Outbreak of *Burkholderia cepacia* bloodstream infections traced to the use of Ringer lactate solution as multiple-dose vial for catheter flushing, Phnom Penh, Cambodia

B. De Smet[1], C. Veng[2], L. Kruy[2], C. Kham[2], J. van Griensven[1,2], C. Peeters[3], S. Ieng[2], T. Phe[2], E. Vlieghe[1], P. Vandamme[3] and J. Jacobs[1]

1) *Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium,* 2) *Department of Infectious Diseases, Sihanouk Hospital Centre of HOPE, Phnom Penh, Cambodia and* 3) *Laboratory of Microbiology, Ghent University, Ghent, Belgium*

Abstract

The *Burkholderia cepacia* complex is a group of Gram-negative bacteria known as respiratory pathogens in cystic fibrosis patients, but also increasingly reported as a cause of healthcare associated infections. We describe an outbreak of *B. cepacia* bloodstream infections in a referral hospital in Phnom Penh, Cambodia. Over a 1.5-month period, blood cultures from eight adult patients grew *B. cepacia*. Bloodstream infection occurred after a median of 2.5 days of hospitalisation. Three patients died: 7, 10 and 17 days after blood cultures were sampled. As part of the outbreak investigation, patient files were reviewed and environmental sampling was performed. All patients had peripheral venous catheters that were flushed with Ringer lactate drawn from a 1 L bag, used as multiple-dose vial at the ward. Cultures of unopened Ringer lactate and disinfectants remained sterile but an in-use bag of Ringer lactate solution and the dispensing pin grew *B. cepacia*. The isolates from patients and flushing solution were identified as *B. cepacia* by *recA* gene sequence analysis, and random amplified polymorphic DNA typing confirmed clonal relatedness. The onset of the outbreak had coincided with the introduction of a dispensing pin with a screw fit that did not allow proper disinfection. Re-enforcement of aseptic procedures with sterile syringe and needle has ended the outbreak. Growth of *B. cepacia* should alert the possibility of healthcare associated infection also in tropical resource-limited settings. The use of multiple-dose vials should be avoided and newly introduced procedures should be assessed for infection control risks.

Keywords: Bacteraemia, bloodstream infection, *Burkholderia cepacia* complex, Cambodia, healthcare associated infection, multiple-dose vial, nosocomial, outbreak, resource-limited setting
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Corresponding author: B. De Smet, Institute of Tropical Medicine, Department of Clinical Sciences, Unit of Tropical Laboratory Medicine, Nationalestraat 155, 2000 Antwerp, Belgium
E-mail: bdesmet@itg.be

Introduction

Healthcare associated infections (HAI) are a worldwide challenge but particularly affect developing countries, where they tend to occur unrecognized or undocumented. The prevalence of HAI in developing countries was found to be 15.5 per 100 patients [1], which is at least double the rates published by the European Centre for Disease Prevention and Control [2].

The *Burkholderia cepacia* complex (Bcc) comprises 17 closely related species of Gram-negative rods that are widely distributed in the environment, one of them is *B. cepacia* [3]. They are a threat to persons with cystic fibrosis and emerge as opportunistic nosocomial pathogens due to their abilities to survive in humid environments with minimal nutrition and their intrinsic resistance to antiseptics.

We describe an outbreak of *B. cepacia* bloodstream infections (BSI) during a 1.5-month period in a referral hospital in Cambodia. The outbreak was alerted by a bacterial surveillance program of BSI and traced to the extrinsic

contamination of Ringer lactate solution used as multiple-dose vial (MDV) to flush peripheral venous catheters. To the best of our knowledge, this is the first report of a common-source outbreak of *B. cepacia* BSI from a resource-limited setting (RLS).

