Tropical Bacteriology in the Top End

Rob Baird
Introduction

Why this E-book guide?

The Top End of the Northern Territory (NT) has a number of clinically significant bacteria that are either unique, or more frequently isolated than in more temperate Australian regions. This guide is for local laboratory staff, scientists, microbiologists, and interested health care workers in the Top End as an introduction to aspects of tropical bacteriology.

Human Infections are generally caused by either an individual’s resident bacterial flora; or from bacteria present in the local environment. The incidence of sepsis in the tropical NT is substantially higher compared to temperate Australia, and this difference is reflected in the high rates of sepsis in Indigenous patients.

This brief overview reviews the:

- Epidemiology
- Microbiology characteristics (& sub species)
- Differential diagnosis from similar pathogens

Bacteria covered include the common and the rare, but focus on aspects of the more frequently isolated bacteria from the Top End of the NT.

Bacteriology identification, is currently undergoing the biggest revolution since Louis Pasteur’s day, with traditional culture, bench tests, and biochemical methods, being augmented or replaced by rapid nucleic acid and protein spectra identification, amongst a number of new technologies becoming utilised. These changes are being driven by the need for rapid information (for identification, susceptibility and epidemiology); in a cost efficient manner, to be delivered, to aid clinical management.
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Tropical Bacteriology in the Top End
Introduction

Common pathogenic bacteria of the Top End
- *Staphylococcus aureus*, *Group A streptococci*, *Neisseria gonorrhoeae*

Marine/Water pathogens (VACS)
- *Burkholderia pseudomallei*
- *Acinetobacter spp.*
- *Corynebacterium diphtheriae*
- *Nocardia spp.*

References

Copyright
Common pathogenic bacteria in the Top End

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<th>Bacteria</th>
<th>% Relative frequency</th>
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Relative frequency of bacterial pathogen isolation at Royal Darwin Hospital; the main tertiary referral hospital for the Northern Territory.

*Staphylococcus aureus* remains our most prevalent pathogen, and along with *E. coli* comprises nearly half the isolates being identified in the microbiology lab.

Significant local aspects of the microbiology and epidemiology of *S. aureus*, Group A streptococci (GAS), and *Burkholderia pseudomallei* are included in this review.

Other pathogens not in the Top 8, but of local significance and isolated in high numbers include: *Neisseria gonorrhoeae*, and cutaneous *Corynebacterium diphtheriae* isolates.

Relevant aspects of local epidemiology, and basic microbiology will be covered and the 2013 local antibiogram is included, as this is part of this general need of the lab to be able to provide timely local epidemiological information.
## Top End Health Service 2015 Antibiogram (% susceptible)

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<th>Gram positive</th>
<th>Number of Isolates</th>
<th>ESBL %</th>
<th>Amp C %</th>
<th>Carbapenemases %</th>
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Data from all isolates susceptibility tests (First 6 months of 2015): duplicates, and multiple patient isolates are deleted from the Gram negative and enterococcal data

ESBL % reported for E. coli and Kleb pneumo and inferred from ceftriaxone and/or cefepime resistance

Amp C % reported for E. coli and Kleb pneumo and inferred from cefoxitin resistance

Colours: green > 90% susceptible, orange > 70% susceptible, red < 70% susceptible

* S. pneumoniae data from MIC's from whole of NT invasive isolates, over the last 2 and half years; 2013-June 2015
S. pneumoniae: meningitis MIC (S=penicillin <0.12 μg/ml, ceftriaxone <1 μg/ml); non meningitis isolates MIC (S= penicillin <4 μg/ml, ceftriaxone <2 μg/ml)
**Staphylococcus aureus**

*S. aureus* is the most commonly isolated bacterial species in the NT. Traditional microbiology phenotypic identification methods such as coagulase testing, latex agglutination, DNA’ase activity and biochemical means (ie Vitek 2); are being augmented by: MALDI-TOF, NAAT, and various typing systems for epidemiological and infection control purposes, and ultimately whole genome sequencing, as it becomes cost effective.

*S. aureus* infection in a haemodialysis fistula site. The NT has a large renal replacement population.

Gram stain microscopy of blood cultures, wounds and aspirates remains a valuable and quick tool to determine the presence of bacteria such as these Gram positive coccis in clusters.
**S. aureus** Culture

Culture on traditional HBA and selective media (ie MSA, mannitol salt agar); and various chromogenic media are the all classical means of bacterial isolation.

**Mannitol salt agar (MSA)** contains a high concentration (~7.5%-10%) of NaCl, making it selective for Gram positive staphylococci, since this level of NaCl is inhibitory to most other bacteria. It is also a differential medium for mannitol fermenters, as it contains mannitol and the indicator phenol red. *S. aureus* produce yellow colonies with yellow zones, whereas other staphylococci produce small pink or red colonies with no colour change to the medium.

Production of DNA’ase is not unique to *S. aureus*, but remains one of a number of useful phenotypic tests. Correct identification is important, and a single phenotypic test alone should not be relied on, but the combination of phenotypic assays improves accuracy.
**S. aureus** Rapid identification by nucleic acid amplification

Confirmation of the identity and likely antimicrobial susceptibility profile can be achieved within 70 minutes by DNA amplification, from either blood culture or plate culture isolates of Gram positive cocci in clusters, or patient specimens.

Confirmation of *S. aureus* comes from amplification of the staph protein A (*spa*) gene.

Antimicrobial resistance pattern comes from *mecA* gene detection (methicillin resistance), and the staphylococcal cassette chromosome *mec* (SCC*mec*).

An internal control is also included and amplifies, if *S. aureus* is not detected.

Both in-house and commercial systems are available.
The epidemiology of *S. aureus* can be considered phenotypically (by antimicrobial susceptibility patterns); or genotypically by DNA profiling bacterial strains by various methods, including whole genome sequencing (WGS) methods, which provide precise information for population studies, and information for use by infection control practitioners.

Phenotypically of the 8 known phenotypes, 4 occur commonly in the NT: penicillin sensitive *S. aureus* (PSSA) 9%; methicillin sensitive *S. aureus* (MSSA) 57%; community or non multi methicillin resistant *S. aureus* (nmMRSA or CaMRSA) 24%; methicillin resistant MRSA (MRSA) 9%.

Of the other 4 described phenotypes: vancomycin intermediate *S. aureus* (VISA), heterogeneous vancomycin intermediate *S. aureus* (hVISA); and borderline oxacillin resistant *S. aureus* (BORSA); are very infrequent. Vancomycin resistant *S. aureus* (VRSA) has not been reported in Australia or the NT.

