

<p style="text-align: center;">Minutes of the 4th Meeting of the International <i>Burkholderia cepacia</i> Working Group (IBCWG)</p>

(7 to 9 am, October 26, 1997, Opryland Hotel, Nashville, Tennessee, held as part of the
11th Annual North American Cystic Fibrosis Conference)

Prepared by Eshwar Mahenthiralingam

Attendees (a complete list of addresses and affiliations of all participants of this IBCWG meeting is provided at the end of this document):

Jane Burns	Peter Gilligan	Cathleen Morrison	David Speert
Peter Bye	John Govan	Philip Murphy	Terry Stull
André Cantin	Keith Grimwood	Warren Regelmann	Peter Vandamme
Fiona Clode	Chip Lambert	Michael Robinson	Alison Wesley
Tom Coenye	John LiPuma	Ute Römling	Paul Whitby
Barb Conway	Noni MacDonald	Lisa Saiman	Susan Whittier
Mary Corey	Barb McKay	Dave Simpson	
Sandra Dalziel	Eshwar Mahenthiralingam	Martin Scott	
Margaret Delano	George Mallory	Anthony Smith	
Stuart Elborn	John Moore	Arnold Smith	

Agenda:

1. General introduction (Jane Burns)

a. Goals and recommendations of the IBCWG set at the 3rd meeting of the group in Victoria, BC (David Speert).

b. Summary of discussion groups:

(i) Group A- Lab diagnosis/speciation (John LiPuma)

(ii) Group B- Virulence determinants of *B. cepacia* (Jane Burns)

(iii) Group C - *B. cepacia* transmissibility/spread (Eshwar Mahenthiralingam)

2. US *B. cepacia* Referral Laboratory (John LiPuma; 20 min)

3. Canadian *B. cepacia* Referral Laboratory (Eshwar Mahenthiralingam; 10 mins)

4. United Kingdom *B. cepacia* situation (John Govan; 10 mins)

5. Open discussion:

(a) Topic 1 (15 mins) - *B. cepacia* and Transplants in CF (John LiPuma)

(b) Topic 2 (15 mins) - Segregation of *B. cepacia* colonised patients - how should strain classification impact on clinic practice ? (Eshwar Mahenthiralingam)

6. Set venue, date, chairs, for the 5th Meeting of the IBCWG (MARCH 1998) (10 mins).

7. Conclude and set goals of IBCWG for the next six months prior to the 5th meeting.

<p style="text-align: center;">1. Introduction to the International <i>B. cepacia</i> Working Group (IBCWG)</p>
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Jane Burns welcomed existing members and new participants to the 4th meeting of the International *B. cepacia* working group (only 10 of the 36 participants had attended a previous meeting of the group).

(a) David Speert reviewed the goals of the working group as they were summarized in the minutes of the 3rd meeting:

Overall objective:

“to promote a coordinated approach to resolving contentious problems related to *B. cepacia* infection/colonisation in patients with CF”.

To that end four specific goals were articulated and three working sub-groups were struck to attempt to achieve the stated objectives of the meeting. The goals of the working sub-groups were to:

- (i) reach consensus on a standard definition of *B. cepacia*;
- (ii) reach consensus on a minimum standard for isolation and identification of *B. cepacia* in clinical laboratories;
- (iii) initiate discussions on strategic approach(es) to identifying virulence determinants of *B. cepacia*; and
- (iv) initiate an international approach to addressing the problem of *B. cepacia* transmission.

