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International Journal of Food Microbiology 59 (2000) 67–72

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Short communication
Recovery of *Burkholderia pseudomallei* and *B. cepacia* from
drinking water

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Received 26 July 1999; received in revised form 20 November 1999; accepted 15 February 2000

Abstract

Samples of drinking water were examined in order to evaluate the occurrence of two Gram-negative bacteria: *Burkholderia pseudomallei* and *B. cepacia*. A total of 85 samples were collected from public and private buildings in the province of Bologna (Italy). Other bacteriological indicators (heterotrophic plate count at 22 and 36°C) were also examined, together with physical and chemical parameters (temperature, pH, residual chlorine, total hardness and chemical oxygen demand (COD)). High levels of *B. pseudomallei* were recovered (mean value = 578 cfu/100 ml) in about 7% of samples, while *B. cepacia* was recovered in 3.5% (mean value = <1) of the samples. The two microorganisms were found to correlate positively with heterotrophic plate counts at 22 and 36°C, but not with the physical and chemical parameters taken into consideration. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Drinking water; Contamination; *Burkholderia pseudomallei*; *Burkholderia cepacia*

1. Introduction

Burkholderia pseudomallei and *B. cepacia* are common environmental Gram-negative bacteria which, according to reports in the literature, may be highly pathogenic.

B. cepacia has a wide geographic distribution, is motile and grows in brain heart infusion at 30°C. *B. pseudomallei* is distinct from *B. cepacia* in its capability to grow under anaerobic conditions, with

positive nitrate respiration, and in its mechanism of pathogenicity (Wongwanich et al., 1996).

Two antigenically and biochemically distinct biotypes of *B. pseudomallei* have been described, only one of which is virulent. The organism is known to cause melioidosis, an infection described for the first time in 1912 by Whittenore in Burma and currently endemic in Southeast Asia (Smith et al., 1995; Yang et al., 1995; Trakulsomboon et al., 1997; Leelarasamee, 1998; Perret et al., 1998), Northern Australia (Brook et al., 1997; Masoud et al., 1997; Sirisinha et al., 1998) and several tropical zones (Perret, 1997). It has also been diagnosed in Europeans returning from India and Thailand (Riecke et al., 1997; Silberman et al., 1997) and, as people

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travel more, will probably be seen with increasing frequency in other parts of the world (Beeker et al., 1999).

Melioidosis is commonly found in many mammals and in endemic areas affects 6–20% of the human population (Perret, 1997). The infection appears to occur fairly commonly from childhood onwards, since in a seroepidemiological survey 80% of children were seen to have antibodies by the age of 4 years (Leelarasamee, 1998). The natural reservoirs for the germ are soil and water and humans are contaminated mainly through skin wounds (Perret, 1997). Conditions favouring infection include diabetes, renal diseases and various forms of immunodepression and the symptoms may vary from pulmonary manifestations to acute septicemia, abscesses affecting almost all the organs (Perret, 1997; Yang et al., 1998) and neonatal meningitis (Halder et al., 1998).

Infection with *B. pseudomallei* has a high mortality rate (Beeker et al., 1999) and the factors significantly associated with a higher case-fatality rate are age (> 55 years), septicemia, smoking and heart or renal failure (Heng et al., 1998).

B. cepacia, on the other hand, is an opportunistic pathogen, being a very important contaminant in the pharmaceutical industry (Gregory and Mc Nabb, 1986) and responsible for nosocomial infections (Roberts et al., 1990; Rodriguez et al., 1995; Rutala et al., 1988; van Laer et al., 1998). The bacteria may be particularly dangerous in cystic fibrosis (CF) patients (Holmes et al., 1998; Hutchison et al., 1998; Lewenza et al., 1999); 38% of Italian CF patients are in fact colonized by *B. cepacia* and this prevalence does not differ significantly from that of other countries (Taccetti and Campana, 1997). In about 20% of these patients the colonization leads to a fulminant pneumonia (cepacia syndrome) sometimes associated with septicemia (Hutchison et al., 1998) and is responsible for significant mortality (Pankhurst et al., 1995; Zughair et al., 1999), therefore having a strong impact on infection control practices (LiPuma, 1998).

