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Patient-to-Patient Spread of a Single Strain of *Corynebacterium striatum* Causing Infections in a Surgical Intensive Care Unit

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Over a 12-month period, *Corynebacterium striatum* strains were isolated from clinical specimens from 14 patients admitted to a surgical intensive care unit. These isolates were identical by morphology and biotype and displayed the same antibiogram. Ten isolates were found to be the sole possible pathogen. These 10 isolates were from six patients, three of whom had signs of infection at the time of positive culture. Further typing was performed by random amplification of polymorphic DNA analysis, by which all strains were identical and were found to differ to various degrees from reference strains and from isolates found in clinical samples from other wards. In a case-control study the only independent risk factor for acquiring the strain was intubation for longer than 24 h (odds ratio, 20.09; 95% confidence interval, 2.29 to 176.09). The same strain was isolated from surfaces and from air sampled in the direct vicinity of infected patients but never from surfaces or air in other places of the ward. The strain was not isolated from the ventilators. The strain was cultured from the hands of personnel attending to infected patients, but no long-term carriers were found among members of the hospital personnel, suggesting transient carriage only. We conclude that *C. striatum* can cause serious nosocomial infections in surgical intensive care unit patients and may spread from patient to patient via the hands of attending personnel.

Coryneform bacteria are gram-positive rods that are common members of the normal flora of human skin and mucous membranes. Apart from infections with *Corynebacterium diphtheriae*, it is difficult to distinguish harmless colonization with coryneform bacteria from infection caused by corynebacteria in clinical samples (4, 6, 15). Certain species of nondiphtheria corynebacteriae like *Corynebacterium jeikeium* and *Corynebacterium urealyticum* are already well recognized as opportunistic pathogens, especially in immunocompromised hosts or patients with intravascular catheters (5, 12).

Corynebacterium striatum, which was first described in 1889 by von Besser (25), is less well known as a cause of infection. Serious infections with *C. striatum* have been reported only sporadically. In case reports, *C. striatum* has been described as the causative agent of pulmonary infections (3, 7, 14), bacteremia (8, 23, 26), keratitis (20), chronic ambulatory peritoneal dialysis peritonitis (1), intrauterine infection (17), wound infection (17), and endocarditis (13, 21). An outbreak with *C. striatum* involving 11 patients on an intensive care unit was recently described by Leonard et al. (11). This strain, which was first recognized as a consequence of its production of a characteristic brown pigment, was involved in the largest nosocomial outbreak reported so far. With the aid of molecular biology-based techniques, the clonal nature of the isolates in that outbreak was unequivocally established, but a common source and the mode of transmission could not be determined.

On the surgical intensive care unit (ICU) of the Dijkzigt University Hospital Rotterdam, *C. striatum* isolates were recovered from clinical samples from 14 patients over a 12-month period. We studied this possible outbreak and attempted to delineate the mode of transmission and the pathological potential of *C. striatum* strains in patients admitted to the ICU.

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MATERIALS AND METHODS

***C. striatum* strains.** All *C. striatum* isolates from patients included in the study were detected in routine diagnostic cultures. In cultures of sputum samples, only colonies representing the dominant growth were further identified. Colonies of catalase-positive gram-positive rods were identified in the API-CORYNE identification system (Bio-Merieux, Lyon, France).

The antibiogram was used as a typing method. The following antibiotics were tested by the disk diffusion method according to the guidelines for aerobic bacteria of the Werkgroep Richtlijnen Gevoeligheidsbepalingen, Bilthoven, The Netherlands (10): penicillin (10 U), amoxicillin (25 μg), sulfonamide (300 μg), erythromycin (15 μg), clindamycin (10 μg), vancomycin (30 μg), tetracycline (30 μg), rifampin (5 μg), and chloramphenicol (30 μg). A 1:100 dilution of a suspension with a turbidity equivalent to that of a 0.5 McFarland standard was used to give a thin confluent layer of growth after 18 h of incubation. Diameters of growth inhibition were interpreted as susceptible (score of 2), intermediate (score of 1), or resistant (score of 0) by using the following cutoff zone diameters: penicillin, $2 > 30$ mm and $0 < 17$ mm; amoxicillin, $2 > 26$ mm and $0 < 16$ mm; sulfonamide, $2 > 13$ mm; erythromycin, $2 > 21$ mm and $0 < 18$ mm; clindamycin, $2 > 17$ mm and $0 < 13$ mm; vancomycin, $2 > 30$ mm; tetracycline, $2 > 28$ mm and $0 < 20$ mm; rifampin, $2 > 19$ mm; and chloramphenicol, $2 > 25$ mm and $0 < 21$ mm.

