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RESEARCH NOTE

Prevalence of *Acinetobacter baumannii* and other *Acinetobacter* spp. in faecal samples from non-hospitalised individuals

L. Dijkshoorn[1], E. van Aken[1], L. Shunburne[2], T. J. K. van der Reijden[1], A. T. Bernards[3], A. Nemec[4] and K. J. Towner[2]

[1]Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands, [2]Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham, UK, [3]Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands and [4]Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic

ABSTRACT

In total, 226 individuals from the community were investigated for faecal carriage of *Acinetobacter* spp. by broth enrichment culture, followed by growth on blood agar and/or Leeds Acinetobacter Medium (LAM). *Acinetobacter baumannii* was isolated on both LAM and blood agar from one of 100 specimens in the UK and one of 126 specimens in The Netherlands. The predominant species were *Acinetobactor johnsonii* and genomic sp. 11, which were cultured from 22 and five specimens, respectively. *A. baumannii* did not seem to be widespread in the faecal flora of individuals in the community.

Keywords *Acinetobacter baumannii*, *Acinetobacter* spp., community, enrichment culture, faecal carriage, Leeds Acinetobacter Medium

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Acinetobacter baumannii is an increasingly important nosocomial pathogen which can cause major outbreaks of infection. The organism is often acquired nosocomially, but can be introduced initially by patients admitted from other hospitals [1]. Certain strains (clones) have been shown to be distributed between hospitals in wide geographical areas [2–4]. However, the origin of a suddenly appearing epidemic *A. baumannii* strain in a hospital is often unknown, and it remains possible that *A. baumannii* has a reservoir in the non-hospitalised human population, from which strains can be introduced into a hospital. The present study investigated the occurrence of *A. baumannii* in faecal specimens from non-hospitalised individuals in order to determine whether there is a significant intestinal reservoir from which spread to hospitals could occur. The study was performed in Nottingham (UK) and Leiden (The Netherlands), thus allowing comparison of populations at two locations. In Nottingham, the investigation focused exclusively on *A. baumannii*, while in Leiden, the prevalence of other *Acinetobacter* spp. was also investigated.

In total, 226 individuals from the community were investigated. One faecal specimen from each individual was received from general practitioners for investigation of parasites or microbial pathogens by the diagnostic laboratories at Leiden University Medical Center and University Hospital, Nottingham. A sterile cotton swab was used to sample the faeces, and the material taken up on the swab was transferred to flasks containing 20 mL of acetate mineral medium [5]. The samples were mixed to resuspend the faecal material, and then incubated with vigorous aeration for up to 48 h at 30°C to enrich for *Acinetobacter* spp. Following incubation, the cultures were streaked on to Leeds Acinetobacter

Corresponding author and reprint requests: L. Dijkshoorn, Department of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands
E-mail: L.Dijkshoorn@lumc.nl

Medium (LAM) [6] and blood agar. The plates were incubated aerobically at 30°C and examined after 24 h and 48 h for putative *A. baumannii* colonies (pink with mauve background on LAM [6]), or other colonies with a morphology on blood agar that was typical of *Acinetobacter* spp. (non-pigmented, white or cream-coloured, smooth to mucoid colonies, 1–2 mm in diameter [7]). One colony of each morphotype was selected for species identification.

Putative acinetobacters were identified presumptively to the genus *Acinetobacter* on the basis of morphology following Gram's stain (Gram-negative coccobacilli), a negative oxidase reaction, and the reactions included in the API 20NE system (bioMérieux, Marcy-l'Etoile, France). Species identification was achieved by amplified ribosomal DNA restriction analysis (ARDRA) [8] and high-resolution genomic fingerprinting using amplified fragment length polymorphism (AFLP™) analysis [9], followed by comparison of the restriction fragments and DNA fingerprints generated by these respective methods to those of 280 reference strains (collection of Leiden University Medical Center) belonging to all known species of *Acinetobacter*. If ARDRA was not conclusive, additional tests based on the biochemical identification system of Bouvet and Grimont [10] were used to achieve a 'consensus' identification [11]. Isolates from the same specimen with an identical API 20NE profile and/or indistinguishable AFLP fingerprints were considered to represent the same strain, and only one of these was included in the final analysis.

