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Research Note

Occurrence of *Burkholderia cepacia* in Foods and Waters: Clinical Implications for Patients with Cystic Fibrosis

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ABSTRACT

Two hundred forty-eight retail "ready-to-eat" foodstuffs in eight food categories and 134 waters categorized into nine types were analyzed for the presence of the *Burkholderia cepacia* complex of organisms. Of these, 14 of 26 (53.8%) samples of raw unpasteurized bovine milk were positive for this organism. Consumption of raw unpasteurized milk may therefore act as a potential source of infection with this organism, which is of particular concern for patients with cystic fibrosis, where colonization and infection with this organism can lead to a fatal necrotizing pneumonia and premature death. In addition to the associated risk of infection from fecal pathogens, patients with cystic fibrosis should therefore avoid the consumption of raw unpasteurized milk to minimize the risk of becoming infected with this organism.

Cystic fibrosis (CF) is the most common genetic disease of Caucasians, with an incidence of 1 in 2,500 live births and a carriage rate of 1 in 20 individuals. CF patients continue to suffer from recurrent and chronic respiratory tract infections, and most of their morbidity and mortality is due to such infections throughout their life *(6).* These infections are usually dominated by gram-negative organisms, especially by the pseudomonads, including *Pseudomonas aeruginosa.*

Burkholderia cepacia is an environmental soil organism found in the rhizosphere of plants and is the cause of "slippery skin" rot in onions. The organism was first described by Walter Burkholder at Cornell University in the late 1940s, where it was demonstrated to be the cause of "sour skin" rot of onion bulbs, which caused major economic loss to the growers of New York State. In addition, it is a causal agent of soft rot in certain vegetables *(13).* Originally named *Pseudomonas cepacia,* it was renamed *B. cepacia* in 1992 *(14),* when taxonomists showed it was sufficiently different from the *Pseudomonas* spp. through a combination of phenotypic and genotypic characterization studies. Although it was first shown to be an important etiological agent in nosocomial infection, this pathogen has particularly serious consequences for CF patients. *B. cepacia* has also been identified as the causative agent in some cases of endocarditis *(5),* catheter-associated urinary tract infections *(9),* and nosocomial infection outbreaks *(8).* Presently, *B. cepacia* is regarded as a complex (the *B. cepacia* complex) of organisms, consisting of five genomovars (GI to GV), of which GI, GIV, and GV are predominantly environmental organisms, with GII and GIII being predominantly of clinical origin *(12).*

Although there have been advances in isolation methodologies relating to this organism *(4),* further work is required to identify environmental reservoirs of this organism and modes of transmission resulting in colonization of the CF patient, and, to date, there have been few reports of this organism being isolated from foodstuffs.

Because the pseudomonads are commonly associated with the spoilage microflora of foods, various studies have previously identified *B. cepacia* as a spoilage organism in food. Blanco et al. *(1)* demonstrated *B. cepacia* to be responsible for "potato defect" taint in raw dry-cured Parma ham. Therefore, the aim of this study was to examine the incidence of *B. cepacia* in several foods and waters, in order to assess the significance of these foods and foodborne transmission via microaspiration of the organism in the mouth and upper gastrointestinal tract, as a potential method of acquisition of this organism in patients with CF.

MATERIALS AND METHODS

Collection of food and water specimens. Two hundred forty-eight food specimens were collected from retail food premises throughout the 26 local council areas in Northern Ireland, as part of the routine food safety surveillance program, in accordance with Food Safety (Northern Ireland) Order 1992. Fresh onions and oranges were collected from a major vegetable and fruit wholesaler for Northern Ireland. All foods, with the exception of the fruit and vegetables, were transported to the laboratory at 4°C and were analyzed within 24 h after collection. Fruits and vegetables were transported to the laboratory at ambient temperature and were analyzed within 4 h after collection. All foodstuffs were categorized into one of eight types, as shown (Table 1).

One hundred thirty-four water specimens were collected and categorized into nine types (Table 1). All water specimens were

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TABLE 1. *Prevalence of* B. cepacia *isolated from foodstuffs and waters*

Specimen category	No. of specimens analyzed	No. (%) positive for *B. cepacia*
Foods		
Cooked meat		
Pork, ham, beef, or lamb	54	0
Poultry, chicken, or turkey	35	0
Salads and rice	23	0
Dairy products (yogurt and cheese)	10	0
Raw (unpasteurized) bovine milk	26	14 (53.8%)
Seafood	30	0
Eggs (chicken)	41	0
Bakery confectionery	2	0
Fresh fruit & vegetables (onions and oranges)	27	0
Subtotal	248	14 (5.6%)
Waters		
Chlorinated water (tap water)	31	0
Unchlorinated water (springs & wells)	26	0
Raw water from reservoirs	18	0
Seawater	8	0
Water used to make ice	2	0
Swimming pools	35	0
Paddling pool	1	0
Hydrotherapy pool	9	0
Jacuzzis	4	0
Subtotal	134	0

transported to the laboratory and analyzed within 24 h after collection.

Bacteriological cultivation of *B. cepacia*. Food specimens (approximately 10 g) were enriched by mixing with 225 ml nutrient broth (Oxoid CM1; Oxoid Limited, Dorset, UK) and were blended by placing in a stomacher for approximately 2 min. The nonselective enrichment broth for each specimen was incubated at 30°C for 24 h before plating onto *B. cepacia* selective agar (BCSA; Mast Diagnostics Ltd., Merseyside, UK). Plates were incubated at 30°C for 48 h, followed by a further incubation at ambient temperature for 5 days.