Over the last few years in the NT, like many other areas of Australia: MRSA rates are relatively decreasing, as CaMRSA rates increase.

Characterisation by phenotype and characterisation of vancomycin MIC for MRSA and CaMRSA isolates is routine.
Local epidemiology of *S. aureus* strains  Genotypes

Of Ca-MRSA strains, the NT has predominantly ST 93 genotypes, similar to Queensland. This is well illustrated in the recent AGAR Australian national genotyping data. CaMRSA strains as percentage of all MRSA strains have been increasing nationally over the last 10 years.

Data AGAR 2012

*Staphylococcus aureus* Programme 2012 (SAP 2012)
Community Survey
MRSA Epidemiology and Typing Report
Local Epidemiology of *S. aureus* strains Genotypes

Of MRSA (HA-MRSA) strains, the NT has predominantly ST 239 genotypes, quite distinct from the rapid spread of ST22 strains around Australia. This is well illustrated in the recent Australian national genotyping data. HA strains as percentage of all MRSA strains have been decreasing nationally over the last 10 years.
**Staphylococcus argenteus**: “silver staph”  
An NT staphylococcal variant

Surveillance of staphylococcal carriage and disease in the tropical northern region of the Northern Territory of Australia has yielded methicillin-susceptible and methicillin-resistant coagulase positive staphylococcus isolates that are divergent from *S. aureus* at the commonly used genotyping loci, and are therefore difficult to classify.

These staphylococcal isolates are closely related to each other and were initially designated as a clonal complex 75 (CC75), and a distinctive phenotype. They accounted for 23% of all staphylococci isolates and 70% of MRSA isolates recovered during a community study of impetigo. Subsequent genome sequencing confirms a new species of staphylococci, related but different from *S. aureus*.

*S. argenteus* isolates lack staphyloxanthin. The *S. aureus* clinical isolate SCC1007 (ST93) (left) produces staphyloxanthin, whereas the *S. argenteus* isolate MSHR1132 (right) does not (Reference: Tong et al)
The 2006 AGAR survey of CaMRSA strains, designated ST75 as a local NT CaMRSA strain. As further genetic information of the strain has become apparent, it has been designated as one of the 50 or so other described staphylococcal species; with a provisional name of *S. argenteus*. This group of bacteria is significant, as it is genotypically distinct from conventional *S. aureus* but is still able to cause *S. aureus*-like disease, and is found in other locations worldwide.

While having many features of *S. aureus* and being genetically close, this story is illustrative of current taxonomical issues in microbiology, where the genotype of the organism is the final arbiter of a bacteria’s designation in a phylogenetic tree and species list.
Streptococcus pyogenes  Group A streptococci (GAS)

The Northern Territory has very high rates on invasive Group A streptococcal disease, predominantly in indigenous patients. In addition, high rates of skin disease, and post streptococcal diseases such as rheumatic fever and subsequent valvular heart disease persist. Rheumatic fever is linked to skin disease rather than streptococcal throat infections as described in other jurisdictions.
Skin sores and secondary Group A streptococcal infection of scabies is common.
S. pyogenes  Group A streptococci (GAS)

Beta haemolysis, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells. There are two types of streptolysin: Streptolysin O (SLO) and streptolysin S (SLS). Streptolysin O is an oxygen-sensitive cytotoxin, secreted by most Group A streptococcus (GAS), and usually results in β-haemolysis under the surface of blood agar. Streptolysin S is an oxygen-stable cytotoxin also produced by most GAS strains which results in clearing on the surface of blood agar, thus displaying beta haemolysis.

GAS as Gram positive cocci in chains on 100x microscopy
Neisseria gonorrhoeae

- The rate of gonococcal infections in the Northern Territory (NT) is high, at 918 cases per 100,000 of population per year, 40 times the national Australian rate. Despite this high rate, resistance to penicillin is uncommon (3.1%), compared to the national average (32%), and amoxicillin remains first line treatment in many indigenous communities.

- Chromosomally mediated penicillin resistance (CMRP) is the most common mechanism of penicillin resistance in Australian N. gonorrhoeae isolates. CMRP mediates a gradual increase in penicillin minimum inhibitory concentrations (MICs) over time by mechanisms including alteration of penicillin binding proteins. Isolates may shift from susceptible (S) (MIC< 0.06mg/L); to less susceptible (LS) (0.06mg/L to 0.5mg/L); to resistant (R) (MIC>0.5mg/L).

- The majority of NT isolates have MICs in the LS range due to CMRP, but are considered penicillin susceptible for treatment purposes.

Penicillin resistance of N. gonorrhoeae isolates geographically, local data 2010.
Traditional microscopy and culture is being rapidly replaced with nucleic acid amplification (NAAT) assays. Conventional *N. gonorrhoeae* antimicrobial resistance surveillance (AMR) is performed by bacterial culture. However, bacterial culture has several major limitations including, the need for stringent sample handling and transport systems to maintain viable organisms, which is a problem in remote and resource limited settings.
Antimicrobial resistance (AMR) in *N. gonorrhoeae*, the causative agent of the disease gonorrhea, is a major public issue and is now recognized as one of the top three urgent AMR threats by the United States Centers for Disease Control and Prevention (CDC). Conventional *N. gonorrhoeae* AMR surveillance is performed by bacterial culture. However, bacterial culture has several major limitations including, the need for stringent sample handling and transport systems to maintain viable organisms, which is a problem in remote and resource limited settings.

Nucleic acid amplification tests (NAATs) are increasingly commonplace for the diagnosis of gonococcal infection in both remote and urban settings. Example of a real-time PCR method to detect penicillinase-producing *N. gonorrhoeae* (PPNG) directly within clinical samples. The method acts as an indirect marker for PPNG by targeting a region of DNA on the gonococcal plasmids carrying the penicillinase gene but not the penicillinase gene itself. The original evaluations of this PPNG-PCR showed high sensitivity and specificity compared to gold standard bacterial culture for the detection of PPNG strains in clinical specimens.
There is a lot of water in Northern Australia. Bacteria present in the local tropical environment include various fresh and salt water bacteria. These bacteria are endemic in the environment and can become human pathogens causing various infections including cellulitis, necrotizing fasciitis, abscesses, septicaemia, and death. Infections are relatively rare, though the bacteria are common and are most often associated with the immuno-compromised hosts, including diabetics and those with alcohol excess challenges. A handy acronym to remember these tropical marine/water bacteria is the VACS organisms.