For genotyping, DNA was isolated by the method described by Boom et al. (2) from pure cultures of *C. striatum* strains, a selection of other *Corynebacterium* isolates from the University Hospital Rotterdam, and reference strains of *C. striatum* obtained from the Centers for Disease Control and Prevention (CDC; Atlanta, Ga.) and Marie B. Coyle of the Harborview Medical Center (HMC), Seattle, Wash.). DNA was typed by random amplification of polymorphic DNA (RAPD) assays with the ERIC 1 and ERIC 2 primers by previously published procedures (16, 24). Strains were considered identical when they showed identical banding patterns on agarose gels or differed by a single band only.

Case-control study. Patients with one or more clinical samples culture positive for the epidemic strain of *C. striatum* were defined as cases. For each case patient two control patients were selected by using the following criteria: one patient admitted to the surgical ICU directly before and one patient admitted directly after the case patient. The medical records of the case and control patients were screened for possible risk factors for aquiring infection with *C. striatum*. Case patients were scored until the date of the first isolation of the epidemic strain; controls were scored for the entire period of admission to the ICU.

Results were analyzed with the SAS statistical package (22). Differences be-

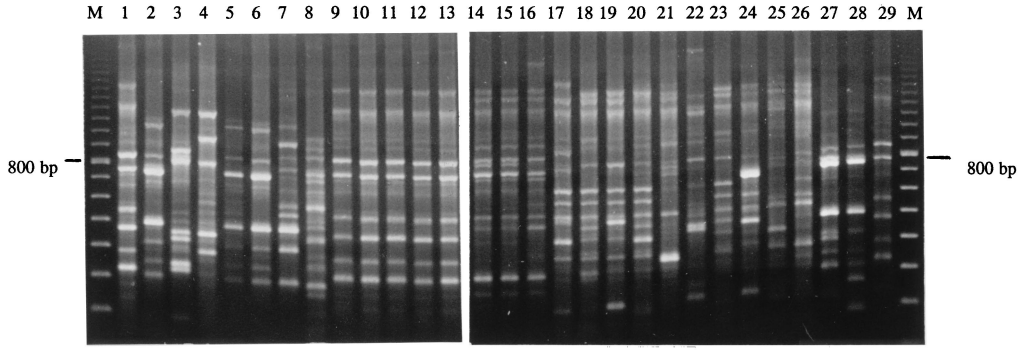

FIG. 1. RAPD typing of *C. striatum* strains with the ERIC 2 primer. Lanes 1 to 13, *C. striatum* isolates from the University Hospital Rotterdam. Lane 1, example of outbreak strain of *C. striatum*; lanes 2, 3, 4, 7, and 8, clinical isolates from patients from other departments; lanes 5 and 6, isolates of *C. striatum* from the hands of one member of the personnel obtained on two separate occasions; lanes 9 to 12, *C. striatum* strains of the outbreak type isolated from the hands of four different personnel on the surgical ICU; lane 13, *C. striatum* strain of the outbreak type isolated from an air sample on the surgical ICU; lanes 14 to 28, reference strains of *C. striatum* from HMC and CDC corresponding (in the same order) to the reference strains in Table 1; lane 29, example of outbreak strain; lane M, 100-bp DNA ladder.

tween groups were tested by the Mann-Whitney U test or the Fisher exact test when applicable. The importance of the risk factor was determined by calculation of odds ratios and 95% confidence intervals. To determine which factors were significantly important, a logistic multivariate analysis was done by using a forward and a backward elimination procedure. Statistical significance was accepted at $P < 0.05$ (two-tailed analysis).

Cultures of environmental samples. Samples from possibly contaminated sources on the ICU, including air and samples from surfaces, were taken from the direct vicinities of the patients and from general areas on the ICU and were cultured. Air samples were taken by opening sedimentation plates for 1 h at several places in the ICU and by taking active air samples with a surface air system (International PBI, Milan, Italy). Active air samples and swabs from several points of the inflows and the outflows of all 10 of the mechanical ventilators (Evita; Dräger) and the two ventilators used during transportation (Oxylog; Dräger) were also taken. Samples only from reusable parts of the ventilators were cultured to find a possible source. Samples were also taken from the balloons used for manual ventilation support by pouring 20 ml of normal saline into the balloon and shaking for a minute. The recovered saline solution was centrifuged at 3,000 × *g* for 10 min, and the sediment was cultured on blood agar.

