

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

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22 Sir,

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

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

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

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

46 Briefly, samples of meat (i.e., pork, n=25; beef, n=25; and chicken, n=25) were purchased
47 during different days and in diverse stores located in Bern (Switzerland) between November
48 2012 and May 2013. Meat (~25 g) was homogenized in Luria-Bertani broth **using standard**
49 **sterile conditions** and incubated over-night at 37°C in a shaking incubator. Ten µl of this
50 culture was streaked onto selective MacConkey, Brilliance CRE (Oxoid, Pratteln,
51 Switzerland), and Chrom ID ESBL agars and incubated at 37°C. Colonies were identified
52 using the matrix-assisted laser desorption ionization–time of flight mass spectrometry
53 (MALDI-TOF MS; microflex LT, Bruker Daltonics, Bremen, Germany). Minimum inhibitory
54 concentrations (MICs) for several antibiotics were obtained with microdilution ESB1F and
55 GNX2F panels (Trek Diagnostics, East Grinstead, England) and results were interpreted
56 according to the 2013 Clinical and Laboratory Standards Institute (CLSI) criteria. Clonality of
57 isolates was determined using MLST (<http://pubmlst.org/bcc/>). The repetitive extragenic
58 palindromic PCR (rep-PCR) was also implemented; isolates with a genetic homology $\geq 85\%$
59 were defined as clonally-related [5].

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

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

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

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

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

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99 **COMPETING INTERESTS**

100 None declared

101

102 **ETHICAL APPROVAL**

103 Not required

104 **REFERENCES**

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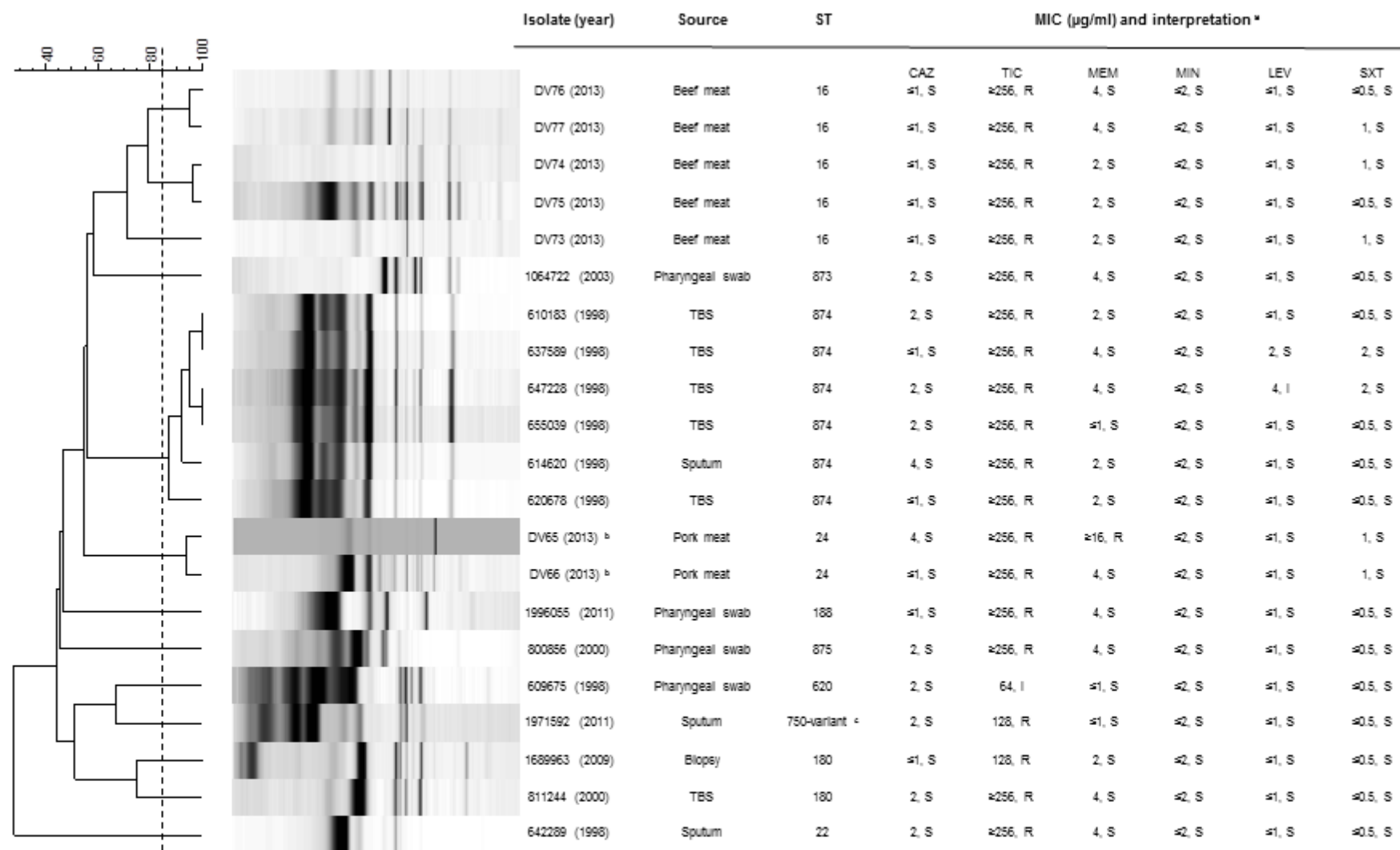


FIGURE 1. rep-PCR, MLST analyses and antibiotic susceptibility of *B. multivorans* isolates found in CF-patients (n=14) and raw meat (n=7). The tree was obtained by UPGMA using Bionumerics 6.6 (Applied Maths, Kortrijk, Belgium) based on Dice bands comparison. TBS, Tracheobronchial secretions; ST, sequence 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