The recovery rates of the different species are shown in Table 1. In Nottingham, faecal specimens from 100 individuals were investigated, and only colonies on LAM that resembled *A. baumannii* were processed further. Only one of the three putative Nottingham *A. baumannii* isolates on LAM was identified definitively to this species; this isolate was resistant only to first-generation cephalosporins, chloramphenicol and trimethoprim. The two other isolates were considered not to be *A. baumannii* following ARDRA and AFLP analysis. In Leiden, 126 faecal specimens were investigated, and all putative acinetobacters from both LAM and blood agar were processed. In Leiden, 31 (24.6%) specimens were positive for a variety of *Acinetobacter* spp. Only one sample yielded an *A. baumannii* isolate (on both LAM and blood agar), which was resistant to first-generation cephalosporins only; the predominant species was *A. johnsonii*, followed by genomic sp. 11. *A. johnsonii* grew mostly (17 of 22 specimens) on blood agar only. Genomic sp. 11 was recovered from five samples, of which four grew on LAM and blood agar, and one on LAM only. Four Leiden specimens yielded two species each. One specimen grew a putative *Acinetobacter* sp. which could not be identified to any of the described named or unnamed species.

Table 1. *Acinetobacter* spp. cultured from faecal specimens of 226 individuals in the UK and The Netherlands

Species	No. (%) of positive specimens	
	Nottingham, UK[a] (n = 100)	Leiden, The Netherlands[b] (n = 126)
A. baumannii	1 (1.0)	1 (0.8)
Genomic sp. 3	–	1 (0.8)
A. junii	1 (1.0)	2 (1.6)
A. johnsonii	1 (1.0)	22 (17.5)
A. lwoffii	–	1 (0.8)
A. ursingii	–	2 (1.6)
Genomic sp. 10	–	1 (0.8)
Genomic sp. 11	–	5 (4.0)

[a]In Nottingham, only putative *A. baumannii* colonies on Leeds Acinetobacter Medium (LAM) were investigated.
[b]In Leiden, all possible *Acinetobacter* spp. colonies on LAM and blood agar were investigated; four specimens each yielded two different species; one specimen yielded a putative *Acinetobacter* colony which could not be identified to any *Acinetobacter* sp. described previously.

The recovery rate (0.8%) of *A. baumannii* in Leiden was in good agreement with that in Nottingham (1.0%), which suggests that the human intestine does not constitute an important community reservoir of this organism. This low isolation rate in the community contrasts with a faecal colonisation rate of 41% among hospitalised patients in an intensive care unit in Spain [12]. However, the present study only included individuals whose faeces were submitted for investigation of possible intestinal pathogens, and this was therefore not a random sample from the community. Nevertheless, the findings were consistent with a previous study in 1978 [13] that failed to find glucose-acidifying acinetobacters (which include *A. baumannii*) following enrichment cultivation in stool samples from 50 individuals.

Several other studies have investigated the possibility that human skin could be a community reservoir of *A. baumannii*. Two studies using enrichment cultivation and validated methods for species identification, one in the UK and one in Germany, found *A. baumannii* carriage in only one (0.5%) of 192 healthy volunteers in the UK [14] and one (2.5%) of 40 healthy volunteers in Germany [15]. A survey in Hong Kong yielded *A. baumannii*, as identified by ARDRA, from

4–12% of individuals in the community [16], and a survey in New York (USA) found a hand carriage rate of 10.4% in individuals from the community [17]. However, these rates may be an overestimation, since species identification was achieved only by phenotypic methods, which are not sufficient for unambiguous identification of *A. baumannii* [18]. In addition, typing of isolates in the New York study [17] showed that the community isolates differed from those in hospitals in the same area. Similarly, the two *A. baumannii* isolates recovered in the present study were far more susceptible to antimicrobial agents than most hospital isolates. Other studies exploring environmental reservoirs have recovered *A. baumannii*, albeit infrequently, from vegetables, meat, fish and soil [19,20]. *A. baumannii* has also been isolated from lice colonising homeless individuals, with the suggestion that the organisms originated from the blood of the host [21].

