Epidemiology and Resistance in *Aspergillus* and other Moulds

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### Invasive Mould Infections

- Often opportunistic
  - Associated with significant morbidity and mortality in immunocompromised hosts
  - Hematologic malignancies
  - HSCT recipients (allogeneic & autologous)
  - SOT recipients (lung, heart, liver, kidney, etc)

- Limited treatment options for some
  - Order Mucorales
  - *Fusarium* species
  - *Scedosporium* / *Lomentospora* species

- Knowledge of fungal taxonomy increasing
  - Diagnostic strategies some what lacking

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### IFIs Organ Transplant Recipients

  - Proven or probable IFIs from 15 U.S. centers

<table>
<thead>
<tr>
<th>Invasive Fungal Infections</th>
<th>Surveillance Cohort (1208 IFI in 1063 pts)</th>
<th>Incidence Cohort (729 IFI in 633 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive candidiasis</td>
<td>52.9%</td>
<td>56%</td>
</tr>
<tr>
<td>Invasive aspergillosis</td>
<td>18.8%</td>
<td>18.8%</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>8.0%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Other moulds</td>
<td>6.5%</td>
<td>8.1%</td>
</tr>
<tr>
<td>Unspecified moulds</td>
<td>2.0%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Endemic fungi</td>
<td>5.3%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>2.3%</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

12-month cumulative incidence for any IFI 3.1% (0.7% for IA; ~0.2% other moulds)


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### IMI Lung Transplant Recipients

- 1173 lung transplant recipients at 11 of 15 TRANSNET SOT centers
- 143 (12.2%) developed invasive mould infections (2001 – 2006)
- IMIs occurred median 11 months post-transplant
  - Majority late onset (>90 days)
  - 26 months for mucormycosis

<table>
<thead>
<tr>
<th>Species</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em></td>
<td>72.7%</td>
</tr>
<tr>
<td>Other moulds</td>
<td>16.8%</td>
</tr>
<tr>
<td>No culture</td>
<td>4.9%</td>
</tr>
<tr>
<td><em>Scedosporium</em></td>
<td>3.5%</td>
</tr>
<tr>
<td>Mucorales</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Other moulds = *Acremonium*, *Alternaria*, *Chrysosporium*, *Cladosporium*, *Exophiala*, *Monosporium*, *Ochroconis*, *Paecilomyces*, *Paraphaenocladium*, *Penicillium*, *Phaeoacremonium*, *Phaeosphaeria*, *Rhinocladiella*, *Scopulariopsis*, *Trichoderma* (several phaeoid species)

IFIs HSCT Recipients

  - Proven or probable IFIs from 22 U.S. centers

<table>
<thead>
<tr>
<th>Invasive Fungal Infections</th>
<th>Surveillance Cohort (983 IFI in 876 pts)</th>
<th>Incidence Cohort (718 IFI in 639 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive aspergillosis</td>
<td>43%</td>
<td>42%</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>8.0%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Other moulds</td>
<td>7.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Unsuspected moulds</td>
<td>6.0%</td>
<td>6.0%</td>
</tr>
<tr>
<td>Fusariosis</td>
<td>3.0%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Endemic fungi</td>
<td>0.6%</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

12-month cumulative incidence for any IFI 3.4% [0.9% - 13.2% range by site]


IFI HSCT PATH Alliance

- 23 medical centers U.S. & Canada, July 2004 to September 2007
  - 250 proven/probable IFI in 234 HSCT patients

<table>
<thead>
<tr>
<th>Invasive Fungal Infection</th>
<th>Proven/Probable</th>
<th>12 Week Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive aspergillosis</td>
<td>59.2%</td>
<td>35.9%</td>
</tr>
<tr>
<td>Invasive candidiasis</td>
<td>24.8%</td>
<td>48.9%</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>7.2%</td>
<td>64.3%</td>
</tr>
<tr>
<td>Other moulds</td>
<td>6.8%</td>
<td>80%</td>
</tr>
</tbody>
</table>


TRANSNET Phaeohyphomycosis

- Included in the “other moulds” category
- 56 of 2191 (2.6%) IFI in TRANSNET cohort
- Similar divided between HSCT & SOT recipients
  - Pulmonary infections in HSCT recipients (57.7% vs. 26.7%)
    - Overall 90 day mortality higher HSCT (42% vs. 10%)  
  - Cutaneous infections in SOT recipients (53.3% vs. 23.1%)