The transmission of the bacteria due to social contact has been reported in CF patients (Ledson et al., 1998), in hospitalized CF patients and non-cystic fibrosis patients (Holmes et al., 1998) and through the hands of medical personnel (Quinn, 1998; Rutala et al., 1998) or respiratory equipment (Pankhurst and

Philpott-Howard, 1996). The prevention of social contact between cystic fibrosis patients by segregation and cohorting of *B. cepacia*-colonized patients has achieved some success in controlling the nosocomial and community spread of the organism (Pankhurst and Philpott-Howard, 1996).

As well as in iodinated drinking water (Pyle and McFeters, 1989) *B. cepacia* producing an antimicrobial substance active against filamentous fungi, yeasts and Gram-positive bacteria has also been found in a water pond (el Banna and Winkelmann, 1998). This characteristic of *B. cepacia* has resulted in its use as a biopesticide for protecting crops against fungal diseases (Holmes et al., 1998). However, given its potential pathogenic action, the use of *B. cepacia* for such purposes should be considered with extreme caution.

Despite the fact that it has been clearly demonstrated that these two bacteria may be responsible for serious infections in humans and are present in water, the Italian law (DPR 236/88) and EU regulations (Direttiva CEE 98/83) regarding water destined for human consumption still do not require the search for *B. pseudomallei* and *B. cepacia*. We therefore decided to investigate the prevalence of these microorganisms in drinking water and how of the occurrence the bacteria is related to certain water characteristics.

2. Material and methods

2.1. Sampling

A total of 85 samples of drinking water were collected from public (44) and private (41) buildings in Bologna and province (Italy) during a 10-month period (about eight samples/month). In order to simulate everyday practice the sampling took place first thing in the morning and was not preceded by heat sterilization of the taps or the running off of water. Favourable conditions were thus created for the development of undemanding bacteria such as those under investigation.

The samples were kept at a temperature of +4°C and were processed within 2 h of sampling. The aliquots of water to be examined were mixed with sodium thiosulphate in order to neutralize the residual chlorine. Water temperature and total residual

chlorine content were measured at the time of collection.

2.2. Examination

For the recovery of the microorganisms of the *Burkholderia* genera, 100-ml water samples were filtered through 0.45- μ m pore-size Millipore membranes. The membranes (four per sample) were placed on MacConkey agar no. 3 (CM 115-Oxoid, Milan, Italy) and *Pseudomonas* CFC agar (CM 559 and SR 103-Oxoid). The plates (two for each medium) were incubated for 24 h at 36°C (MacConkey) and for 24–48 h at 30°C (*Pseudomonas* CFC). All colony types observed on the two media were counted and isolates of each colony type were subcultured onto tryptone soy agar (CM 131-Oxoid). For the identification of the oxidase-positive isolates the API 20 NE system (20050-Biomerieux, Rome, Italy) and the motility test (motility test medium; 105-15-7-Difco, Milan, Italy) were used.

Heterotrophic plate counts at 36 and 22°C were made by the pour plate method (APHA, 1995) with plate count agar (CM 325-Oxoid). Standard method techniques (APHA, 1995) were also used for the physical and chemical parameters (water temperature, pH, residual chlorine, chemical oxygen demand COD, and total hardness).

2.3. Statistical analysis

The effect of the heterotrophic plate counts and the physical and chemical parameters on the counts

of *B. cepacia* and *B. pseudomallei* were calculated by means of correlation test (r). All statistical calculations were made using SPSS Package for Macintosh.

3. Results and discussion

The results of the examination for the two species in question are shown in Table 1. It can be seen that *B. cepacia* was recovered three times and in low numbers while *B. pseudomallei* was found six times and at high levels.

The frequency of isolation of *B. pseudomallei* (7.1%) was slightly lower than that reported in Australia by Merianos et al. (1993) (9%). In the past this microorganism has rarely been found in the waters of the region of Bologna. This fact should be considered carefully, especially since in recent years melioidosis has appeared in areas outside the endemic zones, in particular in France (Perret et al., 1998), Puerto Rico (Dorman et al., 1998), Guadalupe (Perez et al., 1997) and in Queensland (Scott et al., 1997), even though reports in Thailand have shown that the frequency of isolation of *B. pseudomallei* is not directly related to the incidence of melioidosis in an area (Trakulsomboon et al., 1997). Such discrepancies can be partly attributed to phenotypic differences between clinical and some environmental isolates (Anuntagool et al., 1998).