Methods

Setting and patients

The Sihanouk Hospital Centre of HOPE (SHCH) is a 35-bed non-governmental referral hospital in Phnom Penh, Cambodia. The hospital serves a large adult outpatient population with chronic diseases and has four inpatient wards. Bacteriology laboratory services were installed in 2005 and since July 2007 a prospective surveillance study of bacterial pathogens causing BSI is on-going. Ethical approval for this study was granted from the review boards at the Institute of Tropical Medicine and the University Hospital of Antwerp, Belgium and the National Ethical Committee, Phnom Penh, Cambodia.

Bacterial cultures and identification

As part of the on-going surveillance blood cultures are sampled when signs of Systemic Inflammatory Response Syndrome (SIRS) are present [4]. Blood (2 × 10 mL) is cultured in BacT/ALERT bottles (bioMérieux, Marcy l'Etoile, France) and incubated for 7 days at 35°C with daily visual inspection of the chromogenic indicator for growth. According to standard work-up in SHCH, Gram-negative rods are screened for polymyxin resistance (Rosco Diagnostica A/S, Taastrup, Denmark) upon primary subculture. Polymixin resistant oxidase positive isolates are tested with *B. pseudomallei* specific latex agglutination (Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand) and presumptively identified with API 20 NE (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing is performed on Mueller Hinton agar (BIO-RAD, Berkeley, CA, USA) using disk diffusion according to CLSI M100-S22 guidelines [5]. All isolates are stored at −70°C on porous beads (Microbank, Pro-Lab Diagnostics, Richmond Hill, ON, Canada) pending shipment and further analysis. For the purpose of this outbreak, further microbiological assessment was done in batch testing with MicroScan NBC42 panels (Siemens Healthcare Diagnostics, West Sacramento, CA, USA) at the Institute of Tropical Medicine (Antwerp, Belgium) and molecular analyses were done at the Laboratory of Microbiology of Ghent University (Belgium).

Environmental sampling and culture

As part of the outbreak investigation, the following items were cultured: oxygen masks, stock materials of fluids for intravenous (IV) administration, venous catheters, administration sets, antiseptic solutions, an IV catheter removed from one patient as well as the connected NaCl 0.9% solution and a Ringer lactate bag with its dispensing pin used as MDV for flushing catheters. A 100 μL volume of the solutions was aseptically sampled with a syringe, the catheter-tip was aseptically cut off, the dispensing pin (Fig. 1) was flushed with sterile NaCl 0.9% and oxygen masks were swabbed. These environmental samples were primarily cultured in 10 mL Brain Heart Infusion broth (BIO-RAD, Berkeley, CA, USA) and Ashdown selective broth at 35°C for 3 days and subsequently subcultured and worked-up as for clinical samples. Ashdown is a selective broth containing colistine sulphomethate which is in use at SHCH for the recovery of *B. pseudomallei* from non-sterile site specimens, supporting the growth of Bcc as well [6].

Bcc species identification and assessment of clonal relatedness

Isolates were identified by *recA* gene sequence analysis and clonal relatedness was assessed by random amplified polymorphic DNA (RAPD) analysis. DNA was prepared by alkaline lysis [7], PCR amplification and sequencing of *recA* was performed as previously described [8] with primers described by Baldwin *et al.* [9]. Sequences were assembled and compared to those of Bcc type- and reference strains with BioNumerics v5.10 (Applied Maths NV, Sint-Maartens-Latem, Belgium) and sequence identity was confirmed by using the NCBI BLAST server web portal (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

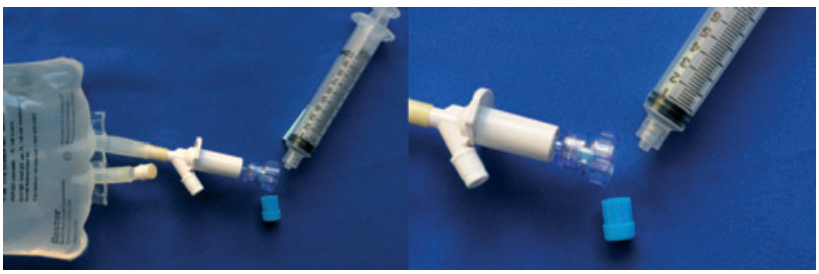

FIG. 1. Left: Needleless dispensing pin connected to 1L bag of Ringer lactate solution used as MDV for catheter flushing, Right: detail of dispensing pin with screw fit that was difficult to disinfect adequately.

