Wisconsin Fungal Case Presentations

WCLN Webinar
February 10, 2016

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Disclosure

Raymond P. Podzorski, Ph.D., D(ABMM)
February 10, 2016

No relevant financial relationships to disclose.
New Pulmonary Nodule-12/2013

- 68 y/o ♀
- Severe rheumatoid arthritis
- Takes multiple immunosuppressive medications
- Quit smoking in 2006 (48 years)
- Right lower lobe (RLL) pulmonary nodule noted on pre-surgical workup
- Images from September 2013 showed no RLL nodule (ct scan)
Past Medical History

- Bladder cancer
- Hysterectomy
- Cholecystectomy
- Cataract surgery
Procedures

- CT of chest w/contrast 12/14/2013
- PET CT scan 12/17/2013
- Demonstrated a 2.5 x 3.3 cm mass at the bottom of the right lower lobe
- Enlarged mediastinal lymph nodes also noted
- Enhanced metabolic activity noted in the large mass and the mediastinal lymph nodes
Procedures

• Video Mediastinoscopy 1/4/2014
  – Biopsies collected
  – Frozen sections showed inflammation and necrotizing granulomas
  – Tissue for routine bacterial culture, fungus culture and AFB culture
    – Tissue Gram stain, rare PMNs, NOS
    – AFB stain, NOS
Pathology Report of Mediastinal Lymph Node

• Multiple caseating granulomas
• Numerous yeast, 3-4 µm
• Yeast consistent in size
Mediastinal Lymph Node
GMS Stain 60X
Mediastinal Lymph Node
GMS Stain 100X
Fig. 4. Algorithm for identifying yeasts on histopathology and cytopathology preparations. The yellow paintbrush denotes a step where a stain may be useful. Note that most yeasts can be differentiated by morphology alone. AB=Alcian blue, MC=Mucicarmine, FM=Fontana Masson.
Sensitivity of laboratory tests for diagnosis of pulmonary Histoplasmosis (Percent positive)

<table>
<thead>
<tr>
<th>Test</th>
<th>Acute or subacute, pericarditis, rheumatological pulmonary</th>
<th>Chronic pulmonary</th>
<th>Mediastinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>25–75</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Fungal stain</td>
<td>10</td>
<td>40</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Culture</td>
<td>15</td>
<td>50–85</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Serology</td>
<td>95</td>
<td>100</td>
<td>67</td>
</tr>
</tbody>
</table>

Adapted from *TRENDS in Microbiology* Vol. 11 No. 10 October 2003 489.

In acute pulmonary disease, the sensitivity of antigen detection ranges from about 25% in patients with local manifestations to over 75% in those who present within the first month of exposure.
### Table 1. Comparison of Diagnostic Tests in All Cases

<table>
<thead>
<tr>
<th>All tests</th>
<th>AIDS (n = 56)</th>
<th>OIC (n = 87)</th>
<th>NIC (n = 15)</th>
<th>All (n = 158)</th>
<th>Pulmonary cases (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>Pathology</td>
<td>Antigen</td>
<td>Antibody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34/48 (70.8)</td>
<td>18/25 (72.0)</td>
<td>53/56 (94.6)</td>
<td>15/19 (78.9)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td></td>
<td>57/75 (76.0)</td>
<td>32/43 (74.4)</td>
<td>81/87 (93.1)</td>
<td>37/53 (71.2)</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11/15 (73.3)</td>
<td>8/9 (83.9)</td>
<td>5/6 (83.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[13.80–7.67]</td>
<td></td>
<td>[2.41–2.26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[10.48–7.62]</td>
<td></td>
<td>[0.53–1.23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[6.92–7.65]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[11.32–7.88]</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98/132 (74.2)</td>
<td>58/76 (76.3)</td>
<td>145/158 (91.8)</td>
<td>60/80 (75.0)</td>
<td>4/6 (66.7)</td>
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<tr>
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<td></td>
<td></td>
<td>39/41 (95.1)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>5/6 (83.3)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.

* Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody tests were positive in 2 (18.2%) of 11 patients who had undergone organ transplantation, 12 (85.7%) of 14 who were receiving tumor necrosis factor antagonists, and 20 (62.5%) of 32 with other causes for immunocompromise.

### Table 2. Comparison of Diagnostic Tests in Proven Cases

<table>
<thead>
<tr>
<th>Proven tests</th>
<th>AIDS (n = 38)</th>
<th>OIC (n = 62)</th>
<th>NIC (n = 11)</th>
<th>All (n = 111)</th>
<th>Pulmonary cases (n = 23)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>Pathology</td>
<td>Antigen</td>
<td>Antibody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34/38 (99.5)</td>
<td>18/23 (78.3)</td>
<td>35/38 (92.1)</td>
<td>9/13 (69.2)</td>
<td>14/17 (82.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>57/61 (93.4)</td>
<td>[14.46–7.34]</td>
<td>16/19 (34.2)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/8 (87.5)</td>
<td>[11.90–7.44]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/8 (100)</td>
<td>[5.11–7.74]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[12.10–7.82]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98/107 (91.6)</td>
<td>58/68 (85.3)</td>
<td>100/111 (90.1)</td>
<td>41/56 (73.2)</td>
<td>12/13 (92.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/12 (66.7)</td>
<td>7/18 (38.9)</td>
<td></td>
<td>3/3 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/4 (75.0)</td>
<td>4/5 (80.0)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[0.62–1.23]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>[0.63–0.54]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of patients with positive test results / no. of patients tested (%). NIC, nonimmunocompromised; OIC, other causes of immunocompromise.

* None of the acute pulmonary cases were proven.

* Mean antigen concentration, standard deviation in ng/mL. Among the OIC group, antibody test results were positive in 2 (20%) of 10 patients who had undergone organ transplantation, 7 (87.5%) of 8 who were receiving tumor necrosis factor antagonists, and 14 (66.7%) of 21 with other causes for immunocompromise.
Laboratory Testing

• Histoplasma Urine Antigen – random 2/3/2014, None Detected
• EIA Histoplasma Ab screen - Equivocal
• CF Histoplasma mycelial Ab – negative
• CF Histoplasma yeast - 1:8
• Histoplasma Immunodiffusion – M Band

Sample Immunodiffusion
NOT FROM THIS CASE
Mediastinal Lymph Node Colony
After 19 Days of Incubation 30° C
Scotch-Tape Preparation of Culture
Identification of *H. capsulatum*

- SLIDE CULTURES SHOULD NOT BE PERFORMED
- Conversion of filamentous form to yeast form in culture (incubate 37°C)
- Exoantigen test
- DNA probe
- Nucleic Acid sequencing
- MALDI-TOF MS
Fungal Sequence Identification

Region of fungal rRNA gene complex PCR amplified and cycle-sequenced by this assay.
Nucleic Acid Sequence

>dbj|AB176510.1| Ajellomyces capsulatus gene for 28S rRNA, partial sequence, isolate:IFM 49110 Length=574 Score = 1051 bits (569), Expect = 0.0

Identities = 571/572 (99%), Gaps = 0/572 (0%) Strand=Plus/Plus

Query 1   ACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAAGAGCTCAAATTTGAAATCC 60
Sbjct 3   ............................................................ 62
Query 61  GGCCCCCCTGGGGGCGCTGAGGTAATGTTGCTCAGAGGAGGGGTGGCGACCGCGGTCC 120
Sbjct 63  ............................................................ 122
Query 121 AAGTCCCCTGGAACGGGGCGTCGTAGAGGGTGAGAATCCCGTCTCCGGCCGGCGTCTC 180
Sbjct 123 ............................................................ 182
Query 181 GCCCGTGTGAAGCTCCTTCGACGAGTCTCAAGTTTGGGAATGCAGCTCCAAATGGGTG 240
Sbjct 183 ............................................................ 242
Query 241 TAAATTTCTCTAAAGCTAAATACTGGTCGGAGACCGATAGCGCACAAGTAGAGTGATCG 300
Sbjct 243 ............................................................ 302
Query 301 AAAGATGAAAAAGCAGTTTTGAAAGAGAGTTAAACAGCATGTGAATTGTTGAAAGGGAAAG 360
Sbjct 303 ............................................................ 362
Query 361 CGCTTGCGATCAGAGTCGGCCGCGGGGGTTCAGCGGGCATTCGTTGCCCGTGCAATCC 420
Sbjct 363 ............................................................ 422
Query 421 CGCGGCCGGGCCAGCGTCGGTTTCGACGGCCGGTCAAAGGCCCCGGGAATGTGTCGCC 480
Sbjct 423 ............................................................ 482
Query 481 TCGGGGCGTCTTATAGCCGGGGTGCAATGCGGCCAGTCGGGACCGAGGAACGCGCTCCG 540
Sbjct 483 ............................................................ 542
Query 541 GCACGGACGCTGGGCTTAATGTCGTCAGCGAC 572
Sbjct 543 ............................................................ 574
Background *Histoplasma capsulatum*

