MELIOIDOSIS:

EPIDEMIOLOGY, PATHOPHYSIOLOGY AND MANAGEMENT

Presented By

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and
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School of Medicine
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DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

______________________________
Allen Cheuk-Seng Cheng
Menzies School of Health Research and
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Australia
“…Shall I demonstrate your own ignorance? What do you know, pray, of Tapanuli fever? What do you know of the black Formosa corruption?”

“I have never heard of either.”

“There are many problems of disease, many strange pathological possibilities, in the East, Watson.”

_The Dying Detective, Sir Arthur Conan Doyle_
SUMMARY

In under a century, melioidosis, the infection due to *Burkholderia pseudomallei*, has emerged from Whitmore’s series of glanders-like infections amongst the morphia addicts in Burma to a major cause of mortality in northeastern Thailand and northern Australia. Also endemic in other parts of south-east Asia, melioidosis may have varied presentations ranging from severe, overwhelming infection to chronic, low grade disease.

Observational evidence had suggested that granulocyte colony stimulating factor (G-CSF), a naturally occurring substance produced by the body in response to infection, may have been useful in reducing the high mortality associated with the more severe forms of this infection. Other observations linked the occurrence of this disease to various environmental factors, such as contamination of drinking water and the annual rainfall. This thesis explores and attempts to quantify these associations.

There are three parts to this thesis. In the first part, I reviewed the epidemiology and management of patients with melioidosis. The use of G-CSF and meropenem was associated with a fall in mortality, although other factors may have at least partially contributed to this effect.

In the second part, I progressed towards a clinical trial of G-CSF. There was no other evidence supporting the use of G-CSF in severe sepsis and ethical issues precluded a trial in Darwin. There was not evidence from laboratory models of G-CSF action in melioidosis to support the use of G-CSF in patients, although there remained some doubt regarding the applicability of such models to human disease. I examined clinical methods to identify patients at high risk of death from melioidosis. A simple scoring system based on clinical and laboratory parameters was developed and externally validated. However, clinical definitions of severe sepsis appeared to be better predictors of mortality. A clinical trial based on clinical definitions was commenced in Thailand.

In the final part, I explored the question of whether different strains or *B. pseudomallei* or different environmental conditions caused different patterns of infection. There was no evidence that strain types of this bacterium determine the pattern or severity of disease, but weather conditions appeared to influence the distribution of disease in northern Australia.
PUBLICATIONS ARISING OUT OF THIS WORK

Published journal articles


13. Cheng AC, Godoy D, Mayo M, Gal D, Spratt BG, Currie BJ. Isolates of Burkholderia pseudomallei from northern Australia are distinct on multilocus sequence typing but are not correlated with clinical presentation. Journal of Clinical Microbiology. 2004 Dec;42(12):5477-83


29. Athan E, Allworth A, Hogg G, Burns K, Bastian I, **Cheng AC**. Tsumani lung: melioidosis is an important cause of aspiration pneumonia in survivors of the tsunami in Aceh, Indonesia. Emerging Infectious Diseases (in press)


melioidosis is often due to re-infection rather than relapse in patients in northeast Thailand. Journal of Clinical Microbiology (in press)

Submitted manuscripts

Unpublished manuscripts


Abstracts
1. **Cheng AC**, Jacups SJ, Currie BJ. A prognostic scoring system for melioidosis. Presentation at Australasian College for Tropical Medicine 11th Annual Scientific Meeting, Cairns, July 8-12, 2002


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At the Menzies School of Health Research, I worked closely with the rest of melioidosis team which included Susan Jacups, Mark Mayo, Daniel Gal, Pallave Dasari and Matty O’Brien. Much of their work, often “behind the scenes”, is represented here. My gratitude to others at the Menzies, including the Ear Team; Peter Morris, Amanda Leach, Cate Wilson, Gabby Mellon, Grant Mackenzie, Pricilla Tipakalippa, Vivianne Latham, Katrina Hodson, Edna and J.R. Gadil, Karin Dunne and Chris Wigger; laboratory and field support including Glenda Harrington, Kalinda Griffiths, Jo Bex and Sue Hutton; other laboratory staff, including Melita McKinnon, Robyn Marsh, Shelley Walton, Heidi Smith-Vaughan and Peter Fagan. I am also indebted to Kerin O’Dea for her advice and to administrative staff including Catherine Richardson, Paul Kelly, Jill Albion, Linda Ward, Robyn Liddle, Gabby Falls, Tracey Burke, Alison Frost, Di Stall, Sanya McLean, David Arthur, Nicki Crute and Ratih Sagung. Thanks also to other students at the Menzies, including Louise Maple-Brown, Matt Stevens and Sheree Cairney, registrars at the Darwin Hospital, including Anna Ralph, Bec Davis, Dorota Long, Laurens Manning and Josh Davis and other infectious diseases physicians including Ric Price, Malcolm McDonald and Dale Fisher.