Hand and throat samples for culture were taken on two occasions from staff attending directly to patients on the surgical ICU (nurses, doctors, and physiotherapists). The swabs containing the samples were streaked onto blood agar, which was made selective by adding 10 μg of chloramphenicol and 10 μg of clindamycin per ml (BAcc), and the agar plates were cultured at 37°C for 3 days. Hand samples for culture were obtained by pouring 30 ml of brain heart infusion (BHI) broth into a sterile latex glove and instructing a person to put his or her hand into the glove for 1 min. The BHI broth was subsequently poured into a centrifuge tube and was centrifuged at 4,000 rpm for 10 min. The sediment was plated onto BAcc and was cultured at 37°C for 3 days. Suspected colonies of gram-positive rods were identified in the API-CORYNE identification system, and if a *C. striatum* strain was isolated, its antibiogram was determined and RAPD analysis was performed as described above. When the outbreak strain was found in a culture of a sample from a member of the staff, repeat set of samples was taken from that person, usually within 7 days from time of collection of the first sample, to study long-term carriage.

From 1 July to 1 September 1995 all sputum samples and wound fluid samples from patients from the surgical ICU were also cultured on the selective BAcc medium to determine if more patients carrying the outbreak strain could be identified.

RESULTS

The use of antibiograms and RAPD typing for identifying the outbreak strain of *C. striatum* was validated by testing a set of isolates of *Corynebacterium* spp. cultured from patients on other wards of the University Hospital Rotterdam and of *C. striatum* reference strains obtained from CDC and HMC. All of the suspected isolates of *C. striatum* from the ICU were found to be identical by each of the typing methods, indicating clonal spread of a single genotype. This clone differed from the reference strains and other strains from our own hospital (Fig. 1 and Table 1). The ERIC 1 and ERIC 2 primers gave the same resolutions.

The API-CORYNE number for of the outbreak strain was 3100104. Additional biochemical reactions were performed, and the results were as follows: oxidase negative, catalase positive, motility negative, growth at 37°C positive, growth at 42°C positive, *o*-nitrophenyl-β-D-galactopyranoside negative, esculine hydrolysis negative, urease negative, methyl red positive, Voges-Proskauer positive, and nitrate reduction positive. Fermentation results were as follows: glucose positive, lactose negative, maltose negative, sucrose negative, arabinose negative, xylose negative, rhamnose negative, mannitol negative, salicine negative, mannose positive, fructose positive, trehalose negative, raffinose negative, and galactose positive.

The outbreak strain was susceptible to penicillin, amoxicillin, erythromycin, and vancomycin and was resistant to sulfonamide, clindamycin, tetracycline, rifampin, and chloramphenicol in the disk diffusion test.

A *C. striatum* strain with a genotype the same as that of the epidemic strain was found in 25 routine cultures of samples taken from 14 patients in the period from June 1994 to May 1995; a summary of the positive samples and infection parameters for patients on the day that the positive sample was obtained is given in Table 2. The outbreak strain was isolated as the sole possible pathogenic organism in 10 cultures of samples from six patients. In three of these six patients (patients 5, 7, and 12) there were signs of infection (fever, leukocytosis, increased erythrocyte sedimentation rate, or pulmonary infiltrate on chest X ray) at the time that the sample that was positive on culture was obtained. Those three samples were all sputum samples. Furthermore patient 1, from whose sputum *Pseudomonas aeruginosa* in addition to *C. striatum*, was cultured in a very small amount, showed leukocytes (4+) and gram-positive rods (4+) and an infiltrate on chest X ray. The patient reacted promptly to monotherapy with amoxicillin. The periods of admission to the surgical ICU and other wards of the hospital for individual patients are provided in Fig. 2. During the whole study period there was always at least one patient on the ICU who had previously been infected with the outbreak strain of *C. striatum* at one or multiple sites.