- Single species in genus, 3 varieties of species
- Endemic in the lower Mississippi river valley and Ohio river valley
- Found in soil enriched with guano of various birds or bats, often found around chicken coups
- Spread though inhalation of microconidia in disturbed soil infected with *H. capsulatum*
- Among Medicare beneficiaries, most common endemic mycosis in the US
- Estimated that 500,000 people are newly infected each year in US
US Distribution of *H. capsulatum*
Hunting for Histo?

Protect yourself – Always follow proper isolation precautions
Painful Hip

- 78 y/o ♂
- Widower, lives alone, does have “lady friends”
- Non-insulin dependent diabetes
- Silicosis diagnosed in 1976
- Atrial fibrillation
- Prostate cancer, post seed implantation
- Not immunosuppressed
- Painful loose left total hip (done 1993/ re-done 2008) Fluoroscopy procedure aspirated 1.5 ml clear yellow fluid in February 2011
Hip Fluid February 2011

• Cytospin Gram stain
  – rare PMNs, NOS

• Hematology
  – No crystals
  – Nucleated cell count 1,115/µl
  – Differential –
    – 45% PMNs
    – 43% Lymphocytes
    – 3% Monocytes
    – 7% Synovial cells
    – 2% Macrophage
Hip Fluid February 2011

• 4 days later culture grew 7 colonies
  – “Very round”, small yeast
  – Rapid urea +
  – Rapid trehalose –
  – Set up DNA sequencing
>gb|FJ534909.1| Cryptococcus neoformans var. grubii strain CBS 8710 large subunit ribosomal RNA gene, partial sequence Length=629 Score = 1146 bits (620), Expect = 0.0 Identities = 620/620 (100%), Gaps = 0/620 (0%) Strand=Plus/Plus

Query 1   GCGGAGGAAAAAGAAACTAACAAGGATTTCCCTTAGTAACGGCGAGTGAACCGGGAAGAGCT 60
Sbjct 4   ............................................................ 63
Query 61  CAAATTTGAAATCTGGCGTCCTCCGGGCGTCCGAGTTGTAATCTACAGAAACGTTCCTCCG 120
Sbjct 64  ............................................................ 123
Query 121 TGCTGGACCGTGTCTAAGTCCCTTGGAATAGGGTATCAAAGAGGGTGACAATCCCGTACT 180
Sbjct 124 ............................................................ 183
Query 181 TGACACGATACCACTGCAGCATCCTGCTGTGATACTGTTTCTACGGAGTCGCTGTTACTTGGGAGTGTA 240
Sbjct 184 ............................................................ 243
Query 241 GCGCAAAATGGGTGGTAAACTCCATCTAAATATTGGTGGAAGACCGATAGCGAA 300
Sbjct 244 ............................................................ 303
Query 301 CAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAA 360
Sbjct 304 ............................................................ 363
Query 361 TTGTTGAAAGGGAAACGATTGAAGTCAGTCGTGTCTATTGGGTTCAGCCAGTTCTGCTGG 420
Sbjct 364 ............................................................ 423
Query 421 TGTATTCCCTTTAGACGGGTCAACATCAGTTCTGATCGGTGGATAAGGGCTGGGGGAATG 480
Sbjct 424 ............................................................ 483
Query 481 TAGCACTCTTCCGGAGTGTGTTTAGCTCTCCTGCTGCGATACACTGTTGGGACTGAGGAAT 540
Sbjct 484 ............................................................ 543
Query 541 GCAGCTCGCCTTTATGGCCGGGGTTCGCCACGTTGGCAGCTTAGGATGTTGACAAAATGG 600
Sbjct 544 ............................................................ 603
Query 601 CTTAAACGACCCGCTCTTGA 620
Sbjct 604 ............................................................ 623
Distance Tree