A summary of the three discussion groups held at the 3rd meeting was then provided by a participant from each group:

- (i) **Group A - Improving the laboratory diagnosis of *B. cepacia* and new methods for speciating *B. cepacia*** (John LiPuma)

This discussion group had proposed several recommendations:

- (a) CF centres should be updated on the optimal methods for identifying *B. cepacia*. Genomovar analysis of the *B. cepacia*-complex and proposal of the new species *B. multivorans* was recently published by Peter Vandamme's research group (Vandamme et al. 1997. Occurrence of multiple genomovars of *Burkholderia cepacia* in Cystic Fibrosis Patients; Proposal of *Burkholderia multivorans* sp. nov. Int. J. Syst. Bacteriol. 47:1188-1200). The new taxonomic descriptions given in this paper will have a major impact on the laboratory diagnosis of *B. cepacia*.
- (b) Laboratory accrediting bodies should be advised to use *B. cepacia* reference strains in their test panels. A national (USA) *B. cepacia* Referral Laboratory should be established to aid in isolate identification - John LiPuma specifically addressed the establishment and preliminary results from this laboratory later in the meeting (see below).
- (d) A collection of "gold standard" *Burkholderia* species should be developed and deposited in the referral/research laboratories - Eshwar Mahenthiralingam addressed this issue later in the meeting.

The following issue was raised and discussed during this summary:

"Many CF centres and associated microbiology laboratories still did not have the information on the

* 136 analyzed: 103 identified as *B. cepacia* by referring lab
33 identified as other than *B. cepacia*

103 referred as *B. cepacia*

86 confirmed as *B. cepacia*
17 (16%) NOT *B. cepacia*
4 *B. gladioli*
1 *R. pickettii*
1 "unidentified"
11 being analysed ^a

33 referred as other than *B. cepacia*

7 (21%) ARE *B. cepacia*
3 *B. gladioli*
2 *P. aeruginosa*
2 *S. maltophilia*
3 "unidentified"
16 being analysed ^a

^a Taxonomic evaluation being performed by Peter Vandamme, University of Gent, Belgium.

The response to the establishment of the *B. cepacia* research laboratory and repository was pleasing, however, John noted that a number of difficulties had been encountered during the first few months. A particular concern was the fact that the response of the *B. cepacia* referral lab was to notify of both the diagnostic laboratories and consulting pulmonologist of the outcome of any strain diagnosis requested. John's concern was that this may lead to an uncomfortable relationship between clinical laboratories, their technologists and the consulting CF pulmonologist. Suggestions from the group (Peter Gilligan) were that the *B. cepacia* referral laboratory reply only to the diagnostic laboratory concerned and leave them to notify the pulmonologist of the final diagnosis. Organisms of the *B. cepacia*-complex are often difficult to identify and John did not want to discourage CF centers and their associated diagnostic laboratories from sending isolates to the *B. cepacia* referral laboratory because the results may reveal an inability to diagnose or misidentification of *B. cepacia* by contributing centres.

An additional concern was that a minority of centres were not performing an adequate diagnosis of the bacterial species prior to sending samples to the *B. cepacia* referral laboratory. John did not want the referral laboratory to be used as a "free" diagnostic service. Overall however, the results of the first few months of strain collection by the *B. cepacia* referral laboratory were encouraging and John noted that the majority of centres providing strains had performed a thorough and correct identification *B. cepacia*. The future aims of the *B. cepacia* referral laboratory were to continue to expand its strain collection and also obtain sequential isolates from patients so the retrospective studies may be carried out in the future.

3. Canadian *B.cepacia* Referral Laboratory - Eshwar Mahenthiralingam

Eshwar Mahenthiralingam provided an update on the progress of *B. cepacia* related research carried out by himself and the research group of David Speert at the University of British Columbia.

The following research initiatives were outlined:

- (i) "*B.cepacia*" Strain Collection. Identification, typing and epidemiological analysis of *B. cepacia* isolates from patients with CF and a number of other sources was ongoing. The *B. cepacia* collection now consisted of approximately 1000 isolates. Over 60 random amplified fingerprint *B. cepacia* strain types, comprising of two or more isolates, had been identified and there were approximately 150 isolates of unique fingerprint. The process of matching fingerprints should become simpler in the future now that sophisticated fingerprint matching software had been purchased.
- (ii) *B. cepacia* Selective Agar (BCSA) (Deborah Henry). The development of the media has now been published (J. Clin. Microbiol. 1997, 35:614-619). The medium is currently being tested by hospital

trials in Vancouver, BC, Seattle, WA, and Denver, CO. To date the clinical results correlate well with research laboratory findings and overall the BCSA is a much more effective selective agar than current commercial formulations.

