Raw Meat Contaminated with Epidemic Clones of *Burkholderia multivorans* Found in Cystic Fibrosis Patients

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Sir,

Burkholderia multivorans is a Gram-negative pathogen belonging to the *Burkholderia cepacia* complex (*Bcc*). Together with *B. cenocepacia* it is the most prevalent *Bcc* species causing respiratory tract infection or colonization in cystic fibrosis (CF)-patients. Due to the natural multidrug resistant (MDR) pattern and the ability to form biofilm, antibiotic treatment of *Bcc* infections is difficult and frequently results in high morbidity and mortality [1].

The implementation of the multi locus sequence typing (MLST) for the *Bcc* species has highlighted that some *B. multivorans* sequence types (STs; i.e., ST16, ST17, ST24, ST181, ST190, ST195, ST274, ST374, and ST439) are internationally distributed among CF-patients [2, 3]. In particular, ST16 is an epidemic clone responsible for outbreaks in at least six different countries (i.e., France, Belgium, Canada, United States, Australia, and New Zeeland), whereas ST24 was reported in Canada and Brazil [3].

Environment, as well as materials and products that come into a hospital, may constitute sources of infection with *B. cenocepacia*. In contrast, it seems that the spread of *B. multivorans* occurs with different dynamics. In particular, *B. multivorans* is rarely isolated from natural environment making the sources of *B. multivorans* infections for CF-patients still unknown [1, 4]. In this context, several analyses have highlighted the importance of the food chain for the dissemination of MDR pathogens (e.g., extended-spectrum β-lactamase producing *Enterobacteriaceae*) [5]. However, this possibility has not yet been explored for the species belonging to the *Bcc*.

During a survey at the Institute of Veterinary Bacteriology (University of Bern) with the aim to examine the presence of cephalosporin-resistant *Enterobacteriaceae* in raw meat retailed in Switzerland, several strains of *B. multivorans* were also recovered. Thus, we aimed to explore their possible clonal relatedness with isolates causing disease in CF-patients.

Briefly, samples of meat (i.e., pork, n=25; beef, n=25; and chicken, n=25) were purchased during different days and in diverse stores located in Bern (Switzerland) between November 2012 and May 2013. Meat (~25 g) was homogenized in Luria-Bertani broth using standard sterile conditions and incubated over-night at 37°C in a shaking incubator. Ten µl of this culture was streaked onto selective MacConkey, Brilliance CRE (Oxoid, Pratteln, Switzerland), and Chrom ID ESBL agars and incubated at 37°C. Colonies were identified using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; microflex LT, Bruker Daltonics, Bremen, Germany). Minimum inhibitory concentrations (MICs) for several antibiotics were obtained with microdilution ESB1F and GNX2F panels (Trek Diagnostics, East Grinstead, England) and results were interpreted according to the 2013 Clinical and Laboratory Standards Institute (CLSI) criteria. Clonality of isolates was determined using MLST (http://pubmlst.org/bcc/). The repetitive extragenic palindromic PCR (rep-PCR) was also implemented; isolates with a genetic homology ≥85% were defined as clonally-related [5].

To study an eventual relationship between strains found in raw meat and those from CF-patients, all non-duplicated *B. multivorans* isolates (n=14) detected from samples of CF-patients processed at the Laboratory of Clinical Microbiology of the Institute of Infectious Diseases (University of Bern) during January 1998 to March 2013 were also analyzed. During this period, unique isolates of *B. cenocepacia* (n=21), *B. cepacia* (n=4), and other *Bcc* species (n=5) from CF-patients were also detected and stored at -80°C. We did not detect clinical *B. multivorans* isolates contemporary with the meat study (i.e., 2012-2013).

Six meat packages were contaminated with *B. multivorans*: one sample of pork meat contained two phenotypically different ST24, whereas five from beef were of ST16. Isolates from CF-patients belonged to ST22, ST180, ST188, ST620, and to new ST873, ST874, and ST875; a ST750 variant was also recorded (Figure 1). The rep-PCR fingerprint revealed four

clonally related groups. Three groups, consisting of two isolates each one, were formed with ST16 and ST24 isolates from meat samples. The fourth group contained six clonally-related isolates of ST874 which were all isolated from unique CF-patients in 1998, indicating a small outbreak. Otherwise CF-patients isolates exhibited distinct profiles. Notably, no other *Bcc* species were found in the different meat samples.

These figures indicate that the isolates found in raw meat are not related to those responsible for infections in the CF-patients referring to our institution. However, the finding that meat retailed in Bern was contaminated with *B. multivorans* of ST16 and ST24 is worrisome because these well-known clones are frequently responsible for chronic infection in CF-patients. In particular, *B. multivorans* ST16 is endemic and responsible for outbreaks in countries surrounding Switzerland (e.g., France) [3]. Fortunately, all *B. multivorans* strains detected during the present study do not possess a MDR phenotype (Figure 1).

Our investigation is the first that points out the attention on the possible role of raw meat as source of *B. multivorans* clones potentially pathogen for CF-patients. It can be speculated that certain *Bcc* species might be transmitted by hands-oral route to CF-patients after the manipulation of at-risk food products. Larger and more systematic studies investigating this aspect and the epidemiology of specific clones of *Bcc* in both CF-patients and food products need to be planned in the near future.