- Tsuijiiaea wingfieldii strain CBS 7118 large subunit ribosomal RNA gene, partial sequence
- Tsuijiiaea wingfieldii 26S ribosomal RNA gene, partial sequence
- Cryptococcus amylolentus strain CBS 6039 large subunit ribosomal RNA gene, partial sequence
- Cryptococcus amylolentus 26S ribosomal RNA gene, partial sequence
- Filobasidiella depauperata strain CBS 7841 large subunit ribosomal RNA gene, partial sequence
- Filobasidiella depauperata strain CBS 7841 26S large subunit ribosomal RNA gene, partial sequence
- Cryptococcus gattii WM276 chromosome 8, complete sequence
- Cryptococcus bacillisporus strain Reg1 26S ribosomal RNA gene, partial sequence
- Cryptococcus gattii gene for large subunit rRNA, partial sequence, strain: IFM 5815
- ldli65177
- Cryptococcus neoformans var. grubii strain CBS 8710 large subunit ribosomal RNA gene, partial sequence
- Cryptococcus neoformans var. neoformans gene for 25S ribosomal RNA, partial sequence, strain: IFM 52379 (= SUM5 0171)
Cryptococcus neoformans
History March 2011

• March 17 hip hardware removed
  – Replaced with an antibiotic-impregnated temporary femoral stem
  – Tissue collected and submitted for culture
  – Few days later tissue grew rare \textit{C. neoformans}
Background *Cryptococcus spp.*

- Genus *Cryptococcus* contains many species (19-30)
- *C. neoformans* and *C. gattii* are the main human pathogens
- *C. neoformans* found in soil enriched with guano from pigeons (and other birds) and aged pigeon guano
- *C. neoformans* worldwide distribution
- *C. gattii* found around flowering *Eucalyptus camaldulenis* trees and coniferous trees native to the pacific northwest
- Spread though inhalation of fungus in disturbed soil infected with *C. neoformans* or plant debris infected with *C. gattii*
- Prior to late 1990’s *C. gattii* found predominantly in the tropics and sub-tropics
Emergence of *Cryptococcus gattii* — Pacific Northwest, 2004–2010

- *C. gattii* previously *C. neoformans var. gattii*
- 1999 *C. gattii* outbreak on Vancouver Island, BC (as of 2007 a total of 218 cases in the area)
- December 2004 a *C. gattii* case in Oregon
- As of July 2011, 96 *C. gattii* cases in Oregon, Washington, Idaho, and California
  - 46 patients had no travel history to Canada
North American of Geography of *C. gattii*
FIGURE. Cases of *Cryptococcus gattii* infection* (n = 51) with known illness onset date,† by quarter — California, Idaho, Oregon, and Washington, 2004–2010

**Source:** *Cryptococcus gattii* Public Health Working Group.
* Defined as illness occurring on or after January 1, 2004, in a U.S. resident with a culture-confirmed isolate of *C. gattii*.
† Includes estimated date for one patient each in 2007, 2008, and 2010, and two patients in 2009.
Possible Areas Endemic for Cryptococcus gattii
## Differences Between *C. gattii* and *C. neoformans*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Cryptococcus gattii</em></th>
<th><em>Cryptococcus neoformans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecological Niche</td>
<td><em>Eucalyptus</em> trees; Native trees of the Pacific NW (e.g. Douglas fir)</td>
<td>Bird Guano</td>
</tr>
<tr>
<td>Location</td>
<td>Tropical &amp; subtropical; Pacific NW; possibility for further spread</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Immune Status of Host</td>
<td>Immuno-competent (&gt;50%)</td>
<td>Immuno-compromised (&gt;80%)</td>
</tr>
<tr>
<td>Lung Manifestations</td>
<td>Commonly nodules</td>
<td>Commonly infiltrates</td>
</tr>
<tr>
<td>Brain Lesions</td>
<td>More common</td>
<td>Less common</td>
</tr>
<tr>
<td>Hospital Stay and</td>
<td>Longer</td>
<td>Shorter</td>
</tr>
<tr>
<td>Duration of Therapy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