<table>
<thead>
<tr>
<th>Species (n = 56 isolates total)</th>
<th>No. Isolates (%)</th>
<th>No. Deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria</td>
<td>18 (32.1%)</td>
<td>6 (33.3%)</td>
</tr>
<tr>
<td>Zopfaphae</td>
<td>10 (10.7%)</td>
<td>2</td>
</tr>
<tr>
<td>Cladophialaphora</td>
<td>5 (8.9%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Scopularispora</td>
<td>5 (8.9%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Fusariosis</td>
<td>4 (7.1%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Phialennsium</td>
<td>4 (7.1%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>Fusariosis</td>
<td>3 (5.4%)</td>
<td>1 (33.3%)</td>
</tr>
</tbody>
</table>


Aspergillosis

- Acute invasive aspergillosis
  - Estimated ~200,000 cases/year
  - Leading cause of invasive mould infections
    - SOT recipients (TRANSNET)
    - Lung transplant recipients (TRANSNET)
    - HSCT recipients (TRANSNET & PATH Alliance)
  - Leading cause IFI in HSCT recipients

- Chronic pulmonary aspergillosis
  - Estimated >3 million people infected worldwide
  - Common with underlying lung disease
    - Tuberculosis, sarcoidosis

Microbiology

- Ubiquitous mould
- >200 spp. Aspergillus
- Common causes of infections in humans
  - A. flavus
  - A. fumigatus
- Conidia inhaled and may evade upper respiratory defenses
  - Germinate into angioinvasive hyphae
    - Local tissue damage
    - Hemorrhage, infarction
    - Coagulative necrosis

Antifungal Activity vs. A. fumigatus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC Range</td>
<td>&lt;0.03 – &gt;16 μg/ml</td>
<td>0.06 – &gt;16 μg/ml</td>
<td>&lt;0.03 – &gt;16 μg/ml</td>
</tr>
<tr>
<td>MIC50</td>
<td>0.5 μg/ml</td>
<td>0.5 μg/ml</td>
<td>0.25 μg/ml</td>
</tr>
<tr>
<td>MIC90</td>
<td>1 μg/ml</td>
<td>1 μg/ml</td>
<td>0.5 μg/ml</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.537 μg/ml</td>
<td>0.586 μg/ml</td>
<td>0.191 μg/ml</td>
</tr>
</tbody>
</table>

Azole Resistance in Aspergillus

**Modification of Target Enzyme**

- Point mutations in CYP51A (gene encoding for 14α-demethylase – azole target) observed in A. fumigatus clinical and laboratory isolates with azole resistance
- Position of point mutation determines azole resistance
  - Some pan-azole, other affect voriconazole & isavuconazole, other affect posaconazole
- Historically observed with chronic azole exposure
  - Patients with chronic pulmonary aspergillosis (Manchester, UK)

Environmental Exposure to Azoles

- Azole-resistant IA identified in patients without prior azole exposure in parts of Europe
  - Indoor environment in hospitals & direct proximity to medical centers
  - Fields where azole fungicides used
    - Used in agriculture to combat crop failure & other products to prevent rotting
- Mechanisms of azole resistance in environmental isolates & azole-naive patients
  - TR_{L98H}
  - TR_{Y121F/T289A}
  - TR_{L3}