At the NT Clinical School, Pauleen Cass was my entry point to the complex Flinders University bureaucracy. David Turner, Simon Conn and the staff at the Flinders Medical Library in Adelaide were all understanding of the issues facing distance students. Also in Adelaide, Adrian Esterman provided substantial biostatistical support, answering numerous queries by e-mail. David Gordon provided Anne Egan’s thesis and an insight into their previous work in this area.

Increasingly, research is being driven by collaboration and I am grateful to the researchers in the field, representing four continents, who have been open with their time, knowledge, data and at times substantial resources in allowing us to work with them. At the Royal Darwin Hospital, I worked closely with Dianne Stephens, Jane
Thomas, Bart De Keulenaer and the staff of the Intensive Care Unit. Other physicians with whom considerable discussions were crystallized in the ethics chapter included Michael Lowe, Tarun Weerathermi, Paul Lawton and Dale Fisher. In Melbourne, the School of Veterinary Science at the University of Melbourne were welcoming and tolerated my clumsy lab and animal handling skills with patience; these included Glenn Browning, Bob Meyer, Jo Allen, Phil Markham and Anna Kanci. Glenn Morahan at the Walter and Eliza Hall Institute also provided support and advice with this work.

Brian Spratt and Daniel Godoy at Imperial College London and Jim Schupp and Paul Keim at Northern Arizona University developed and performed MLST and VNTR typing methods respectively on isolates. Tim Inglis and others at Pathcentre in Perth and Rob Norton in Townsville collaborated on a study examining the role of \textit{B. pseudomallei} in potable water supplies in northern Australia.

At the Wellcome Trust-Mahidol University-Oxford University Tropical Medicine Research Program in Thailand, I am most grateful for the hospitality and generosity of Professor Nick White and Dr Nick Day who supported me during my two seasons there. Their considerable team included Dr Sharon Peacock, Khun Vanaporn Wuthiekanun, Dr Wirongrong Chierakul, Khun Patchari Prakongpan, Khun Kanjana Pongsaswat, Khun Jintana Suwanapruit, Khun Gumphol Wongsuvan, Dr Arjen Dondorp, Dr Stuart Blacksell, Dr Joost Wiersinga, Dr Annemarie Brouwer and Dr Anna Checkley. Special thanks to Khun Nongluk Getchalarat, Khun Premjit Amornchai, Dr Direk Limmathurosakul and Dr Bina Mahajan who worked long hours with me in the small Ubon lab. The staff at Sappasithiprasong Hospital, including Prof Wipida Chaowagul and many others, were most supportive and also tolerated my poor command of Thai with understanding and humor; ขอบคุณครับ (thank you). I am grateful to Ric Price and Sophie Treleaven who gave me the initial contacts there. I also acknowledge a long lineage of luminaries at the Wellcome Unit that have maintained a comprehensive database of clinical details over a 18 year period, including Dr Yupin Supputamongkol, Prof Wipada Chaowagul, Khun Vanaporn Wuthiekanun, Dr David Dance, Dr Andrew Simpson, Dr Mike Smith, Dr Jenny Short, Dr Bina Maharjan and Dr Wirongrong Chierakul.
Much of this thesis was written in spare bedrooms in various cities in the jetlagged early hours; I would like to thank my sister, Lynda, and her family in New York, Libby Dodds Ashley and Tim Ashley in Durham, the Ganovszky family in Vienna, Niklas Lindgårdh and Anna Annerberg in Bangkok, Jonathan Akikusa in Toronto, Alison Ratcliff in Adelaide and Selina Lo in Nanning for their hospitality during my time in their cities.

