Outbreaks of pseudo-infection due to contamination of specimens have been described, often as localised incidents. From August 2006, several English hospital laboratories began to refer an unusually high number of isolates of the fungus Paecilomyces variotii from clinical specimens to the national mycology reference laboratory for microbiological testing. We describe the methods used during the outbreak investigation in order to provide infection control specialists with an overview of how such national incidents may be investigated. We surveyed the hospitals reporting the contamination problem and conducted microbiological and environmental sampling. We applied analytical epidemiology to supply chain data, comparing the supply lines of key equipment to affected and unaffected hospitals in England. The survey was useful to describe procedures and equipment in use in the hospitals reporting the problem. The microbiological aspects of the investigation helped us understand how the fungal spores were distributed in the hospital environment. In the supply chain investigation we used data that was previously only used for logistical purposes. Overall the investigation were methodologically challenging, with no existing protocol to guide the investigators. To our knowledge, this is a novel approach to the investigation of such a widespread contamination problem, affecting geographically disparate hospitals at the same time.

Introduction
Hospital equipment contamination can lead to a so-called pseudo-infection: the isolation of a pathogen in clinical specimens without clinical relevance [1]. Outbreaks of pseudo-infection are referred to as pseudo-outbreaks. Clinical specimen contamination in multiple hospitals occurs, they are however more commonly seen in the form of localised problems due to inadequate sampling techniques, presence of the contaminants (e.g. fungal spores) in the hospital or laboratory environment, or decontamination failures [2-6]. Simultaneous pseudo-outbreaks in multiple hospitals are rare, being more likely the result of contamination in single hospitals or laboratories [7-9]. A pseudo-outbreak involving Ochrobactrum anthropii contamination of blood culture bottles occurred in the United Kingdom in 2001 [10]. It is important to investigate such incidents even in the absence of clinical infections as the laboratory results may lead to patients being treated with drugs which may be toxic or cause side effects and which are often expensive.

Between August and September 2006, 34 laboratories across England and one from Northern Ireland reported identification of 77 isolates of the fungus Paecilomyces variotii from clinical specimens, primarily blood cultures, to the Health Protection Agency (HPA) Mycology Reference Laboratory (MRL) for species confirmation [11]. Given the unusually high number of isolates (the MRL would usually receive only five or six P. variotii isolates per year) [11] the MRL subsequently notified the healthcare-associated infection and antimicrobial resistance department of the HPA Centre for Infections of this increase. Initial communication with referring laboratories indicated that the fungus had been isolated directly from blood culture bottles, that different blood culture systems were used in the hospitals and that, in most instances, the isolates were considered not to be clinically significant. Contamination of blood sampling equipment was therefore hypothesized and a national Incident Management Team (IMT) established. The team included experts in epidemiology of hospital acquired infections, mycology, and laboratory standards [11-13].

We describe the methods used to investigate the outbreak in order to provide infection control and public health specialists with an overview of how such national incidents may be investigated and to provide recommendations for future investigations.

Investigation
To our best knowledge, no standardised or field-tested methods existed to guide the investigation for this multisite outbreak of pseudo-fungaemia. We devised and pursued four investigative strands following active case finding which included:

- constructing and performing a descriptive survey;
- characterising samples microbiologically;
- performing environmental investigations; and
- investigating the supply chain.

Active case finding
The IMT notified the Medicines and Healthcare products Regulatory Agency (MHRA) of the fact that an unusually high amount of clinical samples from across the country were found
positive with Paecilomyces variotii and of the planned investigation. Relevant experts in microbiology, infection control and public health were alerted about the event and the forthcoming investigation through an article in the Communicable Disease Report (CDR) Weekly public health bulletin [12] and an email alert cascaded to all consultant microbiologists in England via the HPA Regional Microbiology Network. All stakeholders were asked to notify the investigation team of any isolates of Paecilomyces variotii after 1 July 2006. Alerts were also transmitted via the relevant public health bodies of Northern Ireland (Department of Health, Social Services and Public Safety), Wales (National Public Health Service for Wales) and Scotland (Health Protection Scotland). Furthermore, an article was published in Eurosurveillance to generate information about whether a rise in Paecilomyces variotii isolates had been noticed elsewhere in Europe [11].

Descriptive survey
Preliminary information indicated that the fungus was being identified directly in blood culture bottles from two different brands of blood culture systems. We conducted a questionnaire survey in order to understand how samples were taken in the affected hospitals and to generate hypotheses on the source of the contamination.

Methods
We sent a questionnaire to staff of every hospital laboratory that reported an isolate of Paecilomyces to collect descriptive data on the contaminated specimens and asked about all species isolated, including non-Paecilomyces contaminants, the specialty from which the contaminated samples were referred and the procedures and equipment used for collection of the samples. We also asked if the laboratory had made any changes in the supplies of equipment or in the standard procedures used for blood sampling and processing the samples. Furthermore, we asked about the assumed clinical relevance of the findings and if antifungal therapy had been initiated for patients that were associated with the positive Paecilomyces samples.