- (iii) **B. cepacia Animal Model** (David Speert). As outlined by Jane Burns, efforts aimed at understanding the virulence and pathogenesis *B. cepacia* require the development of good animal models of infection. David Speert's laboratory has developed a murine intraperitoneal infection model in which *B. cepacia* strains persists significantly longer than *Pseudomonas aeruginosa* strains in the spleen of infected animals (up to 5 weeks). The model is highly reproducible and suggests that *B. cepacia* may be a pathogen capable of intracellular survival. The model also permits the bacterial factors which enable *B. cepacia* to survive in vivo to be examined by a molecular genetic approach.
- (iv) **B. cepacia epidemic strain marker** (BCESM) (Eshwar Mahenthiralingam). The identification and characterization of this marker has now been published (Mahenthiralingam et al. J. Clin. Microbiol. 1997, 35:808-816). The novel DNA marker is associated with several epidemic CF strains and preliminary from studies done in collaboration with Peter Vandamme (University of Gent) suggest it is specific for Genomovar III of the *B. cepacia* complex. The marker has been found to be unstable in vitro in certain strains which suffer a 20-40 kb deletion of DNA when characterized by pulsed field gel electrophoresis. An "isogenic" deletion derivative has been compared against its parental BCESM positive strain in the mouse intraperitoneal infection model described above. During the first 48 hours after intraperitoneal injection the BCESM negative "isogenic" strain is eliminated more rapidly than its BCESM positive parent. This is the first evidence that the genetic locus identified by the BCESM may encode factors which play a role in the virulence of *B. cepacia*. The large size of the BCESM associated DNA, its in vitro instability and its potential requirement for survival in the murine infection model suggest the locus may function as a pathogenicity island in *B. cepacia*.
- (v) **B. cepacia resistance to cationic peptides** (Barbara McKay). *B. cepacia* is highly resistant to cationic peptides and this phenotype correlates with its intrinsic resistance to polymyxin (a cationic antibiotic). *B. cepacia* mutants which were more susceptible to polymyxin were isolated by a transposon mutagenesis strategy. These mutants were also more susceptible to cationic peptides and experiments aimed at identifying the genetic basis for mutant phenotype are currently underway.
- (vi) **B. cepacia-complex experimental strain panel** (Eshwar Mahenthiralingam). A goal outlined at the Third Meeting of the IBCWG was to assemble an experimental strain panel which could be used as experimental standards by investigators studying *B. cepacia*. A provisional list of strains to be included in this panel is shown in Table 1. Several additions to a provisional group of strains (Table 1) had been solicited from members of the IBCWG. The suggestions have now been obtained and will include: *B. vietnamiensis* strains, more genomovar I, II, III and IV CF strains, the first UK ET12/cblA+, and early Toronto ET12/cblA+ strains. In addition, publication of the genomovar classification of members of the *B. cepacia*-complex (Vandamme et al., 1997) provides several standard strains which may be included and are referred to in the published article. Dr. Mahenthiralingam hoped that all the required strains would be deposited in the Canadian *B. cepacia* Referral Laboratory before the end of this year and that distribution of the panel to interested parties could begin in 1998.

4. United Kingdom *B.cepacia* situation - John Govan

- (i) Dr. Govan briefly outlined some issues that had arisen through correspondence with CF patients in the United Kingdom. Patients had expressed a need for more information on *B. cepacia* and John suggested that recommendations and minutes from the IBCWG should be internationally available to the CF community. This issue was addressed after the IBCWG meeting in discussions with Tom

Coenye from Peter Vandamme's research laboratory. Tom Coenye has agreed to set up an internet website for the IBCWG which will be based at the University of Gent in Belgium. Minutes of the IBCWG meetings, a database of members of the working group, and material wishing to be communicated by members of the IBCWG will be posted on the website.