For RAPD, primers 270 and 272 were used as described before [10, 11], and profiles were compared visually. In addition to the clinical and environmental samples, five *B. cepacia* reference strains were included in RAPD analysis: the *B. cepacia* type strain ATCC 25416T, reference strains of the Bcc experimental strain panel LMG 18821 and LMG 2161, R-8575 from rice seeds, and R-20435 from a HAI in Thailand. One additional *B. cepacia* isolate was included isolated from blood culture on the 17th of August 2011 in the Kampong Cham provincial hospital, Cambodia.

Review of the SHCH bacterial strain collection (2007–2011) showed four other Bcc clinical isolates, all recovered in 2008 from blood, urine, pleural fluid and sputum, respectively. These isolates were retrieved for species identification together with the outbreak isolates.

Chart review, corrective and preventive actions

During and after the outbreak, patient charts were assessed for common medications and exposures. Medical, nursing and sampling procedures were reviewed during discussions with the nursing staff, infectious diseases physicians and laboratory staff.

Results

Setting and patients

On July 7th 2011 the bacteriology laboratory of SHCH alerted the clinicians about the growth of two Bcc isolates from the blood of two patients within 1 week time. Over the following 1.5-month period (July – August 2011), a total of eight patients grew *B. cepacia* from blood cultures. All patients were hospitalised at the medical ward (a 14 bed ward) and represented 18% of 49 admissions at this ward during the outbreak period. Median age of the eight patients was 47 years (range 24–71 years), six were female. Table 1 lists the patients' demographics and clinical information and Fig. 2 illustrates the timeline of the outbreak. BSI occurred after a median of 2.5 days of hospitalisation (range 2–7 days) and the median time to positivity of blood cultures was 3 days (range 3–6 days). After blood culture results had become available, all but two patients were treated with appropriate antibiotics (i.e. ceftazidime with or without trimethoprim-sulphamethoxazole). Three patients died: 7, 10 and 17 days respectively after blood cultures were sampled. However, mortality in these patients was most likely not directly attributable to *B. cepacia* BSI as these patients had end stage liver failure.

Environmental sampling

To rule out a pseudo-outbreak, the antiseptic solutions used for blood culture sampling (poviodine-iodine 10% and isopropyl alcohol 70%), were sampled on July the 12th and 22nd, both from the affected ward and the laboratory, but all these cultures remained sterile. Samples of unopened stock materials for IV administration cultured on July the 22nd were sterile and excluded intrinsic (i.e. manufacturing-related) contamination. The cultures of oxygen masks remained sterile. On July 26th, the IV catheter from patient 5 as well as the connected NaCl 0.9% solution were cultured, both grew *B. cepacia*. Cultures of the Ringer lactate solution used as MDV for flushing IV catheters and the connected dispensing pin were taken on August 9th and yielded both *B. cepacia*.

Bacterial cultures and identification

A total of 11 outbreak isolates were available for further analysis: eight clinical isolates (one from each patient) and three environmental isolates (NaCl 0.9% from patient 5, Ringer lactate used as MDV, dispensing pin from MDV). All isolates were yellow pigmented and showed a metallic shine on blood-agar. *B. pseudomallei* specific latex agglutination was negative for all isolates and API 20 NE identified all isolates as *B. cepacia* with profiles 0477577 (nine isolates) and 0467577 (two isolates) after 48 h. Analysis with MicroScan gave two biotypes 0041772 (six isolates) and 4041772 (five isolates). All isolates had identical susceptibility profiles: (natural) resistance to polymyxin, gentamicin and amoxicillin-clavulanic acid with