The questionnaire was sent via email to the reporting laboratories, which then had the option to send it back via email or post. Data from the questionnaire were entered into a customized MS Access database. Analysis was conducted with MS Excel and STATA version 8 (Stata, College Station, TX).

Lessons learned
With the survey we were able to describe how the contaminated samples were collected in the hospitals and how they were processed in the laboratories, although we were not able to formulate hypotheses to test. To collect timely, accurate and comprehensive information to identify the source of a pseudo-infection with a questionnaire is difficult. We speculate that the investigation of pseudo-outbreaks due to contamination of equipment is of little priority for physicians and hospital microbiologists and this leads to a low response rate and delay in responding. In our case the questionnaires provided insufficient answers and further investigation was required. In order obtain results and a high response rate, a web based survey may be more suitable for such incidents instead of sending a questionnaire via email as we did for this investigation.

Microbiological characterisation
Since different species of Paecilomyces present differences in response to treatment (antifungal sensitivity), the differentiation between members of the Paecilomyces complex is clinically relevant [14].

Methods
Isolates received by the MRL were initially characterised using phenotypic identification methods in which the macroscopic and microscopic morphology was examined. Strains were then subjected to broth microdilution susceptibility testing with a range of antifungal agents with systemic activity by means of the National Committee on Clinical Laboratory Standards (NCCLS now Clinical and Laboratory Standards Institute - CLSI) method for filamentous fungi M38-A [15]. Paecilomyces environmental isolates and isolates from clinical specimens were sent by the MRL to a laboratory in the Netherlands (the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre), which specialises in typing mould isolates, for sequencing of part of the beta-tubulin gene in order to compare the profiles of the isolates.

Lessons learned
Molecular typing of these organisms requires highly specialised laboratories. This may present difficulties in logistics, turnaround time and cost. In order to overcome this current limitation we recommend typing a representative sample of isolates received in any similar occurrences.

Environmental investigation
Environmental contamination, such as through Aspergillus spores released during construction work, is known to play a part in fungal infections [7,16]. Information on the ecology of Paecilomyces indicates that it is commonly associated with soil and decaying vegetable matter and has been proven to colonise also plastic surfaces, saline solutions and water damaged organic material like wood, cardboard or fiberboard [17-19]. Consequently our investigation included environmental investigations to assess whether:

- any equipment implicated could be identified;
- evidence could be provided to prove that contaminated equipment had been in, and contaminated the patient care areas sampled, and
- specimens for typing could be provided [20].

Methods
The hospitals that reported isolates (specimens taken less than four weeks before notification to the IMT) were targeted to increase the chance of any contaminated equipment still being present on the premises. We asked laboratories reporting Paecilomyces to undertake microbiological sampling of premises and equipment used when, or associated with, sampling eventually found to be positive. To increase the chance of any contaminated equipment still being present on the premises we asked only the hospitals that had found isolates within four weeks before notification to the IMT to perform microbiological investigations.

Samples of consumable equipment (i.e. syringes, needles, skin swabs, adaptor caps and butterfly needles) obtained from the wards where the blood samples were taken were either sent to the HPA Mycology Reference Laboratory for testing or tested at site of collection. Where possible, equipment belonging to the same batch as that used during the contamination episode was requested, as well as sampling of the outside packaging of these items for testing of fungal contamination (e.g. empty box from skin swabs), especially if there was any suggestion of spoilage. Environmental swabs of the areas surrounding the patient bed or other patient
care areas, where the original positive samples were obtained, were also requested. Settle plates for environmental sampling of fungal spores were also positioned conveniently, depending on the respective location, above head-height in the same areas. The environmental samples were sent for molecular typing to verify if the same strain was implicated both in clinical and environmental isolates.

**Lessons learned**

Environmental investigation was in many cases delayed, because the IMT became aware of the majority of positive isolates only after it had sent out the alerts. However, we recommend keeping the interval between collection of contaminated clinical samples and environmental analysis to a minimum. Environmental sampling in warehouses that supply equipment to affected hospitals and hospital storage areas should also be considered.

**Supply chain investigation**

When using a traditional case-control study to analyse pseudo-outbreaks it may difficult to select the appropriate controls, because all controls may share the same exposures as the cases (i.e. processed by same technician, using same equipment, etc.) thus making internal comparison inappropriate [21]. Other analytical approaches could be employed that compare sites (hospitals or wards) affected by a particular problem with the unaffected ones [22,23].