The VACS organisms have all been isolated in the tropical Top End of Northern Australia, with relative frequencies of *Vibrio* spp. 15%; *Aeromonas* spp. 67%; *Chromobacterium violaceum* 5%; and *Shewanella* spp. 13%.

A strong male predominance is found (M:F ratio 2.3:1). Skin and soft tissue isolations, 79%; from lower limb infections 59% are the most common clinical manifestation. The episodes are usually polymicrobial, 60%. Co-isolates included *S. aureus* 29%, beta-haemolytic streptococci 16%, enterobacteriaceae 24%; and other VACS organisms 8%.
# VACS Bacteria Comparison

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Environmental niche</th>
<th>Relative frequency in the Top End</th>
<th>Gram stain</th>
<th>Culture</th>
<th>Oxidase</th>
<th>Characteristic features</th>
<th>Motile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vibrio spp. (V. vulnificus, V. parahaemolyticus and V. alginolyticus)</td>
<td>salt water (concentrations fluctuate with temperature, salinity and nutrient level)</td>
<td>15%</td>
<td>GNB curved rod</td>
<td>Halophilic species (V. vulnificus &amp; V. alginolyticus), may grow poorly @ 37°C. Non-halophilic (V. cholerae &amp; V. parahaemolyticus)</td>
<td>POSITIVE</td>
<td>*curved rod, growth on TCBS (Thiosulfate-citrate-bile salts-sucrose agar)</td>
<td>YES</td>
</tr>
<tr>
<td>Aeromonas spp. (mainly A. Hydrophilla, &amp; A. Sobla, A. Caviae and 11 others)</td>
<td>fresh or brackish water in tropical &amp; subtropical climates</td>
<td>67%</td>
<td>GNB</td>
<td>grow on blood agar 90% are beta haemolytic, non lactose fermenters</td>
<td>POSITIVE</td>
<td>usually haemolytic on sheep blood agar</td>
<td>YES</td>
</tr>
<tr>
<td>Chromobacterium violaceum</td>
<td>tropical soil and water</td>
<td>5%</td>
<td>GNB slightly curved rods</td>
<td>circular, convex, smooth, pigmented, non lactose fermenter, some strains haemolytic</td>
<td>POSITIVE</td>
<td>violet pigment (most not all), more pigment aerobically than anaerobically</td>
<td>YES</td>
</tr>
<tr>
<td>Shewanella spp. (S.algae and S. putrefaciens)</td>
<td>warm marine environments (including soil and salt water), can tolerate low water temperatures</td>
<td>13%</td>
<td>GNB long, short, filamentous</td>
<td>slightly halophilic S. putrefaciens can be pink, produces H₂S. S. algae haemolytic on sheep blood agar</td>
<td>POSITIVE</td>
<td>S. algae haemolytic on sheep blood agar, S. putrefaciens non haemolytic (but pink and large with brown pigment)</td>
<td>YES</td>
</tr>
</tbody>
</table>

*V. parahaemolyticus* is also associated with the Kanagawa phenomenon, in which strains isolated from human hosts (clinical isolates) are hemolytic on blood agar plates, while those isolated from nonhuman sources are nonhemolytic.
VACS Culture Comparison

A lab clue that you may be dealing with a VACS bacteria is the presence of an oxidase positive bacteria growing on a blood agar plate, in a patient with a compatible disease such as water associated cellulitis.

- **Vibrio spp.**
- **Beta haemolytic Aeromonas spp.**
- Deep purple *Chromobacterium violaceum*
- Tan/orange *Shewanella putrefaciens*
**Vibrio spp.**

Non–cholera vibrios seen in the Northern Territory: *Vibrio parahaemolyticus, Vibrio aglinolyticus and Vibrio vulnificus.*

Clinical: foodborne illness or wound infections – can lead to septicaemia.
- curved GNB, motile by polar flagella
- Ox positive, facultative ANO<sub>2</sub>
- Grow on HBA, choc and TCBS (Thiosulfate-citrate-bile salts-sucrose) agar

Microscopy: slightly curved Gram negative rods seen on Gram stain. Subsequently Isolated from an Achilles heel wound from a patient after a slight disagreement with a mackerel.
**Vibrio spp. comparison**

Typical colonial morphology on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) agar is as follows:

<table>
<thead>
<tr>
<th><em>V. cholerae</em></th>
<th>Large yellow colonies.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>Colonies with blue to green centres.</td>
</tr>
<tr>
<td><em>V. aglinolyticus</em></td>
<td>Large yellow mucoidal colonies.</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>Colonies with blue green centres</td>
</tr>
</tbody>
</table>

TCBS agar is both selective and differential. It is highly selective for *Vibrio spp.* and differential due to the presence of sucrose and the dyes. Sucrose fermentation produces acid, which converts the colour of bromothymol blue or thymol blue. Two dyes rather than one make the medium produce an array of yellow, green or blue so that differentiating among various *Vibrio spp.* is possible.
**Aeromonas spp.**

Found in fresh and brackish water and have been recovered from chlorinated water, including hospital water supplies.

Infection: usually gastroenteritis, cellulitis and wound infections, septicaemia

Important species: *A. hydrophila, A. caviae* and *A. veronii* (*A. sobria*)

Gram negative bacillus
Facultative anaerobe
Beta haemolytic on blood agar
Oxidase POSITIVE
Grow well on MacConkey Agar some strains are lactose fermenters
Chromobacterium violaceum

The colony colour of this striking purple to black Gram negative bacillus (occasionally slightly curved) is characteristic. Up to 10% of colonies however can be non pigmented when grown on media. Water associated, there have been over 30 isolates of this rare bacteria in the NT.
**Shewanella spp. (S. algae & S. putrefaciens)**

*Shewanella* spp. are saprophytic motile Gram-negative rods, belonging to family Vibrionaceae. *Shewanella* spp. are widely distributed in nature, with soil and water being their natural habitat.

Infections of ears, skin or soft tissue, eye, osteomyelitis and bacteraemia, are all described. In the NT, cellulitis, often polymicrobial is the commonest presentation, from water exposure events.

