

Burkholderia cepacia Complex Bacteria from Clinical and Environmental Sources in Italy: Genomovar Status and Distribution of Traits Related to Virulence and Transmissibility

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Sixty-eight *Burkholderia cepacia* complex isolates recovered from the sputum of 53 cystic fibrosis patients and 75 isolates collected from the maize rhizosphere were compared to each other to assess their genomovar status as well as some traits related to virulence such as antibiotic susceptibility, proteolytic and hemolytic activities, and transmissibility, in which transmissibility is determined by detection of the *esmR* and *cblA* genes. Among the clinical isolates, *B. cepacia* genomovar III comprised the majority of isolates examined and only a very few isolates were assigned to *B. cepacia* genomovar I, *B. stabilis*, and *B. pyrrocinia*; among the environmental isolates a prevalence of *B. cepacia* genomovar III and *B. ambifaria* was observed, whereas few environmental isolates belonging to *B. cepacia* genomovar I and *B. pyrrocinia* were found. Antibiotic resistance analysis revealed a certain degree of differentiation between clinical and environmental isolates. Proteolytic activity and onion tissue maceration ability were found to be spread equally among both clinical and environmental isolates, whereas larger percentages of environmental isolates than clinical isolates had hemolytic activity. The *esmR* gene was found exclusively among isolates belonging to *B. cepacia* genomovar III, with a marked prevalence in clinical isolates, whereas only one clinical isolate belonging to *B. cepacia* genomovar III was found to bear the *cblA* gene. In conclusion, the results of the present study show that the species compositions of the clinical and environmental *B. cepacia* complex populations examined are quite different and that some of the candidate determinants related to virulence and transmissibility are not confined solely to clinical isolates but are also spread among environmental isolates belonging to different species of the *B. cepacia* complex.

First described in 1950 by Burkholder (7) as the cause of soft rot in onions, *Burkholderia cepacia* has been recognized since the late 1970s as an opportunistic human pathogen that causes numerous outbreaks, particularly among cystic fibrosis (CF) patients. *B. cepacia* is especially problematic in these patients due to the potential severity of respiratory infections, the easy patient-to-patient spread of the organism, and the organism's innate resistance to a wide range of antimicrobial agents (19). While it has emerged as a human pathogen, *B. cepacia* has attracted increasing interest in agriculture and biotechnology for its potential as a biocontrol and bioremediation agent. The reasons for this interest include the organism's ability to promote plant growth by antagonizing soilborne plant pathogens (5, 6, 21, 30) and to degrade hydrocarbons and thus assist with the bioremediation of contaminated soil and water (16, 23, 25). The mode of acquisition of *B. cepacia* by CF patients remains unclear, and there is a general consensus that the large-scale use of these organisms would be ill advised until more is known about the presence or absence of the pathogenic potentials of environmental *B. cepacia* strains.

Recent taxonomic analyses have demonstrated that bacteria identified as *B. cepacia* actually comprise at least nine distinct

species or genomovars, referred to collectively as the *B. cepacia* complex. These include *B. cepacia* genomovar I, *B. multivorans*, *B. cepacia* genomovar III, *B. stabilis*, *B. vietnamiensis*, *B. cepacia* genomovar VI, *B. ambifaria*, and *B. anthina* (10, 11, 17, 37, 38; P. Vandamme et al., unpublished data); in addition, it was recently shown that the species *B. pyrrocinia* also belongs to the *B. cepacia* complex (12; P. Vandamme et al., unpublished data).

Although all nine species or genomovars have been recovered from CF patients, *B. multivorans* and *B. cepacia* genomovar III account for the majority of isolates from CF patients, especially epidemic clones (26, 29, 35, 37). As far as environmental strains are concerned, in the first extensive study performed with bacterial strains isolated from a single habitat (15), *B. cepacia* genomovar I, *B. cepacia* genomovar III, and *B. ambifaria* have been reported to be the most widespread members of the *B. cepacia* complex in the rhizosphere of maize; moreover, Balandreau et al. (2) found that a group of *B. cepacia*-like organisms isolated from the rhizosphere or tissues of maize, wheat, and lupine belonged to *B. cepacia* genomovar III. However, the risk posed to CF patients by *B. cepacia* strains present in the environment and the relationship between clinical and environmental isolates remain unclear, with contradictory evidence for phenotypic differences between isolates from CF patients and the environment (19). Previous comparative studies of clinical and environmental isolates which have suggested that environmental and clinical