January 18, 2011, ASM
Table 2. Laboratory tests for the differentiation of *C. gattii* from *C. neoformans*

<table>
<thead>
<tr>
<th>Test</th>
<th>Differentiates</th>
<th>Does not differentiate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum tests:</td>
<td></td>
<td>X (positive for both species)</td>
</tr>
<tr>
<td>a) <em>Cryptococcus</em> antigen</td>
<td></td>
<td>X (negative for both species)</td>
</tr>
<tr>
<td>b) β-1-3-D-glucan</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Direct specimen stains:</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>a) Gram</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>b) India Ink</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>c) Calcofluor</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>d) Mucicarmine</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>e) GMS</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Appearance:</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>a) Colony morphology</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>b) Cornmeal-Tween 80 morphology</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>c) Temperature and media requirements</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Commercial biochemical identification systems e.g. VITEK 2 ID-YST, MicroScan</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Biochemical tests:</td>
<td></td>
<td>X (positive for both species)</td>
</tr>
<tr>
<td>a) Urease</td>
<td></td>
<td>X (positive for both species)</td>
</tr>
<tr>
<td>b) Melanin production (phenyloxidase)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>c) CBG agar</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Serological typing of isolates</td>
<td>X (no longer commercially available)</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>X (target-dependent)</td>
<td></td>
</tr>
<tr>
<td>DNA sequencing</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
L-Canavanine Glycine Bromothymol Blue Agar

FIG. 1. Reactions of various cryptococci on CGB agar. The left plate depicts a positive reaction with *C. gattii*; the middle plate shows a weak reaction around the inoculum, which is interpreted as negative; and the right plate shows a negative reaction with *C. neoformans*.

Available from REMEL

47:3669-3672. 2009 JCM
Distance Tree

- *Tsujiisaea wingfieldii strain CBS 7118 large subunit ribosomal RNA gene, partial sequence*
- *Tsujiisaea wingfieldii 26S ribosomal RNA gene, partial sequence*
- *Cryptococcus amylolentus strain CBS 6039 large subunit ribosomal RNA gene, partial sequence*
- *Cryptococcus amylolentus 26S ribosomal RNA gene, partial sequence*
- *Filobasidiella depauperata strain CBS 7841 large subunit ribosomal RNA gene, partial sequence*
- *Filobasidiella depauperata strain CBS 7841 26S large subunit ribosomal RNA gene, partial sequence*
- *Cryptococcus gattii WM276 chromosome B, complete sequence*
- *Cryptococcus bacillisporus strain Reg1 26S ribosomal RNA gene, partial sequence*
- *Cryptococcus gattii gene for large subunit rRNA, partial sequence, strain: IFM 5815*
- *Idi63877*
- *Cryptococcus neoformans var. grubii strain CBS 8710 large subunit ribosomal RNA gene, partial sequence*
- *Cryptococcus neoformans var. neoformans gene for 25S ribosomal RNA, partial sequence, strain: IFM 52379 (= SUM5 0171)*
Nucleic Acid Sequence

gb|CP000287.1|Cryptococcus gattii WM276 chromosome B, complete sequence Length=2187695 Score = 1123 bits (608), Expect = 0.0 Identities = 616/620 (99%), Gaps = 0/620 (0%) Strand=Plus/Plus