TABLE 1. Patients demographics and clinical information

Patient	Age	Sex	Underlying disease	Clinical diagnosis	Day of BSI	Treatment received	Outcome
1	42	Female	DMII, PTB	Pneumonia	7	Ceftazidime + TMP/SMX	Discharged day 18
2	52	Female	Liver cirrhosis	SBP, hepatic encephalopathy	2	Ceftazidime + TMP/SMX	Died day 17
3	53	Female	Liver cirrhosis, HCV infection	SBP, hepatic encephalopathy	2	Ceftazidime + TMP/SMX	Died day 10
4	29	Male	–	Malaria, pneumonia	5	Doxycycline	Discharged day 8
5	41	Female	Liver cirrhosis	Hepatic encephalopathy	2	Ceftazidime	Died day 7
6	71	Female	DMII, chronic renal failure, congestive heart failure	Pneumonia, cardiac decompensation	3	Ceftazidime + TMP/SMX	Discharged day 11
7	53	Female	Goitre, valvular heart disease	Urinary tract infection, cardiac decompensation	2	Cefuroxime	Discharged day 5
8	24	Male	–	Cryptococcal meningitis	3	Ceftazidime	Discharged day 24

BSI, bloodstream infection; DMII, diabetes mellitus type 2; PTB, pulmonary tuberculosis; SBP, spontaneous bacterial peritonitis; HCV, hepatitis C virus; TMP/SMX, trimethoprim-sulphamethoxazole.

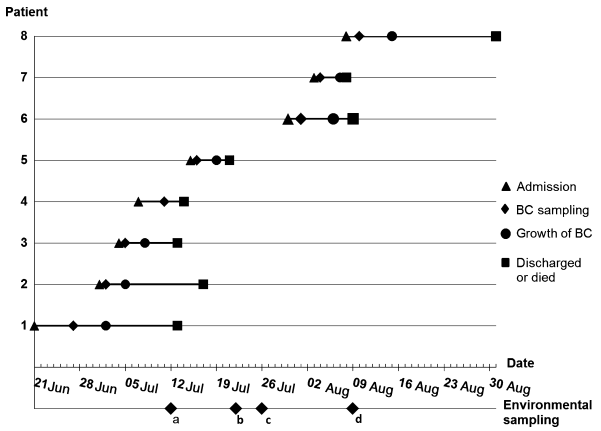

FIG. 2. Timeline of the outbreak. For patient 4 the blood culture (BC) became positive at the day of discharge. Environmental sampling included: (a) antiseptic solutions, (b) unopened stock materials for IV fluid administration and oxygen masks, (c) IV catheter and NaCl 0.9% of patient 5 and antiseptic solutions, (d) Ringer lactate multiple-dose vial and dispensing pin.

susceptibility for trimethoprim-sulfamethoxazole, ceftazidime and meropenem.

Bcc species identification and assessment of clonal relatedness

Sequence analysis of *recA* gene sequence of the outbreak isolates were 98,9% similar to those of the *B. cepacia* type strain, thus identifying them as *B. cepacia* [3, 9]. The four Bcc clinical isolates retrieved from the SHCH bacteria collection were identified as *B. cenocepacia IIIB* ($n = 1$), *B. cenocepacia IIIA* ($n = 2$) and *B. seminalis* ($n = 1$) (data not shown).

RAPD analysis revealed the following results (Fig. 3): for primer 270 all isolates showed identical RAPD profiles, and for primer 272 all but one isolate had identical RAPD profiles. The discrepant isolate (of patient 1) showed a slightly different RAPD profile with three extra bands: a double band around 1000 bp and an extra band between 400 and 200 bp. These differences were interpreted as a normal finding in the short-time evolution of the isolates, and it was concluded that clinical and environmental isolates were clonally related. The reference strains showed clearly distinct RAPD profiles as did the *B. cepacia* isolate from the Kampong Cham hospital in Cambodia.

