

# Dissemination of *Acinetobacter nosocomialis* Clone among Critically Ill Patients and the Environment

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**We examined the environmental dissemination of *Acinetobacter nosocomialis* multilocus sequence typing clonal complex 260/71 in Rio de Janeiro, Brazil, including water from a dam and food samples. The increasing use of sequence based methods has demonstrated a large, previously unpredicted, dissemination of bacteria that may serve as opportunistic pathogens.**

*Acinetobacter nosocomialis* has emerged in the health care setting and may serve as a reservoir of resistance determinants. Clonal spread of *A. nosocomialis* was suspected since the 1980s, but the dissemination of specific types remained debatable until the use of DNA gel band pattern techniques (1–5).

The development of multilocus sequence typing (MLST) schemes evidenced the population structure of *A. baumannii* (6–8). However, only three studies applied MLST to define lineages of *A. nosocomialis* (6, 8, 9). In the first study (8), 20 clinical isolates obtained in Europe and the United States from 1985 to 1996 were included in 18 different sequence types (ST) of the MLST scheme hosted at University of Oxford (described from here on as the UO scheme). The second study typed seven *A. nosocomialis* isolates used as outgroups in phylogenetic analysis of *A. baumannii* by the MLST scheme hosted at Institut Pasteur (described from here on as the IP scheme) (9). Two clusters were observed among the seven *A. nosocomialis* isolates: (i) ST68/IP, including one isolate obtained in Nijmegen, Netherlands, in 1984 and one obtained in Odense, Denmark, during the 1980s, and (ii) ST71/IP, including three isolates from different cities in the Netherlands (Utrecht, Leiden, and Rotterdam) during 1987–2000 (6). The third study included 234 patients admitted to an intensive care unit (ICU) of Rio de Janeiro, Brazil, screened for *Acinetobacter* sp. colonization in 2007–2008 (9). A single *A. nosocomialis* RAPD type was found in surveillance cultures of 24 (10%) patients and caused one episode of bloodstream infection. This type belonged to ST260/UO (9). The aim of the present study was to assess the extent of environmental dissemination of *A. nosocomialis* ST260/UO.

Screening for *Acinetobacter* sp. was performed in samples collected from three sources: inside the ICU ( $n = 28$ ), including the hands of health care staff, sinks, faucets, benches, bed rails, mattresses, and top of tables; lettuce (*Lactuca sativa*) ( $n = 65$ ) bought at different markets in the city of Rio de Janeiro; and water samples ( $n = 4$ ) and swab specimens ( $n = 61$ ) from the surroundings of the main water reservoir of Rio de Janeiro. This reservoir belongs to a complex of dams providing water to almost 90% of the people of Rio de Janeiro city. Imprints from the dominant hand were made on blood agar plates from health care workers. Specimens from environmental surfaces were collected with swabs, inoculated onto MacConkey broth, and incubated at 35°C overnight. Lettuce specimens (10  $\mu$ l each) were obtained from serial dilutions of 10 g of the leaves (dry weight) homogenized in saline solution. Water specimens (100 ml) were membrane filtered.

Samples were subcultured onto MacConkey agar at 35°C for 48 h. Gram-negative coccobacilli, catalase-positive, oxidase-negative, nonmotile and glucose nonfermenters were classified as *Acinetobacter* sp. and speciated by partial *rpoB* gene sequencing analysis as previously described (10). Antimicrobial susceptibility was determined by disk diffusion for amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin, and trimethoprim-sulfamethoxazole and by microdilution method for colistin (11). Isolates were defined as multidrug-resistant (MDR) when resistant to three or more antimicrobial classes (12).

MLST was performed with the UO scheme (<http://pubmlst.org/abaumannii/>), and the IP scheme ([www.pasteur.fr/mlst](http://www.pasteur.fr/mlst)) as described previously (6, 7). goeBURST (<http://www.phyloviz.net/wiki/>) was used to determine relationships between STs in the *Acinetobacter* databases (<http://pubmlst.org/abaumannii/> and [www.pasteur.fr/mlst](http://www.pasteur.fr/mlst)) (13). *A. nosocomialis* isolates were typed by pulsed-field gel electrophoresis (PFGE) as described based on DNA *ApaI* digestion (14). Band patterns were analyzed for similarity with GelCompar version 4.01 (Applied Maths, Kortrijk, Belgium) based on Dice coefficient and the unweighted pair group method with arithmetic averages. The study was approved by the Institutional Review Board of the hospital.

A total of 11 *Acinetobacter* isolates, all non-*A. baumannii* species, were obtained from environmental specimens. Environmental isolates included four from lettuce (two *Acinetobacter berezinae* and one each *A. nosocomialis* and *Acinetobacter rhizosphaerae*); four from ICU nonclinical specimens (two *A. nosocomialis* from furniture surfaces and one from the hand of a staff member, and one *Acinetobacter schindleri* from ICU bench); and three from water (two *A. nosocomialis* and one *Acinetobacter radioresistens*).