Because of the practical constraints discussed above, we decided to take a further investigative approach, analysing the supply chain of consumable equipment to the NHS (National Health Service) Acute Hospital Trusts (hospitals). Supply chain investigations are usually used in outbreaks of foodborne diseases to trace back the affected food items [24,25]. In our investigation we focused on the supply chain of blood taking equipment, but instead of using the standard retrospective approach, we analysed how the supply-chain differed between affected and unaffected hospitals.

In England, hospital equipment is supplied to hospitals via a centralised system, which is managed by the NHS Supply Chain, a subsidiary of DHL (Dalsey, Hillblom and Lynn) express mail services. This agency holds all the information on the equipment supplied to the hospitals in an electronic database, published twice a year. The catalogue has almost 50,000 entries, one for each product supplied. Each product is identified by a unique National Product Code (NPC). The NHS Supply Chain can identify which products are distributed to each hospital, when and how they are transported and in what quantity. The database provides information if specific products were returned to the sender and in what quantity, but information on the reasons for returning is not given. The NHS Supply Chain operates through six different stations.

across England; for each of these stations there is a warehouse that stores all the supplies for the area covered. In general each individual hospital is supplied by one warehouse, although goods may be transferred between warehouses (Figure).

Methods

As contamination of blood sampling equipment was suspected, the investigation focused only on the products involved in blood taking listed in the catalogue. We created a short list from the catalogue of products likely to have been used in or around blood sampling procedures (Table).

For each product, information was obtained about the supplying warehouse, the brand, the supplier, the quantity supplied, when it was delivered and to which hospital. It was therefore possible to identify if a product was supplied to a hospital that reported the isolation of Paecilomyces, or to one that did not report this problem. This information was available only for English hospitals since the NHS Supply Chain operates only in England.

We designed a cohort study including all NHS Acute Hospital Trusts (hospitals) in England.

A case was defined as an affected hospital and a non-case as an unaffected hospital. We were interested in measuring the likelihood of a product being supplied to a hospital, so each one of the single entries (products) in the reduced catalogue was multiplied by each hospital to which it was supplied. According to a hierarchical approach, from large categories to smaller ones, we considered the following risk factors: supplying warehouse, product brand, supplier, product category. Due to the size of the database it was not possible to use every single product as a risk factor. We focused on single products if positive associations were found in the broad categories mentioned above.

Univariate analysis was first undertaken, followed by multivariable analysis (a logistic regression model with random components). We also used a log-linear model to investigate the following variables in the NHS product catalogue: supplier (supplying company if random from NHS supply chain), section (equipment category), and storing warehouse. The model included two correlated random effects corresponding to the two versions of the supply data, one created for the period before the contamination problem was first noticed and one for the period after it became evident. This model allows any possible changes in the supply-chain that may have explained the problem.

We used STATA version 8 (Stata, College Station, TX) and SAS Version 9 (Cary, NC: SAS Institute Inc) for analysis.

Lessons learned

With this investigation we discovered that very accurate and comprehensive data on the supplies to hospitals can be obtained in a timely way in England and possibly elsewhere where a central logistic authority exists in the public health system. We did not have any indication of the source of contamination, so we could not use the supply chain data for tracing back any potentially contaminated equipment. Contaminated equipment was, however, not found. In this investigation it took one month between our first enquiries to NHS Supply Chain and obtaining the data in a format suitable for analysis, but this time could be shortened now that we are aware of what kind of data is available and how to process it.

The involvement of the supply chain authority happened when the outbreak was already tailing off. In case of a similar problem occurring again, we recommend earlier involvement by transmitting alerts not only to health professionals but also directly to the supply chain authorities. Even with detailed analysis of the supply chain, it still can be difficult to identify the exact source of contamination because the transfer of goods between warehouses could spread the contaminant throughout the supply chain. Similarly, cross-contamination of equipment that shared the same storing area for a time may occur, creating multiple sources of contamination which are difficult to disentangle through the use of epidemiological analysis.

Discussion and conclusions

We developed an investigation protocol combining microbiological and epidemiological techniques. When more traditional investigative approaches (descriptive epidemiology and environmental sampling) proved to be insufficient to identify the origin of the contamination problem we applied analytical epidemiology to supply chain data. To our knowledge this use of supply chain data is a novel approach to the investigation of such a widespread contamination problem, affecting geographically disparate hospitals at the same time. We used a traditional cohort study, using the supply catalogue in the same way as the food menu would be used in a “classic” wedding food-poisoning outbreak. The large size of the dataset, with almost one thousand different products possibly implicated, and the fact that these data are normally intended for logistical purposes (e.g. ordering of hospital supplies) made this approach unusual. We experienced some methodological challenges investigating this problem, because there was no existing protocol to guide the IMT. We believe that documenting the methodological and organisational aspects of this investigation could inform future investigation of similar problems in the United Kingdom or elsewhere.

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References


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