Taxonomically, like *Burkholderia* spp., the species can be difficult to split in routine microbiology labs, as many biochemical systems call all isolates *S. algae*. Important differential characteristics between the two species include the ability of *S. algae* to produce mucoid colonies with β-haemolysis on sheep blood agar, to grow at 42°C and in NaCl 6%, which contrasts with *S. putrefaciens*.
**VACS organisms  Antimicrobial susceptibility**

<table>
<thead>
<tr>
<th>VACS Bacteria</th>
<th><em>Vibrio</em> spp</th>
<th><em>Aeromonas</em> spp</th>
<th><em>C. violaceum</em></th>
<th><em>Shewanella</em> spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic</td>
<td>Isolates tested (% susceptible)</td>
<td>Isolates tested (% susceptible)</td>
<td>Isolates tested (% susceptible)</td>
<td>Isolates tested (% susceptible)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>34 (100)</td>
<td>164 (100)</td>
<td>6 (83)</td>
<td>17 (100)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>47 (26)</td>
<td>248 (0.4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amoxycillin-Clavulanate</td>
<td>37 (92)</td>
<td>200 (40)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>37 (100)</td>
<td>198 (91)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>43 (100)</td>
<td>182 (96)</td>
<td>6 (38)</td>
<td>22 (95)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>33 (79)</td>
<td>171 (31)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>41 (27)</td>
<td>185 (9.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>48 (100)</td>
<td>230 (99.1)</td>
<td>11 (100)</td>
<td>27 (96)</td>
</tr>
<tr>
<td>Ceferpine</td>
<td>35 (97)</td>
<td>168 (99.4)</td>
<td>3 (100)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>53 (100)</td>
<td>272 (99.2)</td>
<td>11 (100)</td>
<td>28 (100)</td>
</tr>
<tr>
<td>Meropenen</td>
<td>44 (93)</td>
<td>201 (92)</td>
<td>8 (100)</td>
<td>24 (100)</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>35 (100)</td>
<td>137 (62)</td>
<td>5 (100)</td>
<td>13 (69)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>40 (100)</td>
<td>197 (98)</td>
<td>7 (71)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>49 (100)</td>
<td>256 (97)</td>
<td>7 (86)</td>
<td>22 (100)</td>
</tr>
</tbody>
</table>

* CLSI criteria not available, results inferred from *Pseudomonas aeruginosa* breakpoints.

The antimicrobial susceptibility data is limited by small numbers, but supports the empirical use of meropenem, ciprofloxacin, and gentamicin for *C. violaceum* infections. Earlier in-vitro studies have showed ciprofloxacin to be the most potent of these, and this should therefore be incorporated in first line regimens targeting this organism.

Regarding our inferred antimicrobial susceptibility data for *Shewanella* spp. and *C. violaceum* from *Pseudomonas aeruginosa* results, we believe this is reasonable for the following reasons: (i) relatively low numbers of *Shewanella* spp. and *C. violaceum* make it unlikely independent standards will be developed; (ii) *P. aeruginosa* is also an oxidase positive environmental organism with some similar features to *Shewanella* spp. and *C. violaceum*. It is reasonable to argue that *Vibrio* spp. susceptibility criteria could be used as an alternative.

Moving forward, we believe when these organisms are isolated they should have MIC testing performed against clinically relevant antibiotics, as it is currently the case for other tropical organisms such as *Burkholderia pseudomallei*.
**Burkholderia pseudomallei** “melioidosis”

A serious bacterial infection endemic to Northern Australia, and areas of SE Asia (including Thailand and Malaysia).

Transmission is via direct contact with broken skin and/or inhalation of dust – leading to septicaemia, pneumonia and skin lesions. Abscess formation in the prostate, as well as other sites, is well recognised. The bacteria is isolated from respiratory samples, blood cultures, urine, skin and soft tissue samples and sterile fluids. Risk factors: diabetes, alcoholism, immunosuppression and chronic renal disease.
In the Top End of the Northern Territory, cases of melioidosis are strongly associated with the Wet Season (Dec to March). The distribution of melioidosis endemic areas in Australia (left hand image) closely mirrors the annual Australian rainfall incidence (right hand image).

There is an association between the total monthly rainfall, and the number of cases of melioidosis in the Top End for each month of the year. The correlation between monthly cases of melioidosis and rainfall in the preceding calendar month is slightly tighter than the correlation with rainfall in the concurrent month, suggesting a lag time related to the bacterial incubation period.
The classic characteristics of this organism are: Gram negative bacillus, highly motile, oxidase positive, and strictly aerobic. Identification is then confirmed by culture characteristics, biochemical reactions, and nucleic acid confirmation, and/or mass spectrometry.

Please note the Biohazard status of the organism, therefore handling of this bacteria must be at BSL 2 facilities and above. *Burkholderia spp.* could be potentially cultured in any microbiology lab, and safe handling of blood culture isolates is emphasised. The Biohazard section has details of post exposure prophylaxis for lab staff depending on the risk assessment of the exposure.

Click the links below for additional detailed information, or scroll through:

- Biohazard status
- Gram stain
- Culture characteristics
- Identification
- Susceptibility testing
- Pitfalls in diagnosis
- Therapy
**B. pseudomallei** Biohazard status

Two issues here: Biosafety and Biosecurity. Australia has no overarching national laboratory Biosafety regulations, though the WHO Laboratory Biorisk guidelines are widely available. Biosecurity aspects of handling designated organisms are covered by SSBA (Security Sensitive Biological Agents) regulations. Tier 2 organisms currently include *V. cholera* and *S. typhi*, but not *B. pseudomallei*.

*B. pseudomallei* has been associated with laboratory acquired infections, and specimens from endemic areas should be handled in a minimum of a BSL 2 facility. Blood cultures must be handled in Class 2 cabinets, avoid centrifugation aerosols, and standard safe microbiology laboratory practices followed. Note different countries attribute different risks, and handling procedures to this organism. Post exposure prophylaxis is available for staff.

The following laboratory practices when handling *B. pseudomallei* are considered by risk group:

**Low risk:**
- Inadvertent opening of the lid of an agar plate growing *B. pseudomallei* outside a biologic safety cabinet
- Inadvertent sniffing of agar plate growing *B. pseudomallei* in the absence of contact between worker and bacterium
- Splash event leading to visible contact of *B. pseudomallei* with gloved hand or protected body, in the absence of any evidence of aerosol
- Spillage of small volume of liquid culture (<1mL) within a functioning biologic safety cabinet
- Contamination of intact skin with culture

**High risk:**
- The presence of any predisposing condition without proper personal protective equipment (PPE): diabetes mellitus; chronic liver or kidney disease; alcohol abuse; long-term steroid use; hematologic malignancy; neutropenia or neutrophil dysfunction; chronic lung disease (including cystic fibrosis); thalassemia; any other form of immunosuppression
- Needlestick or other penetrating injury with implement contaminated with *B. pseudomallei*
- Bite or scratch by experimental animal infected with *B. pseudomallei*
- Splash event leading to contamination of mouth or eyes
- Generation of aerosol outside biologic safety cabinet (e.g., sonication, centrifuge incident)
Morphology: Gram negative bacillus, bipolar staining, resembling safety pins. This bipolarity however, can be absent and can occur in other organisms, so though a useful clue, is not totally reliable.
**B. pseudomallei** Culture and identification

**Preliminary identification:**
- Oxidase positive, metallic white on blood agar, highly motile
- Strict aerobe
- Give off an earthy odour (note sniffing of plates is strictly against all lab guidelines)
- Growth on Ashdown’s media

Identification of *B. pseudomallei* can be made presumptively, with the typical antibiogram (R Gentamicin/Colistin: S Amox/Clav)

**Definite identification:**
- Biochemical reactions (ie Vitek 2 or API 20E)
- Nucleic acid amplification
- MALDI-TOF

See section on pitfalls of identification

Typical white colonies on blood agar
Ashdown’s agar contains crystal violet and gentamicin to inhibit the growth of Gram positive bacteria and Gram negative bacilli respectively.