**Worldwide Issue**

<table>
<thead>
<tr>
<th>Continent/Country</th>
<th>Percent Resistance</th>
<th>Isolate Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe (G54, M222, G448S, G138, TR34/L98H, TR46/Y121F, TR53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>5.7%</td>
<td>Clinical</td>
</tr>
<tr>
<td>France</td>
<td>0.9 – 10.6%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Germany</td>
<td>1.1 – 12%</td>
<td>Clinical &amp; Environmental</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2.1 – 20%</td>
<td>Clinical &amp; Environmental</td>
</tr>
<tr>
<td>Poland</td>
<td>2.3%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Spain</td>
<td>1.8%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Turkey</td>
<td>10.2%</td>
<td>Clinical</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>6.6 – 24%</td>
<td>Clinical</td>
</tr>
<tr>
<td>North &amp; South America (G54, M222, G448S, G138, TR34/L98H, TR46/Y121F, TR53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>0.6 – 11.8%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Colombia (TR34)</td>
<td>3.3%</td>
<td>Environmental</td>
</tr>
<tr>
<td>Africa (TR34/L98H, TR46/Y121F, TR53)</td>
<td>13.9%</td>
<td>Environmental</td>
</tr>
<tr>
<td>Tanzania</td>
<td>13.9%</td>
<td>Environmental</td>
</tr>
<tr>
<td>Asia &amp; Australia (G54, M222, G448S, TR34/L98H, TR46/Y121F, TR53)</td>
<td>1.0 – 11.1%</td>
<td>Clinical &amp; Environmental</td>
</tr>
<tr>
<td>Australia</td>
<td>2.6%</td>
<td>Clinical</td>
</tr>
<tr>
<td>China, India, Iran, Japan, Kuwait, Pakistan</td>
<td>1.0 – 11.1%</td>
<td>Clinical &amp; Environmental</td>
</tr>
</tbody>
</table>


**Azole Resistance - FTL Results**

- 20 of 26 isolates confirmed to be *A. fumigatus* & with elevated azole MICs had CYP51A mutations

- TR34/L98H or TR46/Y121F/T289A mutations each found in two isolates
  - First TR46/Y121F/T289A mutation (2008) predates first one found in Europe (the Netherlands, 2009)
  - Patients with proven disease & without travel history

- 6 azole resistant isolates without CYP51A mutation


**Other Mechanisms of Resistance**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Overexpression CYP51B | • Cyp51p encoded by cyp51A & cyp51B
• CYP51B important in regulating 14-α-demethylase activity
• CYP51B associated with growth rate & cell maintenance
• Role CYP51B in azole resistance unclear |
| Efflux pumps | • ~50 ABC family transporters & ~300 MFS genes predicted in *A. fumigatus*
• Overexpression of CDR1B, AtaMDR1, AtaMDR2, AtaMDR3, AtaMDR4 (bdf1 resistance)
• ATRF associated withazole resistance in vitro
• Little known about efflux pumps and in vivo resistance |
| Cholesterol import | • Import of exogenous cholesterol as substitute for ergosterol after azole treatment
• SrbA (sterol regulatory element binding protein) plays role in resistance |
| GOF mutation in HapE | • HapE is a CCAAT binding transcription factor complex subunit
• PB1L mutation in HapE complex & binding to CCAAT box in promoter region can induce CYP51A expression |

**Aspergillus Antifungal Resistance**

Two phenomena associated with resistance to antifungal agents in IFIs caused by *Aspergillus*

1. Secondary azole-resistance in *A. fumigatus* isolates

2. Increase in number of infections due to non-*A. fumigatus* isolates
   - Emerging species with primary resistance to azoles

Cryptic *Aspergillus* Species

- Leading causes of IA:
  - *A. fumigatus* (45%–60%)
  - *A. flavus* (6%–10%)
  - *A. terreus* (4%–10%)
  - *A. niger* (2%–9%)

- Molecular tools led to description of new species
  - Cryptic or sibling species – difficult to differentiate by classic means (phenotype/morphology alone)

  - *A. lentulus* (1.8%), *A. udagawae* (1.4%), *A. tubingensis* (2.8%), *A. calidoustus* (2.8%)

- **FILPOP** (Spain: Oct. 2010 & May 2011) – 14.5% cryptic species among 323 *Aspergillus* isolates
  - *A. lentulus* (1.1%), *A. alliaceus* (1.1%), *A. tubingensis* (7.9%), *A. calidoustus* (1.4%)

*Aspergillus* Section *Fumigati* (aka *A. fumigatus* Species Complex)

- 51 phylogenetically distinct species
  - Difficult to distinguish phenotypically
  - Unstable morphology
  - Identification requires molecular/proteinomic means
  - DNA sequences analysis (β-tubulin, calmodulin)
  - MALDI-TOF

*Aspergillus* Section *Fumigati*

At least 15 reported to have caused disease in humans

**Flavi**

- *A. flavus*
- *A. fumigatiaffinis*
- *A. fumigatus*
- *A. fumisynnematus*
- *A. lentulus*
- *A. novofumigatus*
- *A. parafelis*
- *A. pseudofelis*
- *A. pseudoviridinutans*
- *A. viridinutans*