In conclusion, *A. baumannii* does not seem to be widespread in non-hospitalised individuals, but it can be recovered at low frequencies from skin and faecal flora, and from specific environmental sites. Community-acquired infections with *A. baumannii* in animals and humans have also been reported [22,23]. However, although rare *A. baumannii* strains could be introduced into hospitals from non-hospitalised individuals and/or environmental sources, further work is required to determine whether strains from the community have the capacity to become established and spread among susceptible hospitalised patients.

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RESEARCH NOTE

Macrolide-resistance mechanisms in *Streptococcus pneumoniae* isolates from Belgium

J. Van Eldere[1], *E. Meekers*[1], *K. Lagrou*[1], *C. Massonet*[1], *A. Canu*[2], *I. Devenyns*[1], *J. Verhaegen*[1], *G. Syrogiannopoulos*[3] *and R. Leclercq*[2]

[1]Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium, [2]Laboratoire de Microbiologie, CHU de Caen, Caen, France and [3]Department of Paediatrics, General University Hospital, University of Patras, School of Medicine, Patras, Greece

ABSTRACT

Of 233 erythromycin-resistant pneumococcal isolates collected in Belgium in 1999–2000, 89.7% carried the *erm*(B) gene, 6% the *mef*(A) gene, and 3.5% *erm*(B) plus *mef*(A). Two isolates contained neither *erm*(B) nor *mef*(A); one contained an *erm*(A) subclass *erm*(TR) gene, while the other contained an A2058G mutation in domain V of the 23S rRNA gene. Of 209 *erm*(B)-positive isolates, 191 had clindamycin MICs >16 mg/L and 18 had MICs ≤16 mg/L. *Mef*(A)-positive isolates all displayed the M resistance phenotype. Telithromycin remained active against erythromycin-resistant isolates, with the highest telithromycin MIC_{50} being found in *mef*(A)-positive isolates. No difference in the prevalence of different resistance mechanisms was observed compared to isolates collected in 1995–1997.

Corresponding author and reprint requests: J. Van Eldere, Laboratory of Bacteriology, CDG, 8th floor, UZ Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium
E-mail: johan.vaneldere@uz.kuleuven.ac.be

Keywords *erm*(B), macrolide resistance, *mef*(A), pneumococci, resistance, *Streptococcus pneumoniae*

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Erythromycin resistance in pneumococcal isolates from Belgium increased from 5.5% in 1986 to 37% in 2003 [1]. Macrolides are still used extensively in Belgium, despite a consensus that they are not the first-choice therapy for respiratory tract infections in adults. As the data suggest that macrolides may drive β-lactam resistance, it remains important to monitor macrolide resistance [2].

Pneumococcal macrolide resistance can be caused by *erm*(B) (erythromycin ribosome methylase)-encoded methylation of a residue in the 23S rRNA [3]. Phenotypically, this results in the MLS_B resistance phenotype, with resistance to all macrolides plus lincosamides and streptogramin B antibiotics. An alternative resistance mechanism involves *mef*(A) (macrolide efflux), resulting in M-type resistance to 14- and 15-membered macrolides, but not to 16-membered macrolides, lincosamides or streptogramin B antibiotics. The simultaneous presence of *mef*(A) and *erm*(B) has been reported [4], while other reports have described mutations in regions V and II of the 23S rRNA gene, and also in genes encoding riboproteins L4 and L22. Resistance phenotypes correlating with these mutations may vary [5,6].

Two studies examined erythromycin resistance in pneumococci collected from Belgium in 1995–1997, and showed that resistance was associated predominantly with *erm*(B)- and rare *mef*(A)-positive isolates [7,8]. In the present study, 637 *Streptococcus pneumoniae* isolates obtained from clinical samples (respiratory, blood, cerebrospinal fluid) in Belgium between October 1999 and February 2000 were investigated.

Antibiotic susceptibility was determined by adding doubling dilutions (0.008–16 mg/L) of antibiotics to molten Mueller–Hinton agar (Oxoid, Basingstoke, UK) supplemented with horse blood 5% v/v. Multipoint inoculation was in duplicate, with 10^4 CFU/spot, followed by incubation for 24 h at 36°C in CO_2 5% v/v. NCCLS breakpoints were used for interpretation of the results. Antibiotics were provided by their respective manufacturers.