Plates are incubated for 5 days at 35 deg.

Any suspicious growth is subbed to blood agar.

Ashdown’s agar is part of the routine culture to all respiratory samples and male urines during the wet.

Ashdown agar is only added to other samples if melioidosis is suspected.

Colonies of *B. pseudomallei* also take up neutral red which is present in the medium, and this further helps to distinguish it from other bacteria.

Gentamicin slightly inhibits the growth of *B. pseudomallei* and so specimens inoculated onto Ashdown’s agar needs to be incubated for a minimum of 96 hours instead of 48 hours. The medium is also enriched with 4% glycerol, which is required by some strains of *B. pseudomallei* to grow. *B. pseudomallei* usually produces flat wrinkled purple colonies on Ashdown’s agar.

Colonies are small, light blue and dry on Ashdown agar. After prolonged incubation colonies appear wrinkled.
Ashdown Broth contains colymycin and crystal violet to suppress Gram positive bacteria and common coliforms. Used for screening purposes, and when melioidosis is suspected. Screening is performed mainly on throat and rectal swabs but also on sputum, urine or wound swabs.

Broths are incubated 35 degrees C for 5 days – with lids loosened, then subbed to Ashdown agar. Broths are checked daily.

Note: the positive is suggested by a pellicle growth at the air/broth interface, for this aerobic bacteria.

B. pseudomallei
Selective culture
**B. pseudomallei** Identification

Available methods include: biochemical profiles, antigen detection, PCR or MALDI-TOF

<table>
<thead>
<tr>
<th>Current melioid diagnostics</th>
<th>Rapid</th>
<th>Sensitive</th>
<th>Specific</th>
<th>Cost</th>
<th>Safety</th>
<th>Ease of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Culture</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Biochemical identification</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Agglutination</td>
<td>IH</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>PCR</td>
<td>IH</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>IH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Serology – IHA</td>
<td>IH</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>
**B. pseudomallei** diagnostic algorithms at Royal Darwin Hospital: One for blood cultures, one for plate cultures

**Blood culture signals**

- Microscopy
- Gram stain

**Highly motile, bipolar staining**

- PCR
- Biochemistry ID
- Susceptibility testing

**Plate culture growth**

- Microscopy
- Gram stain
- Oxidase
- Agglutination

**Highly motile, bipolar staining, oxidase positive, agglutinates**

- PCR
- Biochemistry ID
- Susceptibility testing

**Doctor notified**

- "Possible, probable, or confirmed"
Lateral flow antigen assays are in development: they currently require a large bacterial antigen load, but can be quick and accurate.
MALDI-TOF: identification of each isolate is cheap & quick; but still requires a culture isolate, and a local database of local strains needs to be acquired for optimum performance
B. pseudomallei Identification

PCR confirmation: Specific targets make this the current gold standard

Target TTSI-orf2. Kaestli M. JCM June 2012 50:6;2059-2062
IHA Serology: useful but has limitations; due to background Ab level in endemic areas, and low specificity and sensitivity of the current antigen. Improved antigens in development may be beneficial.
Susceptibility testing of *B. pseudomallei* isolates is performed to detect resistance to the antimicrobials used in therapy. Resistance in Northern Territory bacterial isolates is rare but described.

Susceptibility methods for testing *B. pseudomallei* isolates, unlike most common bacteria are more limited, as there are no disc diameter zone sizes available in CLSI and EUCAST methodologies, and automated susceptibility systems such as Vitek 2, produce results which are partially indicative but do not good correlations with formal MIC testing.

The gold standard is broth micro dilution, however E tests are widely available and correlate well with agar microdilution MIC’s. The links below are illustrative:

**Etest MIC testing methods and interpretation for *B. pseudomallei***

**Pooled MIC data for > 230 Northern Territory patient *B. pseudomallei* isolates from 2009-2012**
Routine antimicrobial testing of melioid isolates by Etest is performed for meropenem, ceftazidime, trimethoprim/sulphamethoxazole and doxycycline. Performed by CLSI method. Meropenem, ceftazidime and doxycycline give clear-cut end-points and are read at complete inhibition. Trimethoprim/sulphamethoxazole does not have a clear cut endpoint. The MIC value is taken at 80% inhibition. Picture on left shows the 2 inhibition ellipses. The first one at 0.19 mg/L. The second at about 1.5mg/L. Therefore at 80% inhibition the MIC is 1.0 mg/L. MIC above 2.0mg/L is resistant.

**B. pseudomallei**

![E test interpretation](image)
**B. pseudomallei**  
**E test interpretation**

- **Ceftazidime**  
  E test result 1.5 mg/L

- **Meropenem**  
  E test result 1.0 mg/L

- **Trimethoprim/Sulphamethoxazole**  
  E test result 0.125 mg/L

MIC results interpreted as per CLSI guidelines  
Meropenem, and ceftazidime give clear-cut end-points and are read at complete inhibition, as compared to trimethoprim/sulphamethoxazole.
B. pseudomallei Pooled MIC data 2009-2012

Northern Territory B. pseudomallei isolates SMX/TMP MIC

MIC Interpretative values based on CLSI 2010 M45-A2 Vol 30 No. 18
B. pseudomallei  Pooled MIC data 2009-2012