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isolates of *B. cepacia* represent distinct groups, that clinical strains lack the ability to act as phytopathogens, and that environmental isolates are unlikely to be responsible for human infections have been handicapped by the inclusion of only a few environmental isolates (4, 18, 24, 41). Therefore, more extensive studies with a wider collection of isolates from CF patients and environmental isolates are needed to evaluate the role of natural habitats as potential reservoirs of pathogenic strains and the risks associated with the use of strains belonging to the *B. cepacia* complex for biotechnological purposes.

In the present study, we examined a large collection of clinical and environmental isolates belonging to the *B. cepacia* complex. Sixty-eight isolates were recovered from 53 Italian CF patients living in 11 regions; 75 isolates were collected from the maize rhizosphere, where the *B. cepacia* complex represents one of the predominant bacterial groups (13, 14, 20), in three different locations in northern, central, and southern Italy. By investigating the genomovar distributions of clinical and environmental isolates, as well as some traits related to virulence such as proteolytic and hemolytic activities, antibiotic susceptibility, and genetic markers associated with transmissibility, such as the *B. cepacia* epidemic strain marker (BCESM) encoded by the *esmR* gene and the cable pilus gene (*cblA*), we aimed to assess the differences between clinical and environmental isolates of Italian *B. cepacia* complex bacteria.

MATERIALS AND METHODS

***B. cepacia* complex strains.** Sixty-eight clinical isolates and 75 environmental isolates belonging to the *B. cepacia* complex were investigated in this study. Clinical isolates were recovered from sputum samples from 53 CF patients who attended the Cystic Fibrosis Centre of Gaslini Hospital (Genoa, Italy) between April 1985 and December 1999. The Cystic Fibrosis Centre of Gaslini Hospital is a large specialized unit to which patients from different regions of Italy have been admitted for many years. Clinical specimens were processed by techniques regularly in use for specimens from CF patients (34). The identities of the isolates were confirmed by standard biochemical procedures (API 20 NE; BioMérieux). The environmental strains of the *B. cepacia* complex were isolated from the rhizosphere of maize cultivated in three different fields located in northern, central, and southern Italy (5, 13, 14). Representative strains of the current species of the *B. cepacia* complex were also included (2, 10, 11, 29). All strains were cryopreserved at -80°C in 30% (vol/vol) glycerol.

Species and genomovar identification. (i) **Sample preparation prior to PCR amplification.** Clinical and environmental strains were grown overnight on nutrient agar (NA; Difco Laboratories) at 37 and 28°C, respectively. Lysis of the cells of clinical strains was performed by the digestion technique described by O'Callaghan et al. (33) in 20 μl of lysis buffer (1 \times Polymed PCR buffer, 0.5% Tween 20, 200 μg of proteinase K per ml). Lysis of the cells of rhizosphere *B. cepacia* strains was performed by the procedure described by Di Cello et al. (14).

(ii) **Amplification and restriction analysis of 16S rDNA.** Amplification analysis of 16S rDNAs were performed with 2 μl of each cell lysate suspension in 20 μl of Polymed *Taq* buffer with 0.5 U of *Taq* DNA polymerase (Polymed), as described by Di Cello et al. (14). Preliminary identification was performed on the basis of a previously published PCR-based procedure (15) by digesting each PCR sample in four separate reactions with four of the six enzymes used by Segonds et al. (35) to differentiate *Burkholderia* species or genomovars; these enzymes, i.e., *AluI*, *CfoI*, *DdeI*, and *MspI* (New England Biolabs), were selected because of their discriminatory powers.

(iii) ***recA*-based RFLP analyses and genomovar-specific PCR assays.** Genomovar status was determined by restriction fragment length polymorphism (RFLP) analysis of the *recA* gene, which had previously been amplified with primers selective for bacteria belonging to the *B. cepacia* complex; the PCR products were digested with the endonucleases *HaeIII* and *MnII* (New England Biolabs) (28). Genomovar assignment was confirmed by PCR of the *recA* gene with genomovar-specific primers by previously described procedures (28). The identities of *B. pyrrocinia* isolates were confirmed by DNA-DNA hybridization experiments (P. Vandamme et al., unpublished observations).