Possible risk factors for aquiring the outbreak strain were scored from the files of the case and control patients (Table 3). Compared with the control patients, the case patients remained in the ICU for a significantly longer period of time, were intubated for a longer period, had more bronchoscopies prior to having a positive culture, were more often fed by a nasogastric tube, and had had more wound infections before acquiring the epidemic strain of *C. striatum*. In a multivariate

TABLE 1. Typing of *Corynebacterium* strains

Source and no. of isolates	Species	Clinical source	Source (ward or institution)[a]	Antibiogram[b]	RAPD type
Patients (epidemic strain)					
17	*C. striatum*	Sputum	1	220202000	A[c]
1	*C. striatum*	Blood	1	220202000	A[c]
7	*C. striatum*	Wound fluid	1	220202000	A[c]
Patients (other wards)					
1	*C. xerosis*	Blood	2	22-222222	B
1	*C. xerosis*	Blood	2	000002020	C
1	*C. minutissimum*	Cerebrospinal fluid	3	222222222	D
1	*C. jeikeium*	Blood	4	122222212	E
1	*C. jeikeium*	Blood	5	122222210	F
1	*Corynebacterium* type ANF	Blood	4	022222212	G
1	*C. minutissimum*	Blood	2	122002200	H′
1	*C. minutissimum*	Blood	5	222222202	J
1	*C. striatum*	Wound fluid	6	220002220	H′
1	*C. striatum*	Peritoneal fluid	4	222222212	J
1	*C. striatum*	Ear	7	220222220	K
1	*Corynebacterium* species	Blood	5	120002202	L
Environmental samples					
7	*C. striatum*	Hand	1	220202000	A[c]
2	*C. striatum*	Hand	1	220002220	Y
3	*C. striatum*	Hand	1	220002220	Z
2	*C. striatum*	Hand	1	220002220	BB
1	*C. striatum*	Air sample	1	222222222	X
3	*C. striatum*	Surface	1	220202000	A[c]
5	*C. striatum*	Air sample	1	220202000	A[c]
Reference strains					
1	*C. striatum* HR128		HMC	220102222	M
1	*C. striatum* HR151		HMC	220102222	M
1	*C. striatum* HR109		HMC	220202210	M′
1	*C. striatum* HR201		HMC	222002222	N
1	*C. striatum* HAA59		HMC	222022222	O
1	*C. striatum* HR160		HMC	222222222	P
1	*C. striatum* HR159		HMC	220222222	Q
1	*C. striatum* HR161		HMC	222222222	R
1	*C. striatum* R197 (ATCC 6940)		HMC	222222222	S
1	*C. striatum* R256 (ATCC 4371)		HMC	222222222	O′
1	*C. striatum* R257 (ATCC 43735)		HMC	220202222	T
1	*C. striatum* G7000 (Ohio)		CDC	222222222	U
1	*C. striatum* G5905 (New Zealand)		CDC	220202000	U′
1	*C. striatum* G7961 (South Dakota)		CDC	220002220	V
1	*C. striatum* G8699 (Illinois)		CDC	220002220	W

[a] Locations: 1, surgical ICU; 2, medical ICU; 3, neurological ward; 4, medical ward; 5, cardiologic ICU; 6, hematology ward; 7, pediatric ward.
[b] For the antibiogram 0 is resistant, 1 is intermediate, and is susceptible; 2, the following antibiotics were tested: penicillin, amoxicillin, sulfonamide, erythromycin, clindamycin, vancomycin, tetracycline, rifampin, and chloramphenicol.
[c] Epidemic strain; all strains had the same biotype, antibiogram, and RAPD type.

analysis, all these significant risk factors were correlated to each other. The single most important risk factor was being intubated for more than 24 h.

C. striatum strains of the outbreak type were isolated three times from environmental surfaces and five times from air samples (two times from a sedimentation plate and three times from an active air sample) in the direct environment of case patients. It was never isolated from other areas of the ward and was never isolated from any of the reusable parts of any of the ventilators. The outbreak strain was isolated seven times from the hands of six personnel. However, no long-term carriers were found among the personnel. On inquiry, all personnel with a positive culture for *C. striatum* had been attending to a culture-positive patient on that day prior to having the sample taken.

Over a 2-month period all sputum samples and wound materials obtained from the surgical ICU were also cultured on the selective BAcc medium. By this method, sputum samples from four additional patients were found to be positive for the outbreak strain. In one of these samples, the outbreak strain could be isolated repeatedly during the entire 2-month period.

DISCUSSION

By use of phenotypic and genotypic methods, we established the long-term persistence of the same *C. striatum* strain on a surgical ICU. To our knowledge this is the largest outbreak of *C. striatum* described in the literature. Furthermore, we found that the most likely route of transmission was via the hands of personnel. This particular strain of *C. striatum* posed a pathogenic threat to the patients nursed on this surgical ICU.