B. cepacia (3.5% of the samples), on the other hand, was isolated in much lower numbers despite its proven capacity to multiply in water (Rutala et al.,

Table 1
Bacteriological and physical-chemical characteristics of the samples positive for *B. cepacia* or *B. pseudomallei*

	Sample								
	1	2	3	4	5	6	7	8	9
<i>B. cepacia</i> (cfu/100 ml)	19	1	1	–	–	–	–	–	–
<i>B. pseudomallei</i> (cfu/100 ml)	–	–	–	13 000	10 800	4500	15 000	4800	1020
36°C Heterotrophic plate count (cfu/ml)	40 000	12 050	9	26 560	23 120	3220	2024	2865	370
22°C Heterotrophic plate count (cfu/ml)	50	54	31	22 400	20 000	2406	12000	3501	921
Water temperature (°C)	25.0	24.0	23.9	19.8	19.9	19.7	20.0	19.6	20.8
pH	7.45	7.55	7.90	7.40	7.60	7.81	8.00	7.50	7.82
Residual chlorine (mg/l)	0.06	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02
Total hardness (mmol/l)	1.40	1.51	2.42	2.30	2.61	2.31	1.90	1.61	1.90
COD (mg/l)	3.30	3.96	4.21	9.24	1.32	3.59	3.71	2.05	6.28

1988). In previous studies, moreover, its presence in water supplies was actually higher (5.5%) (Romano et al., 1997).

The two species were never recovered from the same samples. *B. pseudomallei* was isolated exclusively on *Pseudomonas* CFC agar; *B. cepacia* on *Pseudomonas* CFC and MacConkey media (results not shown).

On the whole, considering the sampling methods used, the level of contamination was quite low (Table 2) even if high counts were found in some samples. The heterotrophic plate count at 22°C of environmental origin was on average higher, although only slightly, than the heterotrophic plate count at 36°C. As was to be expected, the heterotrophic counts correlated significantly ($r = 0.44$; $P < 0.0001$).

As far as the other parameters are concerned it is worth noting that, unlike the pH, the interval of temperature ranged quite widely (from 10° to 30.5°C). Levels of chlorine and total hardness were generally quite low, as was the amount of organic matter (COD). With the exception of the chlorine, this can probably be attributed, together with the low seasonal variation, to the frequently deep underground origin of the piped water. Moreover, correlation analysis showed a negative association between temperature and residual chlorine ($r = 0.33$; $P < 0.01$).

While bearing in mind the fact that the organism was recovered on only few occasions, it was nevertheless observed that *B. pseudomallei* was present in

particularly contaminated samples which had been collected, at different times, from private neighbouring buildings served by the same water network (results not shown). *B. pseudomallei* presence, in fact, correlated positively both with the plate count at 36°C ($r = 0.67$; $P < 0.0001$) and with the plate count at 22°C ($r = 0.67$; $P < 0.0001$). *B. cepacia* was found in both public and private buildings, but correlated positively only with the plate count at 36°C ($r = 0.63$; $P < 0.0001$).

The two *Burkholderia* species were seen to be affected differently by temperature. In fact, although 24–32°C is generally considered to be the ideal temperature range for the survival of environmental *B. pseudomallei* strains (Tong et al., 1996), in the water examined in our study the microorganism was isolated at a lower temperature (20°C). These data confirm the findings of Yabuuchi et al. (1993) who maintain that *B. pseudomallei* is able to survive in environmental conditions that are not typically tropical. *B. cepacia*, on the other hand, was found in water with a temperature of around 24°C.

The failure to find any association between the two *Burkholderia* species and residual chlorine are in agreement with the observations of other authors who have reported that *B. cepacia* shows an intrinsic resistance to various disinfectants and antibiotics (Gregory and McNabb, 1986; Anderson et al., 1991; Hancock, 1998; Quinn, 1998).

The microbial contamination did not appear to be associated with the COD, meaning that the bacteria do not necessarily feed on the nutrients dissolved in the water, but most probably use organic matter present in the biofilm normally attached to the inner walls of the waterpipes. Similar results were obtained by Romano et al. (1997).