Analysis of virulence and transmissibility-related traits. (i) **Antibiotic resistance pattern.** Antibiotic susceptibility patterns were determined by the disk diffusion method on Mueller-Hinton medium (Difco Laboratories), according to the criteria of the National Committee for Clinical Laboratory Standards (32). Mueller-Hinton agar plates were inoculated with a bacterial suspension equivalent to a 0.5 McFarland standard, and antibiotic sensitivity disks (Becton Dickinson) were applied. Zones of growth inhibition (in millimeters) were recorded after overnight incubation at 35°C. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218 were used as control strains. The antibiotics tested were amoxicillin, piperacillin-tazobactam, piperacillin, ticarcillin, ceftazidime, ceftoxitin, cefepime, cefotaxime, aztreonam, imipenem, meropenem, nalidixic acid, ciprofloxacin, levofloxacin, tobramycin, amikacin, netilmicin, polymyxin B, minocycline, chloramphenicol, rifampin, and trimethoprim-famethoxazole.

(ii) **In vitro maceration of onion tissue.** A culture (100 μl) of each strain of the *B. cepacia* complex at the log phase of growth (approximately 10^8 CFU ml^{-1}) was inoculated onto the surface of onion slices prepared as described by Wigley and Burton (40). *B. cepacia* LMG 1222 and *E. coli* DH5 α were used as positive and negative controls, respectively. The plates were incubated at 28°C, and readings were taken at 48 h of incubation. Maceration was evaluated on a scale of 0 (no maceration of onion tissue) to 4 (100% maceration of onion tissue).

(iii) **Proteolytic activity.** The proteolytic activities of the strains were evaluated by investigating their caseinolytic capacities. All strains were grown on NA supplemented with 4% (wt/vol) skim milk. The plates were incubated at 28 and 37°C and were examined after 48 h of incubation. Clear zones surrounding the bacterial growth indicated casein degradation. *B. cepacia* LMG 1222 and *E. coli* DH5 α were used as positive and negative controls, respectively.

(iv) **Hemolytic activity.** The hemolytic activities of the strains were assessed at 28 and 37°C on NA supplemented with 5% (vol/vol) ram blood. The plates were examined after 48 h, and clear zones surrounding the bacterial growth indicated hemolytic activity. *E. coli* phy152 and *E. coli* DH5 α were used as positive and negative controls, respectively.

PCR amplification of *esmR* and *cblA* genes. The 1.4-kb *esmR* sequence encoding BCESM was amplified with specific primers BCESM 1 and BCESM 2 by the procedure described by Mahenthiralingam et al. (27). The 664-bp *cblA* DNA encoding the cable pilus was amplified with primers CBL1 and CBL2 by the procedure described by Clode et al. (9). All PCRs were performed with lysate cell suspensions and 0.5 U of *Taq* DNA polymerase (Polymed).

Statistical analysis. Differences between the antibiotic resistance patterns of clinical and environmental isolates were analyzed by one-way analysis of variance. Antibiotic resistance data were converted to a binary code, and interisolate relationships were measured by the Euclidean metric unweighted pair group average method. All analyses were carried out with StatView 512+ software (BrainPower Inc.).

RESULTS

Species and genomovar distributions of clinical and environmental isolates. The identification of the different species and genomovars was accomplished by a combination of PCR-based techniques, such as amplification and restriction analysis of 16S rDNA, genomovar-specific PCR tests, and RFLP analyses based on polymorphisms in the *recA* gene.

As shown in Table 1, not all *B. cepacia* complex species were found within the two groups of isolates. In fact, *B. cepacia* genomovar I, *B. cepacia* genomovar III, *B. stabilis*, and *B. pyrrocinia* were found among the clinical isolates, whereas *B. cepacia* genomovar I, *B. cepacia* genomovar III, *B. ambifaria*, and *B. pyrrocinia* were found among the environmental isolates. It is worth noting that in both the clinical and the environmental groups of isolates, most isolates (86.8 and 53.4%, respectively) belonged to *B. cepacia* genomovar III. Strains belonging to *B. ambifaria* were also recovered in large numbers, but only from among the environmental isolates (37.3%).