Water specimens (about 400 ml) were filtered through a nitrocellulose filter (0.22 μm; Whatman Ltd., Maidstone, UK). Filters were aseptically placed into nutrient broth (9 ml; Oxoid) and were incubated at 30°C for 24 h. The nonselective enrichment broth for each water specimen was incubated at 30°C for 24 h before plating onto BCSA. Plates were incubated at 30°C for 48 h, followed by a further incubation at ambient temperature for 5 days.

Sensitivity of detection assay. An environmental isolate of *B. cepacia* was grown overnight (30°C) on Columbia blood agar (Oxoid), supplemented with 5% (vol/vol) defibrinated sheep blood. Serial dilutions of the organism were prepared in 0.1% peptone saline to 10^{-9}. Approximately 10^1 *B. cepacia* cells were added to nutrient broth (9 ml), as well as 1 ml (approximately 10^8 CFU/ml background flora) of a previous overnight broth culture of a food enrichment, previously demonstrated not to contain *B. cepacia.* The nutrient broth was incubated for 24 h; *B. cepacia* was enumerated on BCSA (30°C, 48 h), and the background flora were enumerated on nutrient agar (Oxoid) (30°C, 24 h).

In addition, for both foods and waters, positive controls (*B. cepacia*) and negative controls (uninoculated diluents and selective and nonselective media) were set up to check integrity of reagents and the process.

Phenotypic and genotypic characterization of *B. cepacia* isolates. Presumptive positive colonies were plated onto Columbia agar base (Oxoid), supplemented with 5% (vol/vol) defibrinated horse blood (Oxoid), and were incubated at 37°C for 48 h. Colonies were confirmed phenotypically by replating onto Columbia agar base (Oxoid) supplemented with 5% (vol/vol) defibrinated horse blood (Oxoid) and placing a polymyxin B antibiotic disk (30 μg ≡ 200 U) onto the surface. Plates were incubated at 37°C for 48 h. In addition, presumptive colonies were checked for their oxidase activity. Those colonies that demonstrated polymyxin B resistance and that were oxidase positive or weakly positive were further confirmed by species-specific polymerase chain reaction, employing the method of Campbell et al. *(2).*

RESULTS AND DISCUSSION

The detection assay was able to detect *B. cepacia* organisms following nonselective enrichment (24 h, 30°C) of 10^1 CFU *B. cepacia* in a background count of 3.43×10^8 CFU/ml. The sensitivity of the polymerase chain reaction assay was 10^2 CFU/ml.

All foodstuffs and waters were negative for the presence of *B. cepacia,* with the exception of raw unpasteurized bovine milk, where 14 of 26 (53.8%) samples were positive (Table 1).

Because *B. cepacia* is an environmental organism, this study was undertaken to examine the incidence of this bacterium as a contaminant in foodstuffs and waters. Only raw unpasteurized milk showed the presence of this organism, out of all the foodstuffs and waters examined, and was probably introduced into the raw milk through contamination of milk with soil, during production. Although there are no data on the heat susceptibility of this organism, it is expected that normal high temperature–short time pasteurization regimes would be of sufficient lethality to inactivate this organism, based on extrapolations of thermal stress data of close phenotypic and phylogenetic neighbors *(10).* Alternatively, this may represent an early report of subclinical mastitis in dairy cattle, with the active shedding of organisms into the milk during the milking process, since closely related gram-negative organisms, in particular, *P. aeruginosa,* are well-established etiological agents of mastitic infection in dairy cattle.

The fact that a large proportion of samples tested were negative for this organism may reflect both the seasonality and local geographical distribution of the organism. Prevalence rates may vary considerably based on agricultural and horticultural methods, including employment of this organism as both a biocontrol and bioremediation agent, as well as geographically, where *B. cepacia* may be more closely associated with the rhizosphere of plants unique to a particular climate of elevation.

In addition to milk, the potential exists for *B. cepacia* contamination of foodstuffs due to its use as a biocontrol agent. This organism has been shown to act as an antifungal agent in order to avoid postharvest loss of fruit and vegetables. Huang et al. *(7)* showed that spraying *B. cepacia* (1.6×10^9 CFU/ml) onto Washington navel oranges reduced postharvest loss mainly due to green mold decay. De Freitas et al. *(3)* showed *B. cepacia* to have antifungal activity against *Rhizoctonia solani* and therefore, to be an effective inoculant as a biocontrol agent of winter wheat. Parke *(11)* showed that *B. cepacia* was an effective biological control of *Pythium* damping-off and *Aphanomyces* root rot of pea.

In conclusion, unlike *P. aeruginosa,* the other most important pathogen of the respiratory tract of the CF patient, *B. cepacia,* does not appear to be as ubiquitous in the environment. This study was only able demonstrate its presence in raw unpasteurized milk and not in any other common foodstuffs or water examined and is the first report of the isolation of the *B. cepacia* complex from a milk source. Consumption of raw unpasteurized milk may therefore act as a potential source of infection with this organism, initially through the potential contamination or colonization of the mouth and throat areas, which, through microaspiration, may act as a source of subsequent infection of the upper and then lower airways. Given the results of raw unpasteurized milk, it would be valuable to examine the prevalence of this bacterium in pasteurized milk and other raw bovine products, particularly in light of the fact that related pseudomonads are a common postpasteurization contaminant of milk.

Therefore, in addition to the associated risks of gastrointestinal infection from fecal pathogens, including *Salmonella* spp., *Escherichia coli* O157, and *Campylobacter,* patients with CF should avoid the consumption of raw unpasteurized milk to minimize the risk of becoming infected with this organism.

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