Chart review, corrective and preventive actions

A standard 1 L bag of NaCl 0.9% solution or, depending on the availability, of Ringer lactate was used as MDV for flushing the non-heparinised peripheral IV catheters from all patients in the medical ward. This MDV was used for an undefined period, frequently till empty. Standard sampling practice included disinfection of the rubber injection ports with isopropyl alcohol 70% and subsequent sampling with sterile syringe and needle. However, shortly before the outbreak the sampling procedure had changed: a needleless dispensing pin with Luer lock and stopper was attached to the Ringer lactate bag and left in place. For sampling, the stopper of the dispensing pin was unscrewed to fit the syringe and re-screwed after fluid withdrawal, no additional sterile stoppers were available. The dispensing pin had been received as part of an in-kind donation of medical supply and had been introduced without adaptation of the nursing care protocol.

Corrective and preventive actions included the discontinuation of the use of a dispensing pin and re-enforcement of aseptic procedures, i.e. swabbing of rubber injection ports with isopropyl alcohol wipe before withdrawal with sterile needle and sterile syringe. In addition, it was decided – in the absence of small volume packaging – to use the 1 L bag as MDV for only 24 h, with a day and time mark as control. Procedures were further adapted to specify NaCl 0.9% and not Ringer lactate or glucose containing solutions for catheter flushing. Finally, monthly surveillance cultures of MDV were installed. Nursing and medical staff were properly informed during the outbreak investigation and procedures were plenary discussed and approved. During the 10 months after the outbreak, no blood cultures with Bcc isolates were noted at SHCH and surveillance cultures of the MDV remained sterile.

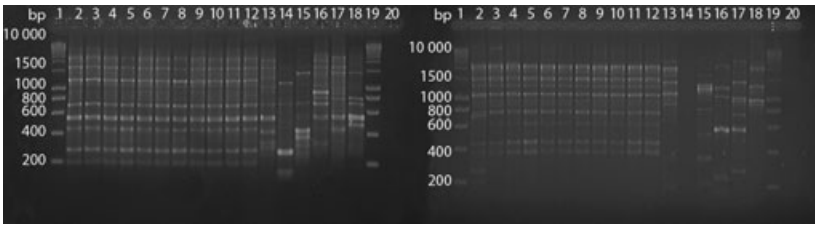

FIG. 3. RAPD profiles primer 270 Left: primer 272 Right: Lanes 1 and 19: molecular size markers; lanes 2–6 and 8, 9, 11: *B. cepacia* blood culture isolates; lanes 7,10,11: environmental isolates (IV catheter from patient 5, Ringer lactate used as MDV, dispensing pin respectively); lane 13: *B. cepacia* blood culture isolate from Kampong Cham hospital; lanes 14–18: *B. cepacia* reference strains (see text, for Right: lane 14 is missing, but showed a distinct profile on repeat testing); lane 20: blank control.

Discussion

Bcc species are frequently reported from outbreaks, often but not exclusively in immune-compromised patients. In a systematic review of HAI related to contaminated substances, Bcc ranked first (together with *Enterobacter* spp.) as contaminating pathogen in substances other than blood [12]. Both intrinsic contamination (during manufacturing) and extrinsic contamination (after opening) of medical solutions and equipment have been reported [13–16].

The use of MDV is common practice in RLS but harbours a high risk for extrinsic contamination. Vonberg *et al.* [12] described that in 49.2% of 130 drug-related outbreaks the use of MDV was reported. When using single dose vials (intended to use only once and consequently without antibacterial preservatives) as MDV, the risk for contamination is even higher. Solutions with a high nutrient content -such as Ringer lactate and glucose containing solutions- will enhance survival and multiplication of bacteria after contamination and are not recommended as flushing solutions [17]. Alcohol hand hygiene, disinfection of vial gums and injection ports, compliance with storage conditions and vial dating are pivotal practices in case the use of MDV cannot be avoided [18]. In 2010 the Cambodian Ministry of Health launched a National Strategic Plan and Guidelines for Infection Control in Health Care Facilities, these guidelines address the risks of MDV, describe aseptic handling and discourage the use of MDV for different patients [19].