Antibiotic resistance. All clinical and environmental isolates were resistant to several antibiotics; the percentages of strains resistant to the various antibiotics varied greatly (Fig. 1). The clinical group of isolates generally had a higher percentage of

TABLE 1. Distribution of genomovars or species among clinical and environmental isolates of the *B. cepacia* complex

Genomovar	% of isolates	
	Clinical	Environmental
<i>B. cepacia</i> genomovar I	2.9 (2) ^a	1.3 (1)
<i>B. multivorans</i>	0.0	0.0
<i>B. cepacia</i> genomovar III	86.8 (59)	53.4 (40)
<i>B. stabilis</i>	7.4 (5)	0.0
<i>B. vietnamiensis</i>	0.0	0.0
<i>B. cepacia</i> genomovar VI	0.0	0.0
<i>B. ambifaria</i>	0.0	37.3 (28)
<i>B. anthina</i>	0.0	0.0
<i>B. pyrrocinia</i>	2.9 (2)	8.0 (6)

^a The numbers in parentheses refer to the number of strains belonging to a particular genomovar or species.

multidrug-resistant isolates than the environmental group did. In fact, significant differences ($P < 0.001$) between the two groups of isolates in terms of the number of drugs to which the isolates were resistant (for clinical isolates, mean, 13.00; standard deviation, 4.94; for environmental isolates, mean, 6.81; standard deviation, 1.32) were found. Within the environmental group of isolates, no significant differences in the number of drugs to which the isolates were resistant among the various species were found ($P > 0.05$), whereas within the clinical group of isolates, most resistant isolates belonged to *B. cepacia* genomovar III (data not shown). When clinical and environmental isolates of *B. cepacia* genomovar III were compared to each other, significant differences ($P < 0.0001$) in the number of drugs to which the isolates were resistant between the two groups of isolates (for clinical isolates, mean, 13.37; standard

TABLE 2. Grouping of *B. cepacia* complex isolates by cluster analysis performed with antibiotic resistance patterns

Isolate source	No. of isolates in cluster:									
	A	B	C	D	E	F	G	H	I	L
CF patients	4	9	13	4	15	1	5	4	4	8
Environment	7	0	57	1	0	0	0	9	0	0

deviation, 4.99; for environmental isolates, mean, 6.47; standard deviation, 1.81) were found.

Altogether, 119 different antibiotic resistance patterns were observed. A total of 106 profiles were unique, but 13 profiles were common to two or more isolates. It is worth noting that clinical and environmental isolates did not share common patterns. Moreover, of the 13 profiles mentioned above, only 2 were for clinical isolates; of the 11 profiles to which only environmental isolates belonged, 1 was shared by 8 isolates. The profiles were compared by numerical methods, and 10 different groups or clusters were found on the basis of 60% similarity (Table 2). A highly uneven distribution of clinical and environmental isolates was observed among the 10 clusters. In fact, the largest group, group C, comprised several clinical isolates ($n = 13$) and most of the environmental ($n = 57$) isolates. Three groups were heterogeneous and contained isolates of both clinical and environmental origins, and as many as six groups were homogeneous, containing only isolates of clinical origin.

Pathogenicity tests. In vitro maceration of onion tissue, casein hydrolysis, and hemolytic activity tests were applied to all isolates to investigate the potential pathogenicities of clinical and environmental isolates for plants and humans.