- (ii) Dr. Govan also reported that a transposon mutant bank of the Edinburgh outbreak ET12/cblA+ *B. cepacia* strain had been created in his laboratory and could be screened for mutant phenotypes that members of the IBCWG were interested in.

5. Open discussion topics:

- (i) *B.cepacia* and Transplants in CF - leader John LiPuma.

John LiPuma outlined that there was still very little new information available on the consequences of lung transplantation of patients colonized with *B. cepacia*. David Speert raised the point that much of the lack of knowledge was due to the fact that *B. cepacia* strains from patients which had undergone transplants had not been stored and were not available for analysis. He suggested that a document be circulated to all transplant centres outlining the current lack of knowledge concerning this subject and requesting that each centre store *B. cepacia* isolates from their patients and if possible deposit them in one of the *B.cepacia* referral laboratories. Warren Regelman, George Mallory, Noni MacDonald and David Speert agreed to follow up on this initiative and draft a document which could be circulated by the IBCWG or the respective CF Foundations.

- (b) Segregation of *B. cepacia* colonized patients - how should strain classification impact on clinic practice? - leader - Eshwar Mahenthiralingam.

Dr. Mahenthiralingam provided preliminary epidemiological data which systematically analysed the prevalence and mortality associated with colonization of CF patients attending clinic in Vancouver with different strains of the *B. cepacia*-complex. Presence of the BCESM and genomovar classification were used to retrospectively analyse strains recovered from 63 CF patients over the last 15 years. *B. cepacia* strains which were epidemic (colonizing 5 or more patients) among CF patients in Vancouver were all BCESM positive and were all members of genomovar III. These strains had been recovered from 39 patients of which 20 had died and nearly all were chronically colonized. Of the remaining 24 *B. cepacia*-complex infected patients; 17 were colonized with *B. multivorans* (genomovar II) and 7 were awaiting genomovar analysis, but all 24 were BCESM negative. Amongst this group of BCESM negative "*B. cepacia*" colonized patients there had been no deaths and no evidence of strain transmission (each patient was colonized with a unique strain type). Ten of these 24 patients also appeared to have been only transiently colonized, with only one positive sputum culture being obtained. Five patients, each of which was colonized with a strain now classified as *B. multivorans*, had subsequently acquired the epidemic *B. cepacia* strains of genomovar III, possibly as a result of cohorting of all "*B. cepacia*" colonized patients.

These data raise the issue of patients segregation and *B. cepacia* strain classification. In Vancouver, colonization BCESM positive genomovar III *B. cepacia* is more problematic than infection with *B. multivorans* or *B. cepacia* of the remaining genomovars. Treatment practices based on cohorting of "*B. cepacia*" colonized patients may have led to spread of genomovar III strains to patients colonized with less problematic *B. multivorans*/other genomovar infection. As a result of these studies infection control cohorting practice in Vancouver will be re-evaluated and may lead to CF patients being further segregated while strain diagnosis is carried out.

These data were briefly debated because of limited time. The overall view of members of the IBCWG was that it was too early to recommend new infection control practices based on the type of

colonizing strain. More studies on the relationship of colonizing strain, clinical outcome, and epidemiology were needed which would require new tools for strain classification since access to Dr. Vandamme's genomovar classification analysis was limited.

6. The 5th Meeting of the IBCWG:

The data and venue for the 5th Meeting of the IBCWG was not finalized at this meeting. David Speert noted that Zeneca, Canada, would be able to provide some funding for the meeting and hoped that the dates, venue and participants would be set early in 1998.

7. Goals to be met prior to the next meeting of the IBCWG:

- (i) Completion of the *B. cepacia*-complex experimental panel and distribution to interested parties (Eshwar Mahenthiralingam).
- (ii) Creation of a website for the IBCWG (Tom Coenye).
- (iii) Circulate a document on the need for transplant centres to provide strains and information on patients colonized with "*B. cepacia*" which have had or are awaiting surgery (Warren Regelman, George Mallory, Noni MacDonald and David Speert).