruginosa* usually do not cause infection in healthy persons, it frequently does in patients with certain underlying diseases, and in patients with disrupted barriers, especially in the ICU. Invasive *P. aeruginosa* infection is a rare disease with an incidence rate of 3.16 per 100 000 pyar or 0.20 per 1 000 hospital stays, but is very serious for those contracting it with a 30 day case fatality rate of 33%. Patients with malignant neoplasms of lymphoid and haematopoietic tissue and other diseases of blood and blood-forming organs have the highest risk of infection. Prudent antibiotic use is one possible explanation for much lower rates of infection in Norway compared with all other published studies from other countries.

Medical devices, moist equipment and solutions and moist environments are frequently associated with outbreaks with *P. aeruginosa* and related moisture-prone bacteria. Lack of

adherence to standard precautions for infection control and prevention by hospital personnel contributes to the propagation of these outbreaks.

Biofilm formation is possibly the more common of the two distinct modes of behaviour for bacteria; the other being the planktonic mode. Biofilm formation is the most plausible explanation for the survival of the bacteria in the production facilities and in the wrapped swabs. Bacterial biofilms are less sensitive to disinfection and make it more difficult to eradicate. Not abiding by the production regulations, e.g. the requirement to have quality assurance systems including an effective microbiological control system, made the contamination possible in the production process.

Outbreak investigations are essential to detect causes of an outbreak and to gain experience in order to prevent their recurrence. Investigating large, multicentre outbreaks is resource demanding and necessitates a defined network structure where everyone know their role and qualifications and try upmost to cooperate. Expertise in a variety of fields is essential. Molecular finger-printing techniques to identify the outbreak strain of the microbe and discriminate against other strains have become an indispensable part of most outbreak investigations.

7.2. Proposed actions and further studies

After the outbreak all parties involved, from producers and private companies to hospitals and all national, administrative bodies for health services, revised their guidelines, made reviews and summaries and proposed action plans. A national "Action plan to prevent hospital acquired infections" was made as a direct consequence of the outbreak (161) and has recently been revised (3).

Medical devices

- The control and audit of producers of medical devices should improve, also of producers of non-sterile medical devices in Class I. Increased resources should be allocated on a national level to support infection control personnel in health care institutions who have enquiries about medical devices.
- The reporting systems for errors in medical devices and other equipment and facilities in the health services are complex and cumbersome. A revision and coordination has started but is still not finalised.
- Moist medical devices are prone to cause infections and outbreaks if not properly manufactured and used. The Council Directive (26) may have been sufficient to prevent

the outbreak if it had been followed by the producer, but it is not optimal. Preservatives with documented effect should be obligatory in all moist, non-sterile medical devices, parallel to what exists for cosmetics and medicinal products.

- A systematic assessment should be made for the level of disinfection or sterility of all devices, other equipment, solutions, food, water and medicines to be used on different groups of infection-prone patients, especially in ICUs. Only documentable quality controlled, high-level disinfected products and items should be used in the oropharynx of susceptible patients.

Outbreak investigations

- Small outbreaks are not very resource-demanding and can in most instances be covered through regular budgets. Large outbreaks can be very costly. The process of securing financing for genotyping of the many hundred strains of *P. aeruginosa* was unnecessarily cumbersome. Streamlining of these processes needs to come in place.

- Infection control personnel in hospitals should have easy access to theoretical and practical training in epidemiology and outbreak investigation.

Infection control and prevention

National action plans to prevent HAIs list many admirable aims and measures and contain descriptions used on festive occasions. However, in contrast to several institutions on a national level, most hospital administrations do not give priority and resources to infection control and prevention. Infection control does not have a high status in the hospital hierarchy, is understaffed, and is not able to fulfil the requirements set up in laws and regulations.

- The four Regional Health Authorities and the administrations of the local Health Trusts need to review the new national strategy, revise their current infection control strategy plans and make detailed plans for the implementation of this new strategy.

Surveillance

There is no national surveillance system for *P. aeruginosa* and other similar opportunistic bacteria. And probably it will be unwise to include them in the current national system (202). Most hospitals have cooperation between infection control and the microbiology laboratories and some have local systems for reporting of microbiological detections in the hospital.

- An assessment should be done at the local or regional level of which microbes that are desirable to watch. Local systems in hospitals need to be developed for efficient surveillance of these microbes.
- On a national level automatic harvesting of data will be possible through the national health net system. When in place NIPH should in collaboration with the microbiology laboratories develop a system for surveillance of indicator microbes.
- One hospital has used a surveillance method from private industry called statistical process control (SPC) on the *Pseudomonas* outbreak to see if the outbreak could have been detected earlier (203). This method and others need to be developed further and implemented if effective.

***Pseudomonas* infections**

- More studies are needed on risk factors for infection caused by opportunistic pathogens among different groups of debilitated patients and how to prevent them from occurring.
- More studies are needed on bacteria forming biofilms and ways of eradicating them from environmental surfaces, medical devices and biofilm forming infections in humans.

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