All clinical isolates were able to macerate onion tissue to

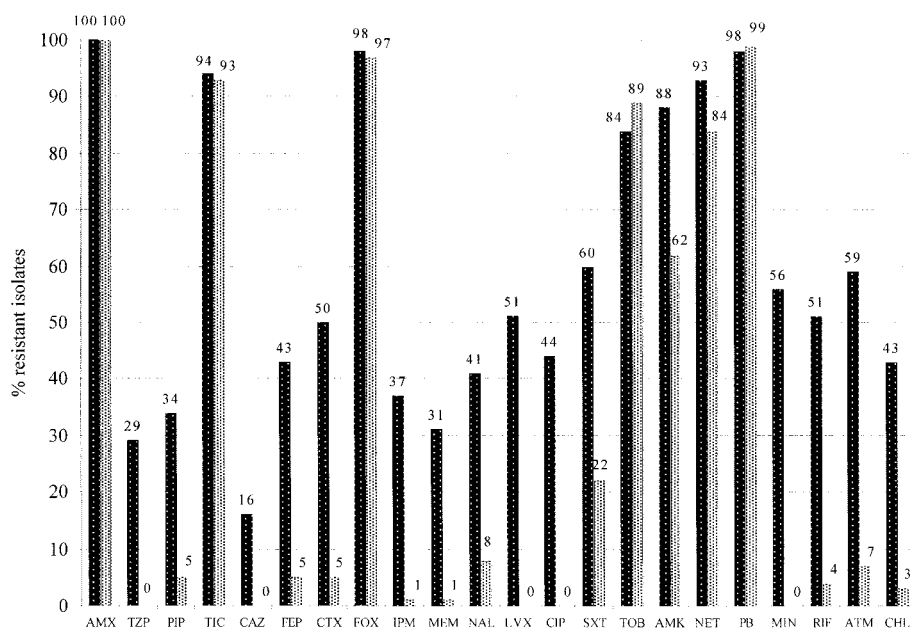


FIG. 1. Percentages of clinical (■) and environmental (▨) *B. cepacia* complex isolates resistant to antibiotics. AMX, amoxicillin; TZP, piperacillin-tazobactam; PIP, piperacillin; TIC, ticarcillin; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; FOX, ceftoxitin; IPM, imipenem; MEM, meropenem; NAL, nalidixic acid; LVX, levofloxacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin; AMK, amikacin; NET, netilmicin; PB, polymyxin B; MIN, minocycline; RIF, rifampin; ATM, aztreonam; CHL, cloramphenicol.

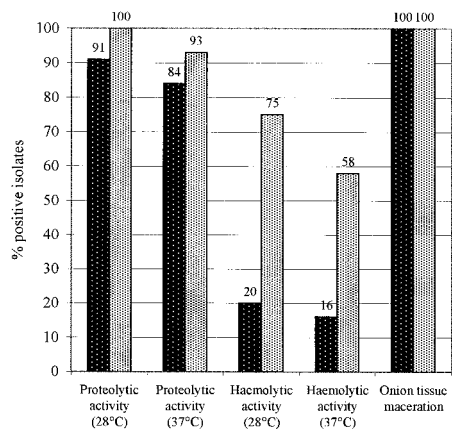


FIG. 2. Percentages of clinical (■) and environmental (▨) *B. cepacia* complex isolates capable of proteolysis, hemolysis, and onion tissue maceration.

some degree, as was the case for all environmental isolates (Fig. 2). No significant differences in the maceration abilities were observed either among clinical and environmental isolates (Fig. 2) or among different species (data not shown).

Most clinical and environmental isolates were able to hydrolyze casein at 28°C (91 and 100%, respectively) and 37°C (84 and 93%, respectively) (Fig. 2). All isolates belonging to *B. pyrrocinia* and clinical strains belonging to *B. cepacia* genomovar I and *B. stabilis* showed marked reductions in their proteolytic abilities at 37°C (Table 3). Among the environmental isolates, *B. cepacia* genomovar III and *B. ambifaria* showed only slightly higher percentages of positive strains at 28°C than at 37°C. It is worth noting that no marked differences between clinical and environmental isolates belonging to *B. cepacia* genomovar III were observed (Table 3).

In contrast to the in vitro maceration of onion tissue and casein hydrolysis, marked differences in hemolytic activities were found between clinical and environmental isolates (Fig. 2). In fact, surprisingly, a much higher percentage of environmental isolates than clinical isolates showed hemolytic activity at both temperatures used. In particular, more than twice as

many environmental than clinical isolates of *B. cepacia* genomovar III exhibited hemolytic activity, a high percentage of *B. ambifaria* isolates were positive for hemolytic activity at both temperatures, and *B. pyrrocinia* isolates showed marked reductions in hemolytic activity at 37°C (Table 3). Furthermore, marked differences were observed when different species were compared to each other, with *B. cepacia* genomovar I and *B. stabilis* being the species with the lowest percentage of strains positive for hemolytic activity and *B. ambifaria* and *B. pyrrocinia* being the species with the highest percentage of strains positive for hemolytic activity.

PCR detection of *esmR* and *cblA*. The potential pathogenicities of clinical and environmental isolates of *B. cepacia* complex were also investigated by evaluating the isolates for the presence of two molecular markers related to transmissibility, *esmR* and *cblA*, which code for BCESM and the cable pilus, respectively.