Northern Territory B. pseudomallei isolates meropenem  MIC

susceptible

intermediate  resistant

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.00%</td>
</tr>
<tr>
<td>0.5</td>
<td>18.03%</td>
</tr>
<tr>
<td>0.75</td>
<td>57.08%</td>
</tr>
<tr>
<td>1</td>
<td>18.88%</td>
</tr>
<tr>
<td>1.5</td>
<td>3.86%</td>
</tr>
<tr>
<td>2</td>
<td>2.15%</td>
</tr>
<tr>
<td>4</td>
<td>0.00%</td>
</tr>
<tr>
<td>8</td>
<td>0.00%</td>
</tr>
<tr>
<td>16</td>
<td>0.00%</td>
</tr>
<tr>
<td>32</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
B. pseudomallei  Pooled MIC data 2009-2012

Northern Territory B. pseudomallei isolates ceftazidime MIC

<table>
<thead>
<tr>
<th>Percentage</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>16</th>
<th>32</th>
<th>&gt;32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series1</td>
<td>0</td>
<td>2.15%</td>
<td>19.31%</td>
<td>60.52%</td>
<td>13.73%</td>
<td>3.86%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.43%</td>
</tr>
</tbody>
</table>

- Susceptible
- Intermediate
- Resistant
B. pseudomallei Pooled MIC data 2009-2012

Northern Territory B. pseudomallei isolates doxycycline MIC

<table>
<thead>
<tr>
<th>MIC</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>&gt; 16</th>
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<td>14.59%</td>
<td>6.87%</td>
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MiC interpretative values based on CLSI 2010 M45-A2 Vol 30. No. 18
**B. pseudomallei**  Pitfalls in diagnosis

**Common phenotypic mis-identifications of B. pseudomallei are primarily due to:**

- Regional biochemical differences in *B. pseudomallei* strains occur. Australian strains are biochemically distinct from some other South East (SE) Asian strains. Incorrect phenotypic identification includes *Burkholderia cepacia* in particular.

- Lack of the regional biochemical profiles in the phenotypic databases. Australian isolates are well represented in the automated biochemical systems databases (ie Vitek 2), but not the biochemically distinct SE Asian isolates. Most mis-identifications reported in the literature are due to SE Asian isolates, not locally acquired NT isolates.

- A proliferation of taxonomic change in the genus, occurring well ahead of the phenotypic database updates. The move towards genotypic and molecular identification, as the gold standard, will reduce this problem for reference labs.

- Local laboratories still require a high index of clinical and microbiological suspicion, when identifying *Burkholderia spp.* isolates from patients with compatible clinical illnesses.

- Ashdown’s media: growth is not limited to *B. pseudomallei*. Coliforms, yeast species, *Stenotrophomonas maltophilia*, *Pseudomonas spp.* and *B. cepacia* can all grow.

- *B. pseudomallei* can survive the decontamination process of tuberculosis (TB) sputum specimens, and can grow quite well in TB liquid broth media.

- Mass spectrometry, while an excellent aid in diagnosis, is dependent on the quality of the protein spectra database of the instrument. Misidentification has been reported in the literature.
A wide spectrum of presentations occurs, and patients with diabetes and heavy alcohol consumption at risk of developing a more severe illness. Pneumonia is the commonest presentation but bacteraemic spread can cause abscesses in any organ, especially the spleen and prostate.

Localised cutaneous ulcers or abscesses are also common and asymptomatic infection, latency and relapse are well recognised.

For initial intensive therapy, use ceftazidime or meropenem

After the initial intensive therapy, eradication is required with trimethoprim/sulfamethoxazole for a prolonged period.

Susceptibility results can aid individual patient therapy, and though local Northern Territory isolates are usually susceptible to first line antimicrobials, isolates from other countries, such as Thailand can have varying susceptibility patterns. Knowledge of local susceptibility patterns is also important in managing any inadvertent laboratory biohazard exposures.
Acinetobacter are known as a cause of tertiary hospital nosocomial infection, more recently with carbapenem resistant *A. baumannii* (CRAB) becoming recognised ICU pathogens.

In the Top End of the Northern Territory a distinct clinical acinetobacter pneumonia syndrome occurs. Although generally community onset *Acinetobacter spp.* infections are much less common than nosocomial infections, they have attracted growing interest in the past decade because of the emerging recognition of their high mortality and their importance as pathogens following trauma in wars and natural disasters.

Community-acquired *Acinetobacter spp.* pneumonia (CAAP) is increasingly recognised as an important entity in tropical and subtropical areas, because usual empirical antimicrobial regimens for pneumonia do not always cover this organism, and its reported mortality is approximately 60%. CAAP almost exclusively occurs in people with one or more of the putative risk factors of hazardous alcohol use, chronic lung disease and diabetes mellitus.

Worldwide, *Acinetobacter* infections predominantly occur in healthcare settings, whereas in tropical and subtropical regions, *Acinetobacter baumannii* and *Acinetobacter nosocomialis* also cause community acquired infections including severe pneumonia. Community- acquired *Acinetobacter* infections generally present as more severe disease and are associated with a higher mortality than nosocomial infections. A recent analysis of a community-acquired *A.baumannii* isolate’s pangenome revealed substantial genotypic and metabolic differences from nosocomial strains.
Acinetobacter spp. appear as short, broad, Gram-negative rods in the rapid growth phase but may assume a more coccobacillary shape in the stationary phase. In direct microscopic preparations the coccobacillar form may appear as a coccus. Growth in fluid media and under influence of cell wall active antibiotics produces rods.
The genus Acinetobacter comprises Gram-negative coccobacilli that are non-motile, strictly aerobic, catalase-positive, and oxidase-negative.

Acinetobacter is easily isolated in standard cultures, although identification can be delayed since it is relatively nonreactive in many biochemical tests commonly used to differentiate among Gram-negative bacilli. (They are indole-negative and do not ferment glucose or reduce nitrate).

Oxidase neg, non lactose fermenting on MacConkey agar, (E. coli on the left, A. baumannii on the right) but often a little bit pink (at late incubation)  This is compared to Stenotrophomonas maltophilia which is often quite clear and a bit smaller.

There are over 30 different species within the genus Acinetobacter. Most are environmental organisms and have not been associated with human disease.

* A. baumannii, and A. calcoaceticus are the species most frequently reported in the clinical literature. The term A. calcoaceticus-A. baumannii complex (ACB) is sometimes used since it is difficult to differentiate among Acinetobacter species on the basis of phenotypic characteristics. The ACB complex includes genospecies 1 (A. calcoaceticus), genospecies 2 (A. baumannii), genospecies 3 (A. pittii), and genospecies 13TU (A. nosocomialis)
*Acinetobacter spp.* Microbiology

*A. baumannii* (genospecies 2 of the ACB complex) is the most resistant of the genospecies and has the greatest clinical importance. This pathogen is the most frequently isolated species in CAAP in the Northern Territory (>90 percent of *Acinetobacter spp.* isolates).