Since *C. striatum* is part of the normal flora of skin and

TABLE 2. Culture characteristics and infection parameters for patients infected with *C. striatum*

Patient no.	Sample source for culture	Gram stain[a]							Organism isolated in addition to *C. striatum*	Parameters of infection on day of positive culture			
		Leu	Ery	Epi	Gr+ coc	Gr− coc	Gr+ rod	Gr− rod		Blood leukocytes (10^9/liter)	ESR (mm/h)[b]	Temp (°C)	Infiltrate on chest X ray[c]
1	Sputum	4+					4+		*Pseudomonas aeruginosa*	14.5	50	38.4	1
	Drain fluid	−							*Pseudomonas aeruginosa*	15.0			
2	Blood culture								*Stenotrophomonas maltophilia*	12.8		37.2	1
	Sputum	3+			4+				*Pseudomonas aeruginosa*	18.9	95		
3	Swab wound fluid								*Enterococcus faecalis*	9.3	44	38.0	0
	Swab wound fluid								*Enterococcus faecalis*	13.7			
	Sputum	2+	1+	1+			4+			17.2			
	Sputum	3+			<1		3+			11.9	49		
	Sputum	2+	1+		3+	1+	3+	<1		15.1			
4	Sputum	2+					4+			13.7		37.3	
5	Sputum	1+					1+	<1		22.2	84	36.8	1
6	Sputum	4+			3+				*Pseudomonas aeruginosa*	9.0		39.0	1
	Punctate rectal abcess	4+						2+	*Klebsiella pneumoniae*, *Escherichia coli*	14.8			
7	Sputum	1+	2+	2+			3+			20.7		37.4	1
	Swab wound fluid								*Enterococcus faecalis*	9.1			
8	Sputum	3+					3+	2+	*Klebsiella oxytoca*, *Pseudomonas aeruginosa*	11.2		38.3	0
9	Sputum	3+	3+	<1	4+	3+	4+	2+	*Klebsiella oxytoca*	18.4		38.3	0
10	Sputum	2+					1+	<1				37.4	1
11	Punctate peritoneal cavity	4+	4+		4+			4+	*Escherichia coli*	14.0		37.0	0
	Punctate peritoneal cavity	4+			4+		3+	4+	*Escherichia coli*, *Pseudomonas aeruginosa*	18.0			
12	Sputum	4+					3+	<1	*Bacillus* spp.	10.5	81	38.1	1
	Swab wound fluid												
	Swab wound fluid												
13	Punctate ascites								*Morganella moragnii*	8.5		38.3	0
14	Sputum	<1		2+			4+		*Pseudomonas aeruginosa*	26.1		37.6	1

[a] Abbreviations: Leu, leukocytes; Ery, erythrocytes; Epi, epithelial cells; Gr+ coc, gram-positive cocci; Gr−coc, gram-negative cocci; gr+ rod, Gram-positive rods; Gr− rod, gram-negative rods.
[b] ESR, erythrocyte sedimentation rate.
[c] 1, yes; 0, no.

mucous membranes (4), infections caused by this organism are usually considered to be of an endogenous origin. However, the patient-to-patient spread of coryneform bacteria has been suggested before (9, 18) and has also been described for *C. striatum* on an intensive care ward (11), but a common source was never found in those studies.

Because it is unusual that *C. striatum* is found to cause infection problems in several patients on a single ward, the idea of the spread of an epidemic strain arose. We first established that a single strain was the cause of this outbreak using the antibiogram and RAPD typing. Strains suspected of being the outbreak strain were found to be identical by these typing methods and differed significantly from reference strains. We concluded that RAPD analysis is a suitable tool for typing *C. striatum* strains and that, indeed, patient-to-patient transmission was occurring on this ward.

Subsequently, we tried to uncover the source and route of infection by establishing common risk factors for the patients positive for *C. striatum* and by culturing samples from all possible sources of contamination. Significant risk factors were all related to the severity of illness of the patients (length of stay, complications of treatment, and artificial ventilation), and they were all related to each other. The only independent and the strongest risk factor in a multivariate analysis was mechanical