Query 1       GCGGAGGAAAAAGAAAACCTAAACAAGGATTCCCTTAGTAACCGGGAAGAGCT 60
Sbjct 1521129 ............................................................ 1521188
Query 61      CAAATTTGAAATCTGGCGTCCTCCGGCGTCCGAGTTGTAATCTACAGAAACGTGGTTCG 120
Sbjct 1521249 ............................................................ 1521308
Query 121     TGCTGGACCGTGTCTAAGTCCCTTGGAATAGGGTATCAAAGAGGGTGACAATCCCGTACT 180
Sbjct 1521309 ............................................................ 1521368
Query 181     TGACACGATCACCAGTGCTCTGTGATACGTTTTCTACGAGTCGCGTTACTTGGGAGTGTA 240
Sbjct 1521369 ............................................................ 1521428
Query 241     GCGCAAAATGGGTGGTAAACTCCATCTAAAGCTAAATATTGGTGGAAGACCGATAGCGAA 300
Sbjct 1521429 ............................................................ 1521488
Query 301     CAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAA 360
Sbjct 1521489 ............................................................ 1521548
Query 361     TTGTTGAAAGGGAAACGATTGAAGTCAGTCGTGTCTATTGGGTTCAGCCAGTTCTGCTGG 420
Sbjct 1521549 ............................................................ 1521608
Query 421     TGTATTCCCTTTAGACGGGTCAACATCAGTTCTGATCGGTGGATAAGGGCTGGGGGAATG 480
Sbjct 1521609 ............................................................ 1521668
Query 481     TAGCACTCTTCGGAGTGTGTTATAGCCTCCTGTCGCATACACTGGTTGGGACTGAGGAAT 540
Sbjct 1521669 ............................................................ 1521728
Query 541     GCAGCTCGCCTTTATGGCCGGGGTTCGCCACGTTCGAGCTTAGGATGTTGACAAAATGG 600
Sbjct 1521729 ............................................................ 1521788
Query 601     CTTAAAACGACCCTGCTTTGA 620
Sbjct 1521789 ............................................................ 1521848
I Got Wacked!

- 16 y/o ♂
- In normal state of good health
- Modest acne
- Mows lawns part time
- Presented with an asymptomatic non-healing “skin tag”-like lesion on left index finger
I Got Wacked!

- Injured finger while operating a gas powered string trimmer
- Spinning string from rotating trimmer sliced finger (he was operating trimmer)
- Accident occurred about 6 weeks ago
- Original wound healed, but “skin-tag” lesion remained
- “Skin-tag”-like lesion removed and send for histology and bacteria, AFB, and fungal cultures
“Skin-Tag” GMS Stain 60X
“Skin-Tag” Fungal Culture after 4 Days
Scotch-tape Preparation of Fungal Culture

*Alternaria sp.*
Table 7. Major Causes of Human Skin, Nail, and Hair Infections by Nondermatophytic Fungi

<table>
<thead>
<tr>
<th>Skin and Nail Infections</th>
<th>Hair Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acremonium</em> spp.</td>
<td><em>Piedraia hortae</em></td>
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<tr>
<td><em>Aspergillus</em> spp.</td>
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<tr>
<td><em>C. albicans</em></td>
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<tr>
<td><em>E. dermatitidis</em></td>
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<tr>
<td><em>Fusarium</em> spp.</td>
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<tr>
<td><em>Hortaea werneckii</em></td>
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<tr>
<td><em>Lasiodiplodia theobromae</em></td>
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<tr>
<td><em>Neoscytalidium</em> spp.</td>
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<tr>
<td><em>Onychocola canadensis</em></td>
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<tr>
<td><em>Pyrenochaeta unguis-hominis</em></td>
<td></td>
</tr>
<tr>
<td><em>Scopulariopsis breviculis</em></td>
<td></td>
</tr>
</tbody>
</table>

CLSI M54-A, 2012, Principles and Procedures for Detection of Fungi in Clinical Specimens- Direct Examination and Culture; Approved Guideline
Background *Alternaria spp.*

- Genus *Alternaria* contains 44 species
- Very common in the environment
- Most are plant pathogens, but a few can be found in the soil
- Commonly considered a saprophytic contaminate of clinical specimens, may occasionally can cause a true infection
- Infections in subcutaneous tissue, eye, nail, skin, nasal sinuses, and peritonitis
- Exposure may play a role in asthma and hypersensitivity pneumonitis
The End