*A. nosocomialis* typing by MLST with the UO scheme showed that one isolate obtained from lettuce belonged to ST260 (UO) and that another obtained from an ICU cabinet was a double-

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TABLE 1 Characteristics of *Acinetobacter nosocomialis* isolates<sup>a</sup>

Type of sample	Isolate	Specimen source	Isolation date (day/mo/yr)	Allelic profile (UO scheme)	ST (UO/IP)	Resistance profile
Human clinical specimen	An2b	Tracheal secretion	16/5/2007	11-65-14-21-37-105-15	260/161	CAZ, CIP, GEN, SXT, TOB
	An2a	Tracheal secretion	6/7/2007	11-67-14-51-37-105-15	NA(260DLV)/161	CIP, GEN, SXT, TOB
	An8	Blood	13/7/2007	11-65-14-21-37-105-15	260/161	AMI, CIP, FEP, GEN, TOB
	An7b	Tracheal secretion	26/7/2007	11-65-14-21-37-105-15	260/161	CIP, GEN, SXT, TOB
ICU	CTI021	ICU cabinet	28/5/2008	11-70-14-21-37-4-15	264(260DLV)	None
	CTI10	Ventilator equipment	28/5/2008	11-27-ND-21-37-113-15	ND	SXT
	CTI15.3	Hand of ICU staff	28/5/2008	11-65-93- 61-25-112-15	263	FEP, SXT, TZP
Environment unrelated to hospital	PCA073	Water reservoir	13/12/2007	49- 68-119-53-41-115-58	262	TOB
	PCA111	Water reservoir	14/3/2008	42-36-92-30-40-114-57	261	None
	A12.1	Lettuce	25/3/2008	11-65-14-21-37-105-15	260/161	CIP

<sup>a</sup> ICU, intensive care unit; UO, University of Oxford MLST scheme; ND, not determined; ST, sequence type; IP, Institut Pasteur; NA, not assigned; DLV, double-locus variant; AMI, amikacin; CIP, ciprofloxacin; FEP, cefepime; GEN, gentamicin; TOB, tobramycin; CAZ, ceftazidime; SXT, sulfamethoxazole-trimethoprim; TZP, piperacillin-tazobactam.

locus variant (DLV) of this ST. For one isolate obtained from ventilator equipment, it was not possible to amplify the *gdhB* gene fragment by PCR, and a complete ST profile was not determined. These results together with data from four *A. nosocomialis* clinical isolates from the previous study (9) are shown in Table 1. The four clinical isolates belong to RAPD [random(ly) amplified polymorphic DNA] genotype A and were obtained from three patients. Isolates An2a and An2b are from a single patient and had RAPD profiles with a slight difference. Two isolates were additionally typed by the IP scheme: one ST260 (UO) and one ST260 (UO) DLV. Both were classified as ST161/IP, a single-locus variant (SLV) of ST71/IP, forming CC71/IP. PFGE was performed on 9 of 10 *A. nosocomialis* study isolates; one was untypeable. Dendrogram of the PFGE bands is shown in Fig. 1. ST260/UO isolates and one of the DLVs of ST260/UO, corresponding to ST161/IP, formed a cluster with >95% similarity. Another DLV of ST260/UO had only 87% similarity with this cluster, and all other isolates had unique band patterns.

*A. nosocomialis* clinical isolates and the isolate from the hand of

ICU staff member were MDR strains. Five isolates obtained from environment were susceptible to all or resistant to only one agent.

This is the first study to describe *A. nosocomialis* MLST typing by both UO and IP schemes. *A. nosocomialis* clone defined by MLST as CC260/UO and ST161/IP spread among several patients admitted to an ICU and its environment over a 9-month period. The clone was also present in the environment outside the hospital. Since the first description of the likely clonal spread of *A. nosocomialis*, its environmental dissemination was suspected (2). Later, dissemination of isolates belonging to a single ribotype and PFGE genotype between patients and the environment was clearly demonstrated during an outbreak in a neurosurgical ICU in the Netherlands (4). In the present study, we describe the environmental dissemination of isolates related to *A. nosocomialis* CC260/UO and ST71/IP. One isolate obtained from lettuce bought in a market unrelated to the hospital was indistinguishable from this clone by MLST and PFGE. The isolate from lettuce was susceptible to several antimicrobials, whereas clinical isolates were all MDR. These findings may indicate CC260/UO is widespread in

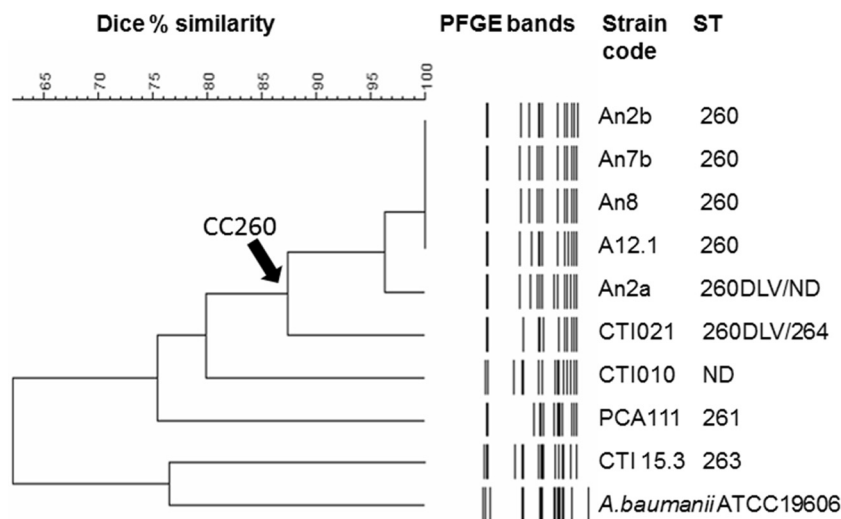


FIG 1 Dendrogram of PFGE band profiles of *A. nosocomialis* isolates and one outgroup *A. baumannii* ATCC 19606 reference strain. DLV, double locus variant; ND, not determined.

the environment but able to colonize and cause human disease, acquiring resistant determinants after exposure to other resistant strains under the selection pressure in the hospital setting.

The observation that CC260/UO-ST161/IP isolates are a SLVs of ST71/IP, obtained in the Netherlands during 1987-2003, is remarkable and shows the intercontinental spread of an *A. nosocomialis* strain (6).

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