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Research costs money. I was grateful to be the recipient of a National Health and Medical Council Scholarship for the past three years. The Royal Australasian College of Physicians and the Flinders Medical Centre Foundation provided funding to purchase G-CSF for the study and airfares were partly supported by an Overseas Field Trip Award from Flinders University. We were fortunate to receive an NH&MRC project grant in support of the genetic typing work. The Menzies School of Health Research and the Co-operative Research Centre for Aboriginal and Tropical Medicine provided some funding to attend conferences. At Flinders University, David Turner facilitated funding to allow me to attend the animal ethics course in Adelaide and the Research Student Maintenance Fund supported travel to Melbourne as well as disbursements.

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and Karen Tymms. Craig Boutlis and Graeme Maguire, both Ph.D students immediately preceding me, provided helpful advice.

Gratitude is also due to my supervisors who responded to thousands of e-mails from afar. Bart Currie is recognized as Australia’s expert in tropical medical issues of local importance; his breadth of knowledge from laboratory science to clinical studies to public health issues, in fields including melioidosis, streptococcal infection, envenomation, parasitic diseases and rheumatic heart disease, is unparalleled. Nick Anstey has performed internationally-recognized work in malaria for many years and both his research and clinical perspectives were invaluable.

My parents have been enormously supportive over the years, work that has taken me far from them to all corners of the globe. They have imparted on me the value of education and hard work in defining one’s place in the world. In some ways a cycle is complete; my father started his life in a rural Chinese village, and this work has taken me back in an effort to improve outcomes from a poorly understood tropical disease.

And to dear Anne, who has traveled a long road with me.
DECLARATION OF THE AUTHOR’S CONTRIBUTION

This thesis is substantially my own work and was implemented under the supervision of Bart Currie and Nick Anstey. I wrote all chapters and manuscripts and analyzed all data.

I acknowledge the contributions to this work made by others:

- The work on an *in vitro* model built on the Honours thesis of Pallave Dasari who had developed the whole blood assay for phagocytosis and bactericidal ability based on a published protocol. The analysis incorporated limited data on healthy controls from her initial experiments. Dr Paul Lawton assisted with the recruitment of dialysis patients from a community dialysis centre. I altered the protocol, performed testing of additional healthy controls and patients and analyzed the data.

- The work on an animal model was carried out with the help of and under the supervision of Dr Glenn Browning at the laboratories at the School of Veterinary Science at the University of Melbourne. The experiments were conceived by Dr Grant Morahan at the Walter and Eliza Hall Institute who also provided the mice. Dr Annette Thomas provided the *B. pseudomallei* strain; Merck Australia donated lenograstim used in this study. Animal house staff at both institutions, particularly Mr Bob Geyer, cared for the mice before and during the experiments.

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- In the review of the role of C-reactive protein, Matthew O’Brien extracted data and performed a preliminary analysis as part of his Bachelor of Medical
Science thesis (University of Melbourne, 2003). Previous data entry had been performed by Dr John Engemann and Dr John Rampton. I re-analyzed the raw data using a multivariate analysis, interpreted the findings and wrote the manuscript for publication.

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1. Declaration of potential conflicting interests

During the course of this research, we received an unrestricted donation of lenograstim (Granocyte, Chugai Pharmaceuticals, Japan) from Merck Australia, who at the time owned Faulding Pharmaceuticals, the Australian distributor of lenograstim. This comprised 50 ampoules of G-CSF valued at approximately A$6000. This donation was used to perform G-CSF replacement studies in G-CSF knockout mice and, in part, the clinical trial in Thailand. The company did not seek, and nor did we offer, a review of our research as a condition of this donation.
# Table of contents

**Section A: A review of the epidemiology, pathophysiology and management of melioidosis**

1. Review of the literature 11
2. Melioidosis in Northern Australia, 2001-02 127
3. Adjunctive granulocyte colony stimulating factor is associated with improved survival in septic shock due to melioidosis 137
4. Outcomes of patients with melioidosis treated with meropenem 150

**Section B: Background, tools and models for a clinical trial** 158

5. A systematic review of G-CSF as an adjunct to antibiotics in the treatment of pneumonia in adults 160
6. An experiment that cannot be done - G-CSF in the treatment of severe melioidosis in Australia 182
7. Granulocyte-colony stimulating factor and an in vitro whole blood model of melioidosis 189
8. C-Reactive protein in the diagnosis of melioidosis 198
9. A proposed scoring system for predicting mortality in melioidosis 206
10. Validation of a prognostic scoring system for melioidosis 218
11. Towards a clinical trial of G-CSF in melioidosis 227