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COMPETING INTERESTS

None declared

ETHICAL APPROVAL

Not required

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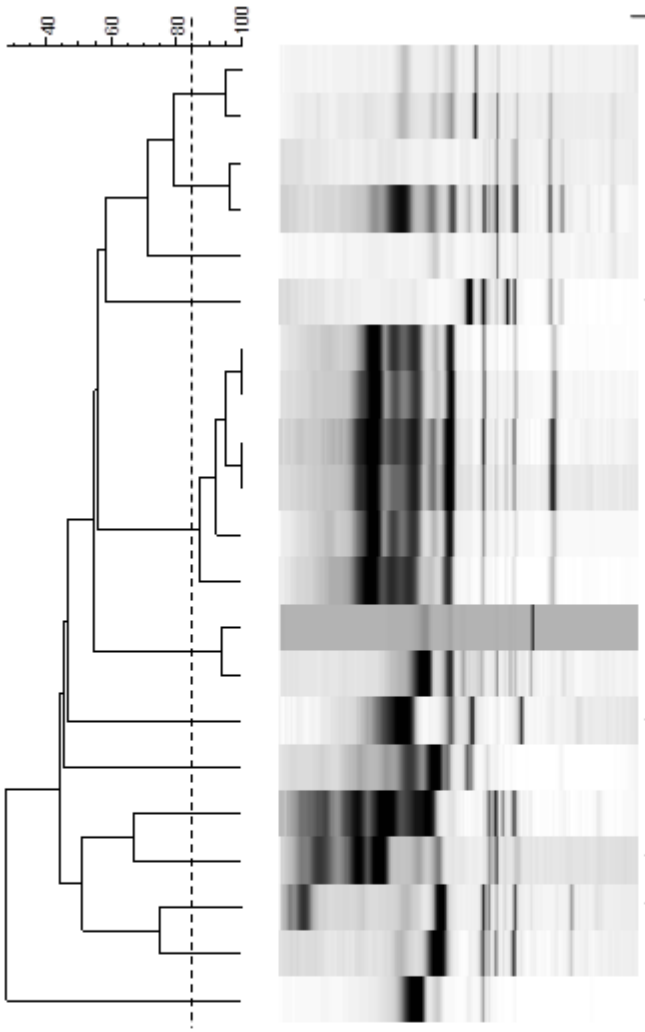

Isolate (year)	Source	ST	MIC (µg/ml) and interpretation [a]					
			CAZ	TIC	MEM	MIN	LEV	SXT
DV76 (2013)	Beef meat	16	≤1, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S
DV77 (2013)	Beef meat	16	≤1, S	≥256, R	4, S	≤2, S	≤1, S	1, S
DV74 (2013)	Beef meat	16	≤1, S	≥256, R	2, S	≤2, S	≤1, S	1, S
DV75 (2013)	Beef meat	16	≤1, S	≥256, R	2, S	≤2, S	≤1, S	≤0.5, S
DV73 (2013)	Beef meat	16	≤1, S	≥256, R	2, S	≤2, S	≤1, S	1, S
1064722 (2003)	Pharyngeal swab	873	2, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S
610183 (1998)	TBS	874	2, S	≥256, R	2, S	≤2, S	≤1, S	≤0.5, S
637589 (1998)	TBS	874	≤1, S	≥256, R	4, S	≤2, S	2, S	2, S
647228 (1998)	TBS	874	2, S	≥256, R	4, S	≤2, S	4, I	2, S
655039 (1998)	TBS	874	2, S	≥256, R	≤1, S	≤2, S	≤1, S	≤0.5, S
614620 (1998)	Sputum	874	4, S	≥256, R	2, S	≤2, S	≤1, S	≤0.5, S
620678 (1998)	TBS	874	≤1, S	≥256, R	2, S	≤2, S	≤1, S	≤0.5, S
DV65 (2013) [b]	Pork meat	24	4, S	≥256, R	≥16, R	≤2, S	≤1, S	1, S
DV66 (2013) [b]	Pork meat	24	≤1, S	≥256, R	4, S	≤2, S	≤1, S	1, S
1996055 (2011)	Pharyngeal swab	188	≤1, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S
800856 (2000)	Pharyngeal swab	875	2, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S
609675 (1998)	Pharyngeal swab	620	2, S	64, I	≤1, S	≤2, S	≤1, S	≤0.5, S
1971592 (2011)	Sputum	750-variant [c]	2, S	128, R	≤1, S	≤2, S	≤1, S	≤0.5, S
1689963 (2009)	Biopsy	180	≤1, S	128, R	2, S	≤2, S	≤1, S	≤0.5, S
811244 (2000)	TBS	180	2, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S
642289 (1998)	Sputum	22	2, S	≥256, R	4, S	≤2, S	≤1, S	≤0.5, S

FIGURE 1. rep-PCR, MLST analyses and antibiotic susceptibility of *B. multivorans* isolates found in CF-patients (n=14) and raw meat (n=7). The tree was obtained by UPGMA using Bionumerics 6.6 (Applied Maths, Kortrjk, Belgium) based on Dice bands comparison. TBS, Tracheobronchial secretions; ST, sequence type; CAZ, ceftazidime; TIC, ticarcillin-clavulanate; MEM, meropenem; MIN, minocycline; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole.

Phenotypic tests were interpreted according to the 2013 CLSI criteria as: S, susceptible; I, intermediate; R, resistant

[a] MIC values of other antibiotics included in the ESB1F and GNX2F panels but not suggested by CLSI have been omitted

[b] These two phenotypically different *B. multivorans* strains were found in the same meat sample

[c] ST not assignable due to the impossibility of amplifying the *gltB* and *phaC* alleles; the remaining alleles are identical to ST750

123