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Epidemiology of multiple *Acinetobacter* outbreaks in The Netherlands during the period 1999–2001

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ABSTRACT

An increase in the number of outbreaks of *Acinetobacter* infection was notified in The Netherlands during 1999–2001. The present study compared the outbreaks at the species and strain levels, and analysed the epidemiology and control measures at the different locations. For each institute, three representative isolates from three patients were identified to the species and strain levels by genotyping methods. A questionnaire investigated the impact of the outbreak, the control measures that were taken, and the possible effects of the measures. Seven outbreaks were associated with *Acinetobacter baumannii* (three outbreaks with a strain designated strain A, two outbreaks with a strain designated strain B, and one outbreak each with strains designated C and D). An additional outbreak was caused by genomic species 13TU, which is related closely to *A. baumannii*. Strains B and D were identified as European clones III and II, respectively. Except for two hospitals with outbreaks caused by strain A, there was no known epidemiological link between the participating hospitals. In all hospitals the outbreak occurred on one or several intensive care units, and spread to other departments was noted in two hospitals. The number of patients affected ranged from six to 66 over a period of 2–22 months. In most outbreaks, patients were the likely reservoir from which spread occurred. In all hospitals, a large panel of measures was required to bring the outbreak to an end. Extensive environmental sampling yielded numerous positive samples in most but not all hospitals.

Keywords *Acinetobacter baumannii*, cross-infection, environmental contamination, genotypes, molecular typing, outbreak management

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INTRODUCTION

Acinetobacter is isolated regularly from clinical specimens in hospitals. Different species are found under endemic circumstances, with species belonging to the *Acinetobacter calcoaceticus–Acinetobacter baumannii* (Acb) complex constituting more than half of the isolates [1,2]. Nosocomial outbreaks of *Acinetobacter* occur frequently and are caused almost always by *A. baumannii* [3]. A common source is found in about half of all outbreaks, and elimination of this source leads rapidly to the end of the outbreak. When no common source is identified and cross-contamination seems to be the driving force of the outbreak, bringing the outbreak to an end is much

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more difficult. It is likely that survival of the microorganism in the environment, as shown by positive environmental cultures, plays an important role in the persistence of outbreaks.

Between 1999 and 2001, eight hospitals in The Netherlands experienced *Acinetobacter* outbreaks. The simultaneous occurrence of so many outbreaks in a relatively short period prompted an investigation with the following questions: (i) were some or all of the outbreaks caused by the same strain of *Acinetobacter*; (ii) what was the consequence for patients, measured as morbidity and mortality; (iii) what control measures were necessary to stop the outbreaks; and (iv) have environmental cultures been helpful in understanding transmission and taking control measures?

MATERIALS AND METHODS

Bacteriological investigations

Isolates were identified presumptively to the species level at each participating hospital using local diagnostic procedures, including use of API 20NE, Vitek 1 or Vitek 2 (bioMérieux, 's-Hertogenbosch, The Netherlands), or identification was limited to the genus level. From each hospital, three representative isolates from three patients (one from the beginning of the outbreak, one mid-way through, and one from the end) were sent to the Department of Infectious Diseases, Leiden University Medical Centre, where species identification was peformed by amplified rDNA restriction analysis (ARDRA) and amplified fragment length polymorphism (AFLP) analysis (see below). AFLP fingerprint analysis was also used to type isolates. Antibiotic susceptibility was determined in the Clinical Microbiology Laboratory of the Leiden University Medical Centre.

ARDRA

ARDRA was carried out as described previously [4]. In brief, separate aliquots of amplified 16S rDNA were digested with five restriction endonucleases (*Cfo*I, *Alu*I, *Mbo*I, *Rsa*I, *Msp*I). Fragments were separated by electrophoresis in agarose 2.5% w/v gels. Species identification was performed by comparing the profiles with those of a library of strains of (genomic) species described previously [4].

AFLP fingerprinting

Selective amplification of genomic restriction fragments using AFLP was performed as described by Nemec *et al*. [5]. Briefly, purified DNA was digested using *Eco*RI and *Mse*I, and this was followed by amplification with a Cy5-labelled *Eco*RI+A primer and an *Mse*I+C primer (A and C are selective bases). The ALFexpress II DNA analysis system (Amersham Biosciences, Roosendaal, The Netherlands) was used for fragment separation. Fingerprints of fragments in the range 50–500 bp were investigated by cluster analysis with Bionumerics v.2.0 (Applied Maths, Sint-Martens-Latem, Belgium), using the Pearson product moment coefficient (r) as similarity measure and the unweighted pair-group method with arithmetic averages (UPGMA) for grouping. For species identification, isolates were compared with a library of strains of all (genomic) species described previously, using a cut-off level of 50%, above which strains are considered to belong to the same species [5]. Isolates were considered to belong to the same strain if they grouped together at ≥90%.

ERIC-PCR fingerprinting

Comparative typing was based on fragments obtained by PCR using enterobacterial repetitive intergenic consensus (ERIC) sequences with two primers: ERIC1R, 5′-ATGTAAGCT-CCTGGGGATTCAC, and ERIC2, 5′-AAGTAAGTGACTGGG-GTGAGCG [6]. Three different PCR amplifications were performed: one with ERIC1R, one with ERIC2, and one with both primers. The results of the three fingerprints were combined to generate a single type, independent of the AFLP fingerprint analysis.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed with the Vitek 2 system according to the manufacturer's instructions. Allocation of the results to the categories S (susceptible), I (intermediately-susceptible) and R (resistant) was according to the Dutch guidelines [7]. One isolate from each centre was tested in an AST-N020 Gram-negative susceptibility card, except for the Utrecht centre, from which three isolates were included.

Questionnaire

A questionnaire was sent to the eight participating hospitals at the end of 2001 to obtain the following information about the outbreaks: number of patients involved; number of patients who had clinical disease or died because of the outbreak strain; times at which the first and the last patient were detected; number and type of departments involved; whether a common source was identified; total number and number of positive environmental cultures, and, if environmental cultures were positive, at which sites *Acinetobacter* was found; total number and number of positive cultures from medical equipment, and, if positive cultures were found, type of medical equipment; and what control measures were taken.

RESULTS

The eight outbreaks occurred in hospitals scattered throughout The Netherlands (Fig. 1). The outbreaks lasted for 2–22 months and the number of patients involved varied between six and 66 per outbreak, with a total number of 169 patients (Table 1). In all hospitals, one or more intensive care units were affected. In two hospitals, *Acinetobacter* had spread to general wards.