The presently used dispensing pen had been introduced without thorough risk analysis and adaptation of the procedures. As for drugs and equipment, in-kind donations of medical devices and consumables should be assessed for relevance and appropriateness [20]. Indeed, concerns have risen about the so-called needleless devices as they have been associated with increased risk for catheter-related BSI due to difficult antisepsis [21]. Likewise, Chodoff *et al.* [22] described a polymicrobial Gram-negative outbreak of BSI with a similar needleless connector used for catheter flushing.

The present study shows evidence of *B. cepacia* as a HA pathogen in tropical RLS. Particularly in South-East Asia, laboratory identification of Bcc bacteria can be challenging as differentiation from *Burkholderia pseudomallei*, the agent causing life-threatening melioidosis, is required. *B. pseudomallei* accounted for 12% of clinically significant bacteria isolated from blood cultures in SHCH [23]. In the present study *recA* gene sequence analysis was used for species identification within the Bcc, because standard or commercial phenotypical tests such as API 20NE are unable to identify Bcc members unequivocally to the species level [24]. RAPD analysis has been used before in Bcc outbreaks to assess clonal relationship because of its high discriminatory power; furthermore it is less complex and faster than the more widely used pulsed field gel electrophoresis [10]. Both recA sequencing and RAPD analysis are techniques not in reach of RLS. However, as shown in the present report, the subsequent recovery of Bcc from clinical samples by itself should point to the possibility of HAI.

Similar to *B. pseudomallei,* Bcc species are resistant to polymyxin and aminoglycosides (with the exception of *B. vietnamensis* for the latter) [26] but unlike *B. pseudomallei,* they are resistant to amoxicillin-clavulanic acid, whereas the vast majority of *B. pseudomallei* isolates are susceptible [25,27]. In addition, cross-reaction of *B. cepacia* with the *B. pseudomallei* specific latex agglutination is not reported [28] and only rarely occurs for Bcc members (personal communication with Vanaporn Wuthiekanun).

Close collaboration between diagnostic laboratory and medical and nursing staff should assure swift communication of healthcare associated infection alerts. The use of multiple-dose vials should be avoided and newly introduced procedures should be assessed for their infection control risks. Without appropriate microbiological culture facilities the rate of healthcare associated infections will remain underestimated in resource-limited settings.

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Transparency Declaration

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No commercial or potential conflict of interest exists.

References

1. Allegranzi B, Bagheri NS, Combescure C *et al.* Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet* 2011; 377: 228–41.

