MALDI-TOF MS Identification of Clinically Significant Yeasts and Moulds in a Global Antifungal Surveillance Program

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ABSTRACT

Background: The spectrum of opportunistic fungi causing invasive mycoses has increased, which makes accurate and rapid fungal identification (ID) essential in guiding appropriate therapy. We assessed the utility of MALDI-TOF MS versus PCR followed by sequencing (SQ) for the ID of moulds and uncommon yeasts in the SENTRY Antimicrobial Surveillance Program.

Methods: Among 2,190 fungal clinical isolates received during 2012, 443 strains from 68 hospitals had ID performed by MS and confirmed with SQ. Uncommon species of *Candida*, non-*Candida* yeasts and all moulds were tested on the MS Biotyper (Bruker Daltonics, Germany) after complete formic acid/acetonitrile extraction. A score value (SV) of ≥2 was considered to be a reliable ID. SQ was performed for one or more of the following genes: ITS, 26/28S, β-tubulin (*Aspergillus* spp