For members of the *A. baumannii* complex (ABC) (*A. baumannii*, *A. calcoaceticus*, *A. nosocomialis* and *A. pittii*) conventional phenotypic identification methods are unable to reliably separate species due to biochemical similarities. Where identification to species level is required, various genotypic methods have been used, although all are time consuming and none are routinely performed in clinical laboratories. Mass spectrophometry accurately identifies community acquired *A. baumannii* complex isolates, despite genotypic and phenotypic differences from nosocomial clones.
**Acinetobacter spp. Therapy**

There have been a number of case series of community onset *Acinetobacter spp.* bacteraemia patients. The consistent features from these reports include a high mortality, lack of the antibiotic resistance usual in nosocomial infections from this genus, and the presence of risk factors including hazardous alcohol use, smoking, chronic lung disease and chronic renal disease.

Many protocols for the empiric treatment of community-acquired pneumonia do not contain antibiotics which reliably cover community *Acinetobacter spp.*, it is important for clinical staff in tropical and sub-tropical areas to try to define which patients should receive broader empiric antibiotics, to cover the possibility of CAAP, such as meropenem in the wet season.

Management of moderate CAP in tropical regions of Australia is the same as for nontropical regions, above, except if risk factors for *B. pseudomallei* and *A. baumannii* are present (eg diabetes, hazardous alcohol consumption, chronic kidney disease, chronic lung disease and immunosuppressive therapy), or if Gram-negative bacilli are identified by cultures of sputum or blood.

If risk factors for *B. pseudomallei* and *A. baumannii* are present, or if Gram-negative bacilli are identified by cultures of sputum or blood, use: ceftriaxone and gentamicin.

### Susceptibility % of 41 community onset bacteraemic isolates

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<th>% Susceptible</th>
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<tr>
<td>Gentamicin</td>
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</tr>
<tr>
<td>Meropenem</td>
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<tr>
<td>Ciprofloxacin</td>
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<tr>
<td>Cefepime</td>
<td>97</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>97</td>
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<td>Ceftazidime</td>
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<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
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<tr>
<td>Cefazolin</td>
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Corynebacterium diphtheriae is regularly isolated from skin lesions in the Northern Territory.

Toxigenic strains of *C. diphtheriae* are the causative agent of respiratory diphtheria, but strains of diphtheria isolated from cutaneous lesions are overwhelmingly non-toxigenic.

Pyoderma or common cutaneous skin sores

Recent Northern Territory experience in assessing the toxin gene status of over 200 cutaneous isolates, since 2005, reveals the current absence of the toxin gene, in this human only pathogen. The absence of this gene, carried on a bacteriophage, has been attributed to high Northern Territory diphtheria toxoid vaccination rates.

Infections with non-toxigenic *C. diphtheriae* occur in patients subsisting in low socio-economic conditions regardless of the climate. Rare recent Australian cases of clinical disease attributed to toxigenic *C. diphtheriae* have been acquired overseas.
Mixed bacterial species are often present on the primary plate from cutaneous skin sores, and can include haemolytic streptococci, *S. aureus* and the non-haemolytic corynebacteria.

Laboratory scientific staff do not always follow-up all mixed bacterial colonies, from skin lesions. Colonies with typical morphology, if heavy or predominant, are screened for *C. diphtheriae*, by purity plating on HBA, & have a Hoyles plate set up, (characteristic black colouration); and a Gram stain examined for the characteristic Gram positive bacilli morphology.

Representative HBA plate of common cutaneous skin sore flora. Beta Haemolytic streptococci, haemolytic staph aureus and non haemolytic corynebacteria
C. diphtheriae can be misidentified as a non haemolytic staph species, and can give a positive staph latex reaction, and they can also be DNA’ase positive. Gram stain is therefore still very important, in differentiation.

Full identification is by Vitek or API Coryne, (which also provides traditional biotype identification .ie var gravis, var mitis) or mass spectrometry.

Gram stain C. diphtheriae from blood agar. Small Gram positive bacilli. Gram stain shows Gram-positive pleomorphic rods arranged in perpendicular, parallel, and pallisade formations. eg “Chinese letters”
Potassium tellurite ($K_2TeO_3$) is used together with agar as part of a selective medium for growth of some bacteria (Hoyle's medium). Corynebacteria and some other species reduce $TeO_3^{2-}$ to elemental $Te$, which stains the bacteria black. 

- 48 hours incubation

On Tellurite Agar (Hoyle), *C. diphtheriae* produces gray to black colonies on the medium. Tellurite agar is not completely selective for *C. diphtheriae*. Other corynebacteria and other bacterial species may grow on this medium. Colonies may be inspected for an indicative determination of the biovars.

The potentially toxigenic corynebacteria comprise *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans*. *C. diphtheriae* traditionally consists of four biotypes: gravis, mitis, intermedius and belfanti.

Once a potentially toxigenic corynebacteria is identified, then both PCR for toxin gene detection, or a phenotypic immunodiffusion test (Elek’s test) are considered, depending on the clinical circumstance.
In the past, toxigenic *C. diphtheriae* have been isolated from patients living in the NT. In the Central Australian desert, 38% of *C. diphtheriae* isolates tested over a 7-year period from 1985, were found to be toxigenic by Elek testing. (*Elek’s test*, also known as the immuno-diffusion technique, is an in vitro virulence test performed upon *C. diphtheriae* and *C. ulcerans*).

The last reported case of locally-acquired diphtheria was a fatal respiratory adult case from the north of the NT in 1992. Sporadic cases of toxigenic cutaneous diphtheria have since occurred, but all have been acquired from outside of Australia.

In 2011, two-hundred and nineteen isolates of *C. diphtheriae* were obtained from clinically infected skin lesions over the period from 2005 to 2010; 197 were isolated at Royal Darwin Hospital, 18 were isolated at Alice Springs Hospital, 3 were isolated at Gove District Hospital and 1 was isolated at Tennant Creek Hospital. Coryneform bacteria were identified as *C. diphtheriae* using the API (RAPID) Coryne system (bioMérieux). The biotypes identified were as follows; var mitis 127 (58%), var gravis 57 (26%), ambiguous biotype, listed as *C. diphtheriae* 35 (16%). Over the same period, *C. diphtheriae* was isolated from blood cultures taken from 9 patients, and was not isolated from any respiratory specimens.