All *esmR*-positive strains belonged exclusively to *B. cepacia* genomovar III (Table 3). Interestingly, *esmR* was not confined solely to clinical isolates but was also found among environmental isolates; however, the proportion of clinical isolates positive for *esmR* (61%) was significantly higher than the proportion of environmental isolates positive for *esmR* (15%).

Of all the clinical and environmental isolates evaluated for the *cblA* gene, only one clinical isolate belonging to *B. cepacia* genomovar III was found to harbor the *cblA* gene (Table 3).

DISCUSSION

For the first time, to our knowledge, two large sets of clinical and environmental isolates belonging to the *B. cepacia* complex were compared to each other in the same study to assess their species and genomovar compositions and the presence of traits related to virulence and transmissibility.

The species compositions of the two groups of isolates were found to be quite different. Clinical isolates were assigned almost exclusively to *B. cepacia* genomovar III, and only a few clinical isolates were assigned to *B. cepacia* genomovar I, *B. stabilis*, and *B. pyrrocinia*. Among the environmental isolates, the predominant species were *B. cepacia* genomovar III and

TABLE 3. Virulence and transmissibility-related traits in different genomovars or species of the *B. cepacia* complex

Test	% of isolates positive by specimen type within each genomovar or species ^a									
	<i>B. cepacia</i> gen. I		<i>B. cepacia</i> gen. III		<i>B. stabilis</i>		<i>B. ambifaria</i>		<i>B. pyrrocinia</i>	
	Clin (2) ^b	Env (1)	Clin (59)	Env (40)	Clin (5)	Env (0)	Clin (0)	Env (28)	Clin (2)	Env (6)
Onion maceration	100	100	100	100	100	—	—	100	100	100
Proteolysis										
	28°C	100	100	90	100	100	—	—	100	100
37°C	50	100	93	95	0	—	—	96	50	67
Hemolysis										
	28°C	50	0	19	42	0	—	—	100	100
37°C	0	0	14	35	20	—	—	96	100	83
<i>esmR</i>	0	0	61	15	0	—	—	0	0	0
<i>cblA</i>	0	0	2	0	0	—	—	0	0	0

^a Abbreviations and symbols: gen., genomovar; Clin, clinical isolates; Env, environmental isolates; —, isolates were not found.

^b The numbers in parentheses refer to the number of strains belonging to a particular genomovar or species.

B. ambifaria, whereas only a few isolates were assigned to *B. cepacia* genomovar I and *B. pyrrocinia*. Previous reports (2, 15) have already shown that a significant proportion of isolates from the maize rhizosphere are actually members of *B. cepacia* genomovar III, the genomovar seemingly best adapted to the lungs of CF patients and the one that includes the major epidemic strains of the *B. cepacia* complex. Thus, the wide diffusion of *B. cepacia* genomovar III in both clinical and soil habitats raises serious concern that natural habitats may be potential reservoirs for *B. cepacia* genomovar III and therefore sources of infection for vulnerable humans. However, the virulence of environmental *B. cepacia* genomovar III isolates remains to be determined. On the contrary, we found that *B. ambifaria*, a species that comprises several plant-growth promoting strains (11, 22; L. Chiarini et al., unpublished data), was represented in large numbers among our environmental isolates but was absent from among the clinical isolates. This finding is in agreement with recent results obtained by Fiore et al. (15), who also reported the presence of *B. ambifaria* strains among environmental strains isolated in Italy, and by Agodi et al. (1), who did not find *B. ambifaria* within a group of Italian isolates from CF patients. Moreover, LiPuma et al. (26) examined 606 clinical isolates of the *B. cepacia* complex and found that only 0.7% belonged to *B. ambifaria*. Assuming that CF patients become infected with *B. cepacia* complex strains not only by patient-to-patient transmission but also by occasionally picking up strains from the environment, the unequal distribution of *B. ambifaria* in the clinical and environmental group of isolates suggests that this species may play a minor role as a human pathogen either because its pathogenicity or transmissibility may not be very high or because it may be confined to habitats where the probability of being picked up by humans is very low. In general, the different distributions of species in clinical and natural habitats suggest that there may be differential capacities for pulmonary colonization or interpatient spread, as already pointed out by LiPuma et al. (26).