- Reduced susceptibility to AMB and echinocandins

**Nidulantes**

- *A. nidulans*
- *A. delacroixii*
- *A. quadrilineatus*
- *A. falcisporioides*
- *A. phoenicisporioides*
- *A. pseudoviridinutans*
- *A. viridinutans*

- Variable AMB susceptibility & reduced azole susceptibility

**Nigri**

- *A. niger*
- *A. tubingensis*
- *A. citrinoterreus*

- Reduced AMB and azole susceptibility

**Terrei**

- *A. terreai*
- *A. alabamensis*
- *A. citrinoterreus*

- AMB and azole susceptibility

**Versicolors**

- *A. sydowi*
- *A. versicolor*

- Reduced susceptibility & reduced azole susceptibility

**Flavo**

- *A. flavus*
- *A. fumigatus*

- Reduced susceptibility to AMB and echinocandins

**Nidulantes**

- *A. nidulans*
- *A. obliquus*
- *A. qaudrirenatus*

- Variable AMB susceptibility

**Nigri**

- *A. niger*
- *A. tubingensis*

- Variable azole susceptibility

**Terrei**

- *A. terreai*
- *A. alabamensis*
- *A. citrinoterreus*

- AMB and azole susceptibility

**Versicolors**

- *A. sydowi*
- *A. versicolor*

- Reduced susceptibility & reduced azole susceptibility

*NOT A COMPREHENSIVE LIST!*
Mucormycosis

<table>
<thead>
<tr>
<th>Study</th>
<th>Prevalence</th>
<th>IMI Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRANSNET - SOT</td>
<td>2.3%</td>
<td>2</td>
</tr>
<tr>
<td>TRANSNET - Lung SOT</td>
<td>2.1%</td>
<td>3</td>
</tr>
<tr>
<td>TRANSNET - HSCT</td>
<td>8.0%</td>
<td>2</td>
</tr>
<tr>
<td>Johns Hopkins (HSCT &amp; SOT)</td>
<td>2.3 - 12.9%</td>
<td>2 &amp; 3</td>
</tr>
<tr>
<td>UT MD Anderson (Hematol. malignancies - autopsy)</td>
<td>11.5%</td>
<td>2</td>
</tr>
</tbody>
</table>

France 1.2 cases/million population/year
Spain 0.4 cases/million population/year
California 1.7 cases/million population/year


Causative Agents Mucormycosis

<table>
<thead>
<tr>
<th>Genera</th>
<th>Representative Species (not comprehensive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apophysomyces</td>
<td>A. elegans, A. trapeziformis, A. variabile</td>
</tr>
<tr>
<td>Cunninghamella</td>
<td>C. bertholletiae, C. achrotaile</td>
</tr>
<tr>
<td>Lichtheimia (formerly Absidia)</td>
<td>L. corymbifera, L. ornata, L. ramosa</td>
</tr>
<tr>
<td>Mucor</td>
<td>M. circinelloides f. circinelloides &amp; f. janssenii, M. mucedo</td>
</tr>
<tr>
<td>Rhizomucor</td>
<td>R. paucus</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>R. arrhizus, R. microsporus, R. oryzae</td>
</tr>
<tr>
<td>Saksenaea</td>
<td>S. erythrospora, S. oblongispora, S. vasiformis</td>
</tr>
</tbody>
</table>

France 1.2 cases/million population/year
Spain 0.4 cases/million population/year
California 1.7 cases/million population/year


Rhizopus arrhizus (oryzae)

- Major cause of mucormycosis in United States
  - 55% mucormycosis infections in PATH Alliance HSCT patients
  - Very aggressive; high morbidity & mortality (immunocompromised)
- Difficult to treat with limited options (increasing)
- R. arrhizus var. arrhizus & R. arrhizus var. delemar

Is Rhizopus delemar a Separate Species?
(R. arrhizus var. delemar OR R. delemar)

- Phylogenetically distinct
  - ITS & multilocus analysis
    - In agreement with other studies
- R. arrhizus able to produce lactic acid
- R. delemar lack idhA gene; cannot produce lactic acid
  - fumaric and malic acid

Abe et al. Mycologia 2007;99:714-722

Clinical Significance?