**Section C: Understanding the epidemiology of melioidosis** 239

12. Chlorination and pH of drinking water do not correlate with rates of melioidosis in the Northern Territory, Australia 241
13. Extreme weather events and environmental contamination are associated with outbreaks of melioidosis in northern Australia 246
14. Burkholderia pseudomallei strain type, based on pulsed field gel electrophoresis, does not determine disease presentation in melioidosis 260
15. Isolates of Burkholderia pseudomallei from northern Australia are distinct on multilocus sequence typing but are not correlated with clinical presentation 273

**Section D: Conclusions and future directions** 290

**Appendices** 293

Appendix A: Incident cases of melioidosis and median annual rainfall 293
Appendix B: Public domain software used in this thesis 293
Appendix C: Protocol and consent forms for proposed trial of G-CSF in patients with septic shock due to melioidosis in Ubon Ratchathani 295

Vita 311
**Tables**

Table 1-1: Worldwide distribution of melioidosis based on reported cases............ 12

Table 1-2: Genes associated with survival and virulence identified in the *B. pseudomallei* genome .................................................................................................................................................. 32

Table 1-3: Clinical risk factors for melioidosis.......................................................... 41

Table 1-4: Variation in clinical pattern of melioidosis worldwide............................. 44

Table 1-5: Sensitivity and specificity of diagnostic tests for culture-confirmed
melioidosis.................................................................................................................... 53

Table 1-6: In vitro activity of selected antibiotics against *B. pseudomallei*............ 60

Table 1-7: Clinical trials of intensive phase intravenous antibiotics in severe
melioidosis..................................................................................................................... 72

Table 1-8: Clinical trials of eradication phase oral antibiotics in treatment of
melioidosis..................................................................................................................... 74

Table 1-9: Intensive phase antibiotic regimens used for patients with normal renal
function......................................................................................................................... 75

Table 1-10: Eradication phase antibiotic regimens used for patients with normal
renal function................................................................................................................. 76

Table 1-11: Interventions demonstrated to reduce mortality in critically ill patients
(supported by clinical trials evidence)........................................................................ 81

Table 2-1: Cases of melioidosis by State/Territory; 1 November, 2001 to 31
October, 2002........................................................................................................... 128

Table 3-1: Summary of results comparing G-CSF and historical control groups .... 141

Table 4-1: Characteristics of meropenem- and ceftazidime-treated patients ......... 153

Table 5-1: Search strategies for clinical trials of G-CSF in pneumonia................. 164

Table 5-2: Characteristics of included studies............................................................ 170

Table 8-1: Clinical features and C-reactive protein levels during admission period 200

Table 9-1: Univariate and multiple variable logistic regression of predictors for
mortality......................................................................................................................... 208

Table 9-2: Melioidosis score worksheet .................................................................... 210

Table 10-1: Comparison of clinical definitions of sepsis severity against melioidosis
score thresholds and observed mortality....................................................................... 223

Table 11-1: Table of relative costs: (in ThB) per day/per treatment course .......... 232

Table 11-2: Baseline data for patients enrolled in G-CSF study .............................. 237
Table 13-1: Categories of cyclones and a list of cyclones in the Top End region between 1990 and 2002. .................................................................248
Table 13-2: Outbreaks identified and probable explanatory events ..................251
Table 14-1: Dice coefficients for within group and between group comparisons (median, interquartile range and number of isolate pairs).................................266
Table 15-1: Isolates not epidemiologically linked but clonal on PFGE: comparison with MLST.................................................................279
Table 15-2: Isolates epidemiologically linked but polyclonal on PFGE: comparison with MLST.................................................................280
Figures

Figure 1-1: Worldwide distribution of melioidosis .....................................................14

Figure 2-1: Geographic distribution of cases of melioidosis and average annual rainfall in Australia .................................................................................................131

Figure 2-2: Monthly number of cases in northern Australia (November 2001 – October 2002) ........................................................................................................132

Figure 3-1: Kaplan Meier survival curve from day of admission for G-CSF and historical control groups .................................................................................................140

Figure 5-1: G-CSF in pneumonia meta-analysis: 28 day mortality forest plot .......176

Figure 5-2: G-CSF in pneumonia meta-analysis: all adverse events forest plot .....176

Figure 5-3: G-CSF in pneumonia meta-analysis: Incident acute respiratory distress syndrome forest plot ..........................................................................................177

Figure 5-4: G-CSF in pneumonia meta-analysis: disseminated intravascular coagulation forest plot .................................................................................................177

Figure 5-5: G-CSF in pneumonia meta-analysis: acute renal failure forest plot ......178