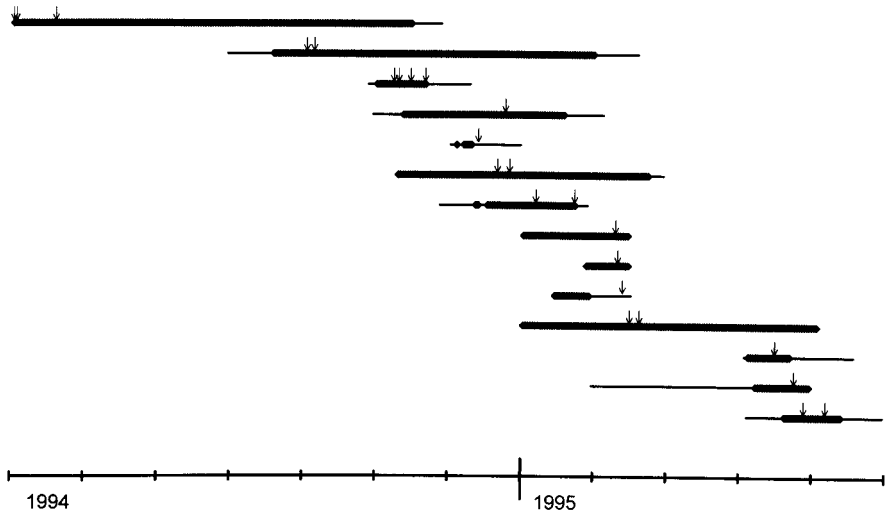

FIG. 2. Period of hospital admission for individual patients with samples culture positive for the outbreak strain of *C. striatum*. Thick line, patients admitted to surgical ICU; thin line, patients admitted to another ward; arrow, patients culture positive for *C. striatum*.

TABLE 3. Univariate risk factor analysis of characteristics of the population for aquiring the epidemic strain of *C. striatum*

Characteristic	Case patients (n = 14)	Control patients (n = 28)	Odds ratio (95% CI[a])	P value[b]
Age (yr)	62.9	62.8		0.82
Gender (no. [%] female)	7 (50)	7 (25)	1.67 (0.42–6.68)	0.49
Median (range) no. of days admitted	37 (11–410)	24 (1–160)		0.70
Median (range) no. of days on surgical ICU	16 (5–45)	7 (1–95)		0.03
Underlying illnesses (no. of patients)				
Chronic pulmonary disease	4	2	5.20 (0.82–32.99)	0.16
Cardiovascular disease	1	9	0.16 (0.02–1.44)	0.13
Diabetes mellitus	1	0		
Malignancy	5	14	0.56 (0.15–2.10)	0.52
Autoimmune disease	0	0		
Chronic renal failure	1	2		
Chronic liver failure	1	0		
Corticosteroid use during admission (no. of patients)	9	11	2.78 (0.74–10.52)	0.19
Intubation for >24 h (no. of patients)	13	11	20.09 (2.29–176.09)	<0.01
Median (range) no. of days intubated	12 (1–55)	0 (0–46)		<0.01
No. of patients with:				
Bronchoscopy	5	0	33.00 (1.66–654.08)	<0.01
Gastroscopy	5	5	2.56 (0.59–11.00)	0.26
Feeding by nasogastric tube	13	17	8.41 (0.96–73.73)	0.04
Central venous lines	14	23	6.79 (0.35–132.05)	0.15
Previous airway infection	9	9	3.8 (0.99–14.67)	0.10
Previous wound infection	12	12	8.00 (1.50–42.65)	0.01
Bed sore pressure (Therapulse)	6	4	4.50 (1.01–20.11)	0.06
Mean (SD) no. of days on antibiotics (SDD[c] excluded)	15.4 (7.1)	11.5 (12.3)		0.09
Mean (SD) no. of days on antibiotics (SDD included)	16.5 (5.6)	12.4 (11.8)		0.10

[a] 95% CI, 95% confidence interval.
[b] Mann-Whitney U test and Fisher's exact test were used for statistical analysis where applicable.
[c] SDD, selective decontamination of the gut.

ventilation. Thus, extensive sampling of the ventilation equipment was conducted, and samples from the hands and throats of staff directly involved in the care of these patients were also cultured.

During the entire period at least one patient previously positive for the outbreak strain on culture was present on the ICU. Long-term carriage in a patient could be confirmed when samples from the patient were examined specifically by selective bacterial culture. These two facts suggest that an infected patient may be a long-term source for such strains. Since no long-term carriers were found among the personnel and no other source was found, we conclude that the only possible reservoir for this strain is the colonized patient on the ward.

Contaminated surfaces and air samples were only found in the direct surroundings of patients with a positive culture and never on other places on the ward. On seven occasions the outbreak strain was found on the hands of members of the hospital personnel, all of whom were caring for a patient known to be positive for the outbreak strain on that day. Since no other contaminated sites were found outside the direct environments of culture-positive patients, the most probable route of transmission is from patient to patient via the hands of personnel transiently contaminated with the organism. Educating personnel on the proper application of hand disinfectants and making them more aware of the problem were successful, halting the epidemic in our institution.

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