Such considerations indicate that there is a need for more detailed knowledge about the distributions of each of the species of the *B. cepacia* complex in different natural habitats and, in particular, for a more precise evaluation of the pathogenicities of environmental and clinical isolates of each species belonging to the *B. cepacia* complex. Therefore, the distributions of some phenotypic traits and genetic markers related to virulence and transmissibility were investigated in an effort to evaluate the potential pathogenicities of *B. cepacia* complex isolates in relation to their origins and genomovar status. *B. cepacia* is endowed with a wide range of potential virulence determinants, but so far none has been proved to play an important role in enhancing disease in humans. Among the candidate virulence determinants, we chose antibiotic susceptibility, proteolytic and hemolytic activities, and two transmissibility markers, *esmR* and *cblA* (36). In addition, we investigated the abilities of the isolates to macerate onion tissue, which is a trait related to pathogenicity for plants.

Our results suggest that clinical and environmental *B. cepacia* strains might be functionally equivalent in terms of several traits related to virulence for humans and plants. In fact, proteolytic activity was found to be equally spread within both groups of isolates, as was onion tissue maceration ability. The latter property, which is related to pathogenicity for plants, was

previously thought to be characteristic only of environmental strains (42), but at present most clinical isolates including epidemic strains readily cause soft rot of onions (8). On the contrary, the antibiotic resistance profiles of the *B. cepacia* complex isolates were associated with their origins; in fact, clinical isolates generally exhibited resistance to larger numbers of antibiotics and a higher degree of resistance to the single antibiotics tested than the environmental isolates did. In particular, clinical isolates of *B. cepacia* genomovar III were far more resistant than environmental isolates belonging to the same genomovar. In a recent study, Berg et al. (3) analyzed the antibiotic susceptibilities of *Stenotrophomonas maltophilia* isolates from CF patients and the environment and found no significant differences between the two groups of isolates, suggesting that *S. maltophilia* isolates do not acquire antibiotic resistance during antibiotic therapy in the clinical or hospital environment. In the case of *B. cepacia* complex isolates, our results suggest that antibiotic therapy may play a fundamental role in the acquisition of antibiotic resistance.

When looking for the hemolytic activity of *B. cepacia* complex isolates, a characteristic considered to be typical of virulent strains, we found that it was partially associated with their origin; i.e., more environmental isolates than clinical isolates showed hemolytic activity. In particular, the percentage of hemolytic environmental *B. cepacia* genomovar III isolates was markedly higher than the percentage of clinical ones. Furthermore, it is worth noting that almost all isolates belonging to *B. ambifaria* were hemolytic. The low percentage of clinical isolates positive for hemolytic activity correlates well with earlier findings of Nakazava et al. (31) and Vasil et al. (39), whereas, to our knowledge, this is the first extensive survey of environmental isolates for hemolytic activity. The reason for such marked differences between clinical and environmental isolates is unclear. Vasil et al. (39) observed that the loss of hemolytic activity in clinical isolates belonging to the *B. cepacia* complex is associated with genetic rearrangements. Therefore, we hypothesize that hemolytic activity may not be a primary virulence factor in patients with CF.

The *esmR* marker, related to strain transmissibility (27), was found not only in a large proportion (61%) of clinical isolates belonging to *B. cepacia* genomovar III but also in a large proportion of environmental isolates (15%) of the same genomovar. The presence of BCESM DNA among environmental *B. cepacia* genomovar III isolates reinforces the hypothesis expressed above that the environment may serve as a reservoir of pathogenic strains belonging to this genomovar. In the case of the cable pilin subunit gene (*cblA*), only one isolate from a CF patient contained the *cblA* gene. The low frequency of the *cblA* gene casts further doubts on its reliability as a marker for virulence or transmissibility, as already pointed out by LiPuma et al. (26).

In summary, our results suggest that there may be differences in the pathogenic potentials of the various species of the *B. cepacia* complex. Moreover, we observed that some of the candidate determinants related to virulence and transmissibility are not confined solely to clinical isolates but are spread also among environmental isolates belonging to different species of the *B. cepacia* complex. A fuller appreciation of the species-related differences in pathogenicity and of the risks posed by strains likely to be encountered in the natural envi-

ronment implies a more in-depth comprehension not only of the biological mechanisms involved in pathogenicity but also of the ecophysiology of each species belonging to the *B. cepacia* complex.

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