Figure 5-6: G-CSF in pneumonia meta-analysis: incident septic shock forest plot ..178

Figure 6-1: Who wouldn't want this? ........................................................................184

Figure 7-1: Whole blood bactericidal activity, measured by reduction in bacterial counts, of control, dialysis and diabetic subjects without/with co-incubation with G-CSF ..............................................................................................................191

Figure 8-1: Distribution of C-reactive protein levels (median and interquartile range (IQR)) by time from admission ..............................................................................201

Figure 9-1: Generalized additive model plots examining non-linear relationships between continuous variables and mortality .................................................................211

Figure 9-2: Receiver-operator characteristic curve, examining the sensitivity and specificity of the total score for mortality .................................................................212

Figure 9-3: Melioidosis score; distribution of patients, deaths and mortality rate ....213

Figure 10-1: Melioidosis score and mortality (complete set analysis) ......................220

Figure 10-2: Receiver operator curve for melioidosis score vs death (complete set analysis) .................................................................................................................221

Figure 10-3: Melioidosis score and mortality (imputed values analysis) ..............222

Figure 10-4: ROC curve for melioidosis score vs death (complete set analysis) ....222

Figure 11-1: Survival of patients with melioidosis and septic shock in Ubon Ratchathani, 2002 .........................................................................................................228
Figure 11-2: Survival of patients enrolled into G-CSF trial .....................................236
Figure 12-1: Crude rate of melioidosis and drinking water pH .................................242
Figure 12-2: Chlorinated water supplies and melioidosis rate .................................243
Figure 13-1: Top End region of the Northern Territory ............................................247
Figure 13-2: Incident cases of melioidosis in relation to TC Thelma, December 1998
(clusters 1 and 2) ................................................................................................252
Figure 13-3: Incident cases of melioidosis in relation to Katherine floods in January,
2000 (cluster 3) ................................................................................................253
Figure 13-4: PFGE gel for isolates in cluster 3: Isolates 3a, 3b, 3c, 3d, 3f and 3g
were from patients involved in cluster 3; other isolates were taken from patients in
the same geographic area outside the outbreak period. .......................................254
Figure 14-1: Dendrogram based on UPGMA and Dice coefficient from SpeI-
restricted B. pseudomallei isolate DNA .............................................................263
Figure 14-2: Distribution of major strain types by (a) site of infection and (b)
severity of infection. ...........................................................................................264
Figure 14-3: Distribution of Dice coefficients for within group (solid line) and
between group isolates (dashed line) for (a) severe sepsis, (b) neurological
disease, (c) genitourinary disease, (d) pneumonia, (e) skin/soft tissue infection
(f) Darwin urban region. Within group comparisons are between isolates
derived from patients with the same presentation/locality and between group
comparisons are between isolates from patients with the presentation and those
without the presentation ......................................................................................267
Figure 15-1: Comparison of PFGE and MLST for isolates not epidemiologically
linked but clonal on PFGE (PFGE clone 1 and PFGE clone 2) and a case cluster
from a remote community .................................................................................278
Figure 15-2: Relatedness among isolates was displayed as a dendrogram by the
UPGMA method using the matrix of pair-wise differences between the allelic
profiles of the isolates. Clusters and arrows indicate Australian isolates from
this study. Number of Australian isolates from this study indicated in
parentheses; single unless otherwise indicated .................................................284
Figure 15-3: Tree constructed from the concatenated sequence of the seven MLST
loci from B. pseudomallei isolates illustrating distribution of Australian
sequence types (clusters indicated by brackets and unclustered strains by
arrows) and those from other endemic areas .....................................................286