2. European Centre For Disease Prevention And Control. Annual epidemiological report on communicable diseases in Europe 2008. 2008, Stockholm.
3. Vandamme P, Dawyndt P. Classification and identification of the *Burkholderia cepacia* complex: past, present and future. *Syst Appl Microbiol* 2011; 34: 87–95.
4. Levy MM, Fink MP, Marshall JC *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International sepsis definitions conference. *Intensive Care Med* 2003; 29: 530–8.
5. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement, M100-S22*, Vol. 32; Number 3. Wayne: Clinical and Laboratory Standards Institute, 2012.
6. Wuthiekanun V, Smith MD, Dance DA, White NJ. Isolation of *Pseudomonas pseudomallei* from soil in north-eastern Thailand. *Trans R Soc Trop Med Hyg* 1995; 89: 41–3.
7. Storms V, Van Den Vreken N, Coeyne T *et al.* Polyphasic characterisation of *Burkholderia cepacia*-like isolates leading to the emended description of *Burkholderia pyrrocinia*. *Syst Appl Microbiol* 2004; 27: 517–26.
8. Mahenthiralingam E, Bischof J, Byrne SK *et al.* DNA-Based diagnostic approaches for identification of *Burkholderia cepacia* complex, Burkholderia vietnamiensis, *Burkholderia multivorans, Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* 2000; 38: 3165–73.
9. Baldwin A, Mahenthiralingam E, Thickett KM *et al.* Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. *J Clin Microbiol* 2005; 43: 4665–73.
10. Mahenthiralingam E, Campbell ME, Henry DA, Speert DP. Epidemiology of *Burkholderia cepacia* infection in patients with cystic fibrosis: analysis by randomly amplified polymorphic DNA fingerprinting. *J Clin Microbiol* 1996; 34: 2914–20.
11. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 1990; 18: 6531–5.
12. Vonberg RP, Gastmeier P. Hospital-acquired infections related to contaminated substances. *J Hosp Infect* 2007; 65: 15–23.
13. Mangram A, Jarvis WR. Nosocomial *Burkholderia cepacia* outbreaks and pseudo-outbreaks. *Infect Control Hosp Epidemiol* 1996; 17: 718–20.
14. Govan JR, Hughes JE, Vandamme P. *Burkholderia cepacia:* medical, taxonomic and ecological issues. *J Med Microbiol* 1996; 45: 395–407.
15. van Laer F, Raes D, Vandamme P *et al.* An outbreak of *Burkholderia cepacia* with septicemia on a cardiology ward. *Infect Control Hosp Epidemiol* 1998; 19: 112–3.
16. Gravel-Tropper D, Sample ML, Oxley C, Toye B, Woods DE, Garber GE. Three-year outbreak of pseudobacteremia with *Burkholderia cepacia* traced to a contaminated blood gas analyzer. *Infect Control Hosp Epidemiol* 1996; 17: 737–40.
17. Centre for Disease Control. *Guidelines for prevention of intravascular catheter-related infections*. Atlanta: Centre for Disease Control, 2011.
18. Mattner F, Gastmeier P. Bacterial contamination of multiple-dose vials: a prevalence study. *Am J Infect Control* 2004; 32: 12–6.
19. Cambodia Ministry of Health. *Infection prevention and control guidelines for health care facilities*. Phnom Penh: Cambodia Ministry of Health, 2010.
20. World Health Organization. *Guidelines for medicine donations*. Geneva: World Health Organization, 2011.
21. Btaiche IF, Kovacevich DS, Khalidi N, Papke LF. The effects of needleless connectors on catheter-related bloodstream infections. *Am J Infect Control* 2011; 39: 277–83.
22. Chodoff A, Pettis AM, Schoonmaker D, Shelly MA. Polymicrobial gram-negative bacteremia associated with saline solution flush used with a needleless intravenous system. *Am J Infect Control* 1995; 23: 357–63.
23. Vlieghe E, Kruy L, De Smet B *et al.* Melioidosis, phnom penh, Cambodia. *Emerg Infect Dis* 2011; 17: 1289–92.
24. Henry DA, Mahenthiralingam E, Vandamme P, Coenye T, Speert DP. Phenotypic methods for determining genomovar status of the Burkholderia cepacia complex. *J Clin Microbiol* 2001; 39: 1073–8.
25. Trunck LA, Propst KL, Wuthiekanun V *et al.* Molecular basis of rare aminoglycoside susceptibility and pathogenesis of *Burkholderia pseudomallei* clinical isolates from Thailand. *PLoS Negl Trop Dis* 2009; 3: e519.
26. Jassem AN, Zlosnik JE, Henry DA, Hancock RE, Ernst RK, Speert DP. *In vitro* susceptibility of *Burkholderia vietnamiensis* to aminoglycosides. *Antimicrob Agents Chemother* 2011; 55: 2256–64.
27. Wuthiekanun V, Amornchai P, Saiprom N *et al.* Survey of antimicrobial resistance in clinical *Burkholderia pseudomallei* isolates over two decades in Northeast Thailand. *Antimicrob Agents Chemother* 2011; 55: 5388–91.
28. Amornchai P, Chierakul W, Wuthiekanun V *et al.* Accuracy of *Burkholderia pseudomallei* identification using the API 20NE system and a latex agglutination test. *J Clin Microbiol* 2007; 45: 3774–6.