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate Number</th>
<th>VT-1161 HBC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. arrhizus var. arrhizus</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. delemar</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. arrhizus</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. delemar</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. arrhizus</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. delemar</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. arrhizus</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. delemar</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. arrhizus</td>
<td>&gt;32</td>
<td></td>
</tr>
<tr>
<td>R. arrhizus var. delemar</td>
<td>&gt;32</td>
<td></td>
</tr>
</tbody>
</table>

Antifungal

- Amphotericin B 0.42 0.39
- Itraconazole 0.78 0.91
- Posaconazole 0.44 0.91

Fungus Testing Laboratory Internal Results
(10 months data - 31 R. arrhizus var. arrhizus & 29 var. delemar)
**Scedosporiosis**

- Invasive infections primarily in immunocompromised hosts
  - Breakthrough infections in persistently neutropenic and/or lymphopenic hosts
  - Enters through sinopulmonary route & causes pulmonary infection; difficult to distinguish from invasive aspergillosis
- Common colonizers of cystic fibrosis patients
- Infections can occur in survivors of near-drowning events
  - Pulmonary infections with dissemination to the CNS

**Scedosporium Species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Antifungal Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F901318 Amphotericin Caspofungin Posaconazole Voriconazole</td>
<td></td>
</tr>
<tr>
<td>S. apiospermum (43)</td>
<td>0.079 3.40 5.70 1.94 1</td>
</tr>
<tr>
<td>S. boydii (15)</td>
<td>0.046 5.59 7.64 1.83 0.630</td>
</tr>
<tr>
<td>S. aurantiacum &amp; S. dehoogii (6 &amp; 2)</td>
<td>0.193 4.76 2.00 1.00</td>
</tr>
<tr>
<td>S. prolificans (1)</td>
<td>0.168 &gt;10 &gt;8 &gt;10 &gt;10</td>
</tr>
</tbody>
</table>

*Fothergill et al. ECCMID 2016.*

**Scedosporium Antifungal Activity**

**Fusariosis**

- Significant cause of morbidity and mortality in immunocompromised hosts
  - Usually invasive and disseminated (bloodstream)
  - Neutropenic patients with acute leukemia
  - Severe T-cell deficiency (corticosteroids for GVHD)
- Poor prognosis (although improving)


*F901318 Amphotericin Caspofungin Posaconazole Voriconazole
0.004 0.008 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32
MIC (mg/mL)
L. prolificans
Scedosporium Species
MALDI-TOF Dendrogram (FTL Internal Data)
**Fusarium Species**

Human infections can be caused by 8 species complexes

- **Fusarium solani** spp. Complex (FSSC)
- **Fusarium oxysporum** spp. Complex (FOSC)
- **Fusarium fujikuroi** spp. Complex (FFSC)
- **Fusarium chlamydosporum** spp. Complex (FCSC)
- **Fusarium dimerum** spp. Complex (FDSC)
- **Fusarium incarnatum-equiseti** spp. Complex (FIESC)
- **Fusarium sambucinum** spp. Complex (FSAMSC)
- **Fusarium tricinctum** spp. Complex (FTSC)

*Etiologic agents for majority of infections*

**Fusarium solani** Species Complex

- Composed of numerous haplotypes, many of which are now considered separate species
  - F. petrophilum (haplotype 1)
  - F. keratoplasticum (haplotype 2)
  - F. falciforme (haplotype 3+4)
  - F. solani (haplotype 5)
- Other species/haplotypes cause disease in animals & plants
  - Major plant pathogen
- Identification to species level requires MLST
  - EF1α, RPB2
  - [www.fusariumdb.org/](http://www.fusariumdb.org/)

Clinical relevance of different species in FSSC unknown (most clinical laboratories do not identify to this level)

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**Summary**

- Invasive mould infections significant causes of morbidity & mortality in immunocompromised patients
  - Aspergillus, Mucorales, Scedosporium, & Fusarium species
  - Other moulds can also cause disease in these populations

- Although new antifungals are available & under development, continued work needed due to resistance (intrinsic & acquired)

- Epidemiology & clinical understanding of causes of IMI infections changing due to use of molecular/proteinomic assays for species identification

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