Of these cutaneous *C. diphtheriae* isolates, tox testing, by PCR of the *tox* gene, of 192 isolates was negative. No positive tox genes from cutaneous isolates were present. So the bacteria remain common, but the bacteriophage carrying the *tox* gene is not currently present in these isolates.
Clinical diphtheria is very rare in Australia but may occur in recent overseas travellers or their contacts. It may presents as gross membranous pharyngitis, with or without airway obstruction, associated with obvious systemic toxicity.

The mainstay of treatment for diphtheria is diphtheria antitoxin. Its use can be associated with acute allergic reactions, and hence it is given in a hospital setting.

Parenteral penicillin is frequently used as adjunctive therapy. Contacts of proven cases should be given oral penicillin or erythromycin.

Diphtheria antitoxin and penicillin should be given immediately to suspected cases. Do not wait for bacteriological confirmation of the disease. Diphtheria antitoxin derived from horse serum is used because sera of sufficient titre are not available from humans. Due to the presence of foreign protein, diphtheria antitoxin may provoke acute, severe, allergic reactions or serum sickness. Consequently, a test dose should be administered, and if there is evidence of hypersensitivity, it may be necessary to administer diphtheria antitoxin under corticosteroid, adrenaline, and antihistamine cover. The therapeutic dose of antitoxin will depend on the clinical condition of the patient, and may be given either intramuscularly or diluted for administration in an intravenous infusion.

Nocardia spp. are opportunistic Gram positive branching bacteria. In the Northern Territory (NT), on average 3 cases/year are identified. An estimated population based incidence of 1.7 per 100,000/year is documented, but this correlates with a rate of > 4/100,000/year in indigenous patients. This is one of the highest rates recorded in the literature. Major risk factors are: chronic lung disease, alcohol use, steroid use and diabetes. The three year survival is low at 48%, reflecting the presence of co-morbidities.

Clinical presentations in the Top End are predominantly pulmonary disease (75% of all infections), then subcutaneous nodules and disseminated disease; compared to the classic presentations in tropical areas of cutaneous abscesses.

Over 30 species are now identified with > 16 associated with human disease.

Microbiologically, Nocardia spp. classic characteristics are those of an aerobic actinomycete, environmental organism (soil, organic), animal & human pathogen; with no person-person spread; and a reputation as “suppurative organism” which does not respect anatomic boundaries.

Species of Nocardia identified in a recent review of 45 Northern Territory cases, in which 38 isolates were speciated included: N. asteroides complex, 47.3%; N. otitidiscavarium, 18.4%; N. cyriacigeorgia, 10.5%; N. veterana, 10.5%; N. pseudobrasiliensis, 5.2%; and one isolate each of N. elegans; N. beijingensis and N. testacea.
Three quarters of the presentations in the Northern Territory are pulmonary, on the background of chronic lung disease and other co-morbidities; rather than the more classically described cutaneous lesions in the tropics.

Multiple cutaneous abscesses of Nocardia. Photo courtesy of Whiting S. in: An Audit of Nocardia Infections at the Royal Darwin Hospital 1997 - 2010
Gram positive branching filaments in the microscopy of the clinical specimen. Gram staining usually provides the first clue that *Nocardia spp.* are present in clinical specimens. Modified acid fast stains are then used to confirm the Gram stain findings.

*Nocardia* like organisms seen in Gram stain, do not always culture due to bacterial overgrowth.
**Nocardia spp.**

**Microscopy**

*Nocardia spp.* are classically described as modified acid fast stain positive.

This is confirmed by using 0.5% to 1% H₂SO₄. Modified acid fastness can be dependent on age of culture and media, and is ideally best performed on the same clinical specimen as the Gram stain.

Regular Ziehl-Neelsen staining (using 3% hydrochloric acid) is negative for nocardia acid fastness.
Nocardia grow in 2-7 days on most routine media. (not particularly fastidious). Selective media including buffered charcoal yeast extract (BYCE), Thayer Martin and some fungal media, are used to suppress normal flora. Some species can take 2-3 weeks to grow. These selective media can suppress oral and colonising sputum bacteria.

Usually filamentous white to yellow colonies, often hard, indenting the media and chalky.
There is a current taxonomical state of flux.

Essentially an isolate can be classified as probably in the Nocardia genus, on the basis of colony morphology, presence of aerial hyphae, typical Gram stain morphology, and partial acid fastness. Classic phenotypic classifications of the Nocardia species type based on biochemical reactions such as hydrolysis of casein, L-tyrosine and xanthine, are not accurate enough; and should be supplemented by 16SrRNA gene sequences, and/or partial gene sequencing which is now essential for species identification. The taxonomy continues to evolve.

Classically; human infections are often caused by inhalation of members of the Nocardia asteroides group, which includes three subgroups:

- *N. asteroides* complex (which contains multiple subspecies)
- *Nocardia farcinica*
- *Nocardia nova*.

Species of Nocardia identified in a recent review of 45 Northern Territory cases, in which 38 isolates were speciated included: *N. asteroides* complex, 47.3%; *N. otitidiscavarium*, 18.4%; *N. cyriacigeorgia*, 10.5%; *N. veterana*, 10.5%; *N. pseudobrasiliensis*, 5.2%; and one isolate each of *N. elegans; N. beijingensis* and *N. testacea*.

*N. brasiliensis* is the most important classical isolate in tropical areas; it is most often seen as a cutaneous infection that can affect individuals with normal immune function, but was not isolated in the last 15 years in the Northern Territory.
Susceptibility testing is problematic. Issues include:

- no truly validated method
- correlations with patient outcomes for individual species are lacking data
- significant technical problems exist including:
  - uniform inocula, slow growth, hazy growth with non-bactericidal antimicrobials

Microbroth dilution is the standard method.

*Nocardia spp.* isolates usually demonstrate 100% in vitro susceptibility to, amikacin, and linezolid, and > 90% susceptibility to trimethoprim/sulphamethoxazole. Variable susceptibility to ceftriaxone and meropenem can be species dependent.

**Standard therapy** for nocardial infections includes trimethoprim/sulphamethoxazole for skin infections; and combined therapy for severe and disseminated infections in immunocompromised patients.

The recommended combined acceptable therapy for severe infections includes trimethoprim/sulfamethoxazole combined with impenem/meropenem or amikacin. Other drugs with activity include ceftriaxone & linezolid. Drug therapy is guided by the susceptibility results and species identification.

Due to the rapid changes in taxonomy within the various species, and the difficulties in susceptibility testing, large scale therapy trials are not available.
References

General

S. aureus

Group A strep

N. Gonorrhoeae

VACS organisms
Burkholderia pseudomallei

Acinetobacter

Diphtheria

Nocardia