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>Micrograms</td>
</tr>
<tr>
<td>129/OLA</td>
<td>Inbred mouse strain 129, substrain OLA</td>
</tr>
<tr>
<td>x° y’ W</td>
<td>x degrees, y minutes west of Greenwich meridian</td>
</tr>
<tr>
<td>x° y’ S</td>
<td>x degrees, y minutes south of the equator</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>95% CI</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>A$</td>
<td>Australian dollars</td>
</tr>
<tr>
<td>ACCP/SCCM</td>
<td>American College of Chest Physicians/Society of Critical Care Medicine</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APACHE</td>
<td>acute physiology and chronic health evaluation score</td>
</tr>
<tr>
<td>Ara⁺</td>
<td>Arabinose assimilating</td>
</tr>
<tr>
<td>ARDS</td>
<td>Acute respiratory distress syndrome</td>
</tr>
<tr>
<td>ARF</td>
<td>Acute renal failure</td>
</tr>
<tr>
<td>AZT</td>
<td>Azidothymidine; zidovudine</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td><em>Burkholderia pseudomallei</em></td>
</tr>
<tr>
<td>Balb/c</td>
<td>Inbred mouse strain Balb/c</td>
</tr>
<tr>
<td>BKA</td>
<td>Below knee amputation</td>
</tr>
<tr>
<td>C57B6</td>
<td>Inbred mouse strain C57 black, substrain 6</td>
</tr>
<tr>
<td>CD-ROM</td>
<td>Compact disc read only memory</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COAD</td>
<td>Chronic obstructive airways disease</td>
</tr>
<tr>
<td>CVVHF</td>
<td>Continuous veno-venous haemofiltration</td>
</tr>
<tr>
<td>D</td>
<td>Simpson’s index of diversity</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>ECMO</td>
<td>Extra-corporeal membrane oxygenation</td>
</tr>
<tr>
<td>EMBASE</td>
<td>Excerpta Medica database</td>
</tr>
</tbody>
</table>
GAM  Generalized additive model
G-CSF  Granulocyte colony stimulating factor
G-CSF -/-  Homozygous G-CSF gene knockout mice
GI bleed  Gastrointestinal tract bleeding
GIS  Geographical information systems
HBA  Horse blood agar
HBV  Hepatitis B
HEPA  High efficiency particulate air filtration
HIV  Human immunodeficiency virus
HTLV-1  Human T-cell leukaemia virus
ICU  Intensive Care Unit
IFN  Interferon
Ig  Immunoglobulin
IHD  Ischaemic heart disease
IL  Interleukin
IV  Intravenous
LB  Luria Bertani
LD₅₀  Lethal dose for 50%
LOD  Logistic Organ Dysfunction
Log  Logarithm (base 10 unless specified)
LR⁺  Positive likelihood ratio
MEDLINE  Medical Literature Analysis and Retrieval System Online
MIC  Minimum inhibitory concentration
mL  Milliliters
MLEE  Multi-locus enzyme electrophoresis
MLST  Multi-locus sequence testing
mmol  Millimoles
MODS  Multiple Organ Dysfunction Score
MRSA  Methicillin-resistant Staphylococcus aureus
n=  Number in sample
ng  Nanograms
nm  Nanometers
NT  Northern Territory, Australia
NZ  New Zealand
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>p=</td>
<td>Probability equals</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed field gel electrophoresis</td>
</tr>
<tr>
<td>pH</td>
<td>Power of hydrogen ion concentration</td>
</tr>
<tr>
<td>PT</td>
<td>PFGE strain type</td>
</tr>
<tr>
<td>Qld</td>
<td>Queensland, Australia</td>
</tr>
<tr>
<td>RAPD</td>
<td>Randomly amplified polymorphic DNA</td>
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<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
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<tr>
<td>RDH</td>
<td>Royal Darwin Hospital</td>
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<tr>
<td>ROC</td>
<td>Receiver operator characteristic</td>
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<tr>
<td>S. pyogenes</td>
<td>Streptococcus pyogenes</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>SOFA</td>
<td>Sequential Organ Failure Assessment</td>
</tr>
<tr>
<td>SpeI</td>
<td>Restriction enzyme SpeI</td>
</tr>
<tr>
<td>ThB, a</td>
<td>Thai baht (A$1≈ThB23-26 at the time of writing)</td>
</tr>
<tr>
<td>TM</td>
<td>Trademark</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>Trimethoprim-sulphamethoxazole (cotrimoxazole)</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>t-test</td>
<td>Student’s t-test</td>
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<tr>
<td>UPGMA</td>
<td>Unweighted pair group matching band average</td>
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<tr>
<td>US$</td>
<td>United States dollars (A$1≈US$0.60-0.65 at the time of writing)</td>
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<tr>
<td>WA</td>
<td>Western Australia</td>
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<tr>
<td>WCC</td>
<td>White cell count</td>
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<tr>
<td>yr</td>
<td>Year</td>
</tr>
<tr>
<td>Σ</td>
<td>Sum of</td>
</tr>
<tr>
<td>σ²</td>
<td>Variance</td>
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<tr>
<td>χ²</td>
<td>Chi-squared statistic</td>
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</table>