As far as the overall level of microbial contamination is concerned, the stagnation of the water in the pipes for at least a night, together with the fact that the water was not run off before sampling, may explain the values of the heterotrophic counts (especially at 36°C), which were higher than the limits established by Italian guidelines (DPR 236/88). In a study by Barbeau et al. (1996), it was observed that after a run off of only 2 min the load was reduced by 96%.

In conclusion, the results of our study underline the importance of extending investigations of drinking water to include microorganisms belonging to the

Table 2
Mean value and range of bacteriological and physico-chemical characteristics of the 85 drinking water samples

	Mean	Min–Max
<i>B. cepacia</i> (cfu/100 ml) ^b	< 1	0–19
<i>B. pseudomallei</i> (cfu/100 ml) ^b	578	0–15 000
36°C Heterotrophic plate count (cfu/ml) ^a	172	1–40 000
22°C Heterotrophic plate count (cfu/ml) ^a	178	1–22 400
Water temperature (°C) ^b	20.8	10.0–30.5
pH ^b	7.76	7.45–8.10
Residual chlorine (mg/l) ^b	0.03	0.01–0.20
Total hardness (mmol/l) ^b	2.12	1.40–2.61
COD (mg/l) ^b	5.33	1.32–14.32

^a Geometric mean.

^b Arithmetic mean.

Burkholderia species, given the extent of their wide occurrence and their potential pathogenicity.

References

- Anderson, R.L., Vess, R.W., Carr, J.H., Bond, W.W., Panlilio, A.L., Favero, M.S., 1991. Investigations of intrinsic *Pseudomonas cepacia* contamination in commercially manufactured povidone-iodine. *Infect. Control Hosp. Epidemiol.* 12, 297–302.
- Anuntagool, N., Intachote, P., Wuthiekanun, V., White, N.J., Sirisinha, S., 1998. Lipopolysaccharide from nonvirulent Ara + *Burkholderia pseudomallei* isolates is immunologically indistinguishable from lipopolysaccharide from virulent Ara-clinical isolates. *Clin. Diagn. Lab. Immunol.* 5, 225–229.
- APHA, 1995. In: Standard Methods for the Examination of Water and Wastewater, 19th Edition, American Public Health Association, Washington.
- Barbeau, J., Tanguay, R., Faucher, E., Avezard, C., Trudel, L., Coté, L., Prévost, A.P., 1996. Multiparametric analysis of waterline contamination in dental units. *Appl. Environ. Microbiol.* 62, 3954–3959.
- Beeker, A., Van de Stadt, K.D., Bakker, K., 1999. Melioidosis. *Neth. J. Med.* 54, 76–79.
- Brook, M.D., Currie, B., Desmarchelier, P.M., 1997. Isolation and identification of *Burkholderia pseudomallei* from soil using selective culture techniques and the polymerase chain reaction. *J. Appl. Microbiol.* 82, 589–596.
- Direttiva 98/83/CEE del Consiglio del 3.11.1998 concernente la qualità delle acque destinate al consumo umano (GU della Repubblica Italiana, 2° serie speciale, del 1.2.1999).
- Dorman, S.E., Gill, V.J., Gallin, J.I., Holland, S.M., 1998. *Burkholderia pseudomallei* infection in a Puerto Rican patient with chronic granulomatous disease: case report and review of occurrence in the Americas. *Clin. Infect. Dis.* 26, 889–894.
- DPR 24 maggio 1988, n. 236: Attuazione della direttiva CEE n.80/778 concernente la qualità delle acque destinate al consumo umano, ai sensi dell'art. 15 della legge 16 aprile 1987, n.183 (Supplemento della GU della Repubblica Italiana n. 152 del 30.6.1988).
- el Banna, N., Winkelmann, G., 1998. Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against streptomycetes. *J. Appl. Microbiol.* 85, 69–78.
- Gregory, W.J., McNabb, P.C., 1986. *Pseudomonas cepacia*. *Infect. Control* 7, 281–284.
- Halder, D., Zainal, N., Wah, C.M., Haq, J.A., 1998. Neonatal meningitis and septicaemia caused by *Burkholderia pseudomallei*. *Ann. Trop. Paediatr.* 18, 161–164.
- Hancock, R.E., 1998. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative Gram negative bacteria. *Clin. Infect. Dis.* 27 (Suppl. 1), S93–S99.
- Heng, B.H., Goh, K.T., Yap, E.H., Loh, H., Yeo, M., 1998. Epidemiological surveillance of melioidosis in Singapore. *Ann. Acad. Med. Singapore* 27, 478–484.
- Holmes, A., Govan, J., Goldstein, R., 1998. Agricultural use of *Burkholderia (Pseudomonas) cepacia*: a threat to human health? *Emerg. Infect. Dis.* 4, 221–227.
- Hutchison, M.L., Poxton, I.R., Govan, J.R., 1998. *Burkholderia cepacia* produces a hemolysin that is capable of inducing apoptosis and degranulation of mammalian. *Infect. Immun.* 66, 2033–2039.
- Ledson, M.J., Gallagher, M.J., Corkill, J.E., Hart, C.A., Walshaw, A.U., 1998. Cross infection between cystic fibrosis patients colonised with *Burkholderia cepacia*. *Thorax* 53, 432–436.
- Leelarasamee, A., 1998. *Burkholderia pseudomallei*: the unbeatable foe? *Southeast Asian J. Trop. Med. Public Health* 29, 410–415.
- Lewenza, S., Conway, B., Greenberg, E.P., Sokol, P.A., 1999. Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. *J. Bacteriol.* 18, 748–756.
- LiPuma, J.J., 1998. *Burkholderia cepacia*. Management issues and new insights. *Clin. Chest Med.* 19, 473–486.
- Masoud, H., Ho, M., Schollaardt, T., Perry, M.B., 1997. Characterization of the capsular polysaccharide of *Burkholderia (Pseudomonas) pseudomallei* 304b. *J. Bacteriol.* 179, 5663–5669.
- Merianos, A., Patel, M., Lane, J.M., Noonan, C.N., Sharrock, D., Mock, P.A., Currie, B., 1993. The 1990–1991 outbreak of melioidosis in the Northern Territory of Australia: epidemiology and environmental studies. *Southeast Asian J. Trop. Med. Public Health* 24, 425–435.
- Pankhurst, C.L., Philpott-Howard, J., 1996. The environmental risk factors associated with medical and dental equipment in the transmission of *Burkholderia (Pseudomonas) cepacia* in cystic fibrosis patients. *J. Hosp. Infect.* 32, 249–255.
- Pankhurst, C.L., Harrison, V.E., Philpott-Howard, J., 1995. Evaluation of contamination of the dentist and dental surgery environment with *Burkholderia (Pseudomonas) cepacia* during treatment of children with cystic fibrosis. *Int. J. Paediatr. Dent.* 5, 243–247.
- Perez, J.M., Petiot, A., Adjide, C., Gerry, F., Goursaud, R., Juminer, B., 1997. First case report of melioidosis in Guadeloupe, a French West Indies archipelago. *Clin. Infect. Dis.* 25, 164–165.
- Perret, J.L., 1997. Melioidosis: a tropical time bomb that is spreading. *Med. Trop. Mars.* 57, 195–201.
- Perret, J.L., Vidal, D., Thibault, F., 1998. Pulmonary melioidosis. *Rev. Pneumol. Clin.* 54, 365–372.
- Pyle, B.H., McFeters, G.A., 1989. Iodine sensitivity of bacteria isolated from iodinated water systems. *Can. J. Microbiol.* 35, 520–523.
- Quinn, J.P., 1998. Clinical problems posed by multiresistant nonfermenting Gram negative pathogens. *Clin. Infect. Dis.* 27 (Suppl. 1), S117–S124.
- Riecke, K., Wagner, S., Eller, J., Lode, H., Schaberg, T., 1997. Pulmonary melioidosis in German Southeast Asia tourist. *Pneumologie* 51, 499–502.
- Roberts, L.A., Collignon, P.J., Cramp, V.B., Alexander, S., McFarlane, A.E., Graham, E., Fuller, A., Sinickas, V., Hellyar, A., 1990. An Australia-wide epidemic of *Pseudomonas pickettii* bacteraemia due to contaminated 'sterile' water for injection. *Med. J. Austr.* 152, 652–655.
- Rodriguez, P., Fourmaux, S., de Barbeyrac, B., Rogues, A.M., Parneix, P., Leger, A., Bebear, C., 1995. Molecular typing by pulsed field gel electrophoresis of *Pseudomonas (Burkholderia)*

- cepacia* isolated from a nosocomial infection. *Pathol. Biol.* 43, 352–357.
- Romano, G., Stampi, S., Zanetti, F., De Luca, G., Tonelli, E., 1997. Occurrence of Gram-negative bacteria in drinking water undergoing softening treatment. *Zbl. Hyg. Umweltmed.* 200, 152–162.
- Rutala, W.A., Weber, D.J., Thomann, C.A., John, J.F., Saviteer, S.M., Sarubbi, F.A., 1988. An outbreak of *Pseudomonas cepacia* bacteremia associated with a contaminated intraaortic balloon pump. *J. Thorac. Cardiovasc. Surg.* 96, 157–161.
- Scott, I.A., Bell, A.M., Staines, D.R., 1997. Fatal human melioidosis in south-eastern Queensland. *Med. J. Aust.* 166 (4), 197–199.
- Silbermann, M.H., Gyssens, I.C., Endtz, H.P., Kuijper, E.J., Van der Meer, J.T., 1997. Two patients with recurrent melioidosis after prolonged antibiotic therapy. *Scand. J. Infect. Dis.* 29, 199–201.
- Sirisinha, S., Anuntagool, N., Intachote, P., Wuthiekanun, V., Puthucheary, S.D., Vadivelu, J., Withe, N.J., 1998. Antigenic differences between clinical and environmental isolates of *Burkholderia pseudomallei*. *Microbiol. Immunol.* 42, 731–737.
- Smith, M.D., Wuthiekanun, V., Walsh, A.L., White, N.J., 1995. Quantitative recovery of *Burkholderia pseudomallei* from soil in Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 89, 488–490.
- Taccetti, G., Campana, S., 1997. Microbiologic data overview of Italian cystic fibrosis patients. *Eur. J. Epidemiol.* 13, 323–327.
- Tong, S., Yang, S., Lu, Z., He, W., 1996. Laboratory investigation of ecological factors influencing the environmental presence of *Burkholderia pseudomallei*. *Microbiol. Immunol.* 40, 451–453.
- Trakulsomboon, S., Dance, D.A., Smith, M.D., White, N.J., Pitt, T.L., 1997. Ribotype differences between clinical and environmental isolates of *Burkholderia pseudomallei*. *Med. Microbiol.* 46, 565–570.
- van Laer, F., Raes, D., Vandamme, P., Lammens, C., Sion, J.P., Vrints, C., Snoeck, J., Goossens, H., 1998. An outbreak of *Burkholderia cepacia* with septicemia in a cardiology ward. *Infect. Control Hosp. Epidemiol.* 19, 112–113.
- Wongwanich, S., Chotanachan, P., Kondo, E., Kanai, K., 1996. Multifactorial pathogenic mechanisms of *Burkholderia pseudomallei* as suggested from comparison with *Burkholderia cepacia*. *Southeast Asian J. Trop. Med. Public Health* 27, 111–118.
- Yabuuchi, E., Wang, L., Arakawa, M., Yano, I., 1993. Survival of *Pseudomonas pseudomallei* strains at 5°C. *Kansenshogaku Zasshi (J. Jpn. Assoc. Infect. Dis.)* 67, 331–335.
- Yang, S., Tong, S., Lu, Z., 1995. Geographical distribution of *Pseudomonas pseudomallei* in China. *Southeast Asian J. Trop. Med. Public Health* 26, 636–638.
- Yang, S., Tong, S., Mo, C., Jiang, Z., Yang, S., Ma, Y., Lu, Z., 1998. Prevalence of human melioidosis on Hainan Island in China. *Microbiol. Immunol.* 42, 651–654.
- Zughaier, S.M., Ryley, H.C., Jackon, S.K., 1999. A melanin pigment purified from an epidemic strain of *Burkholderia cepacia* attenuates monocyte respiratory burst activity by scavenging superoxide anion. *Infect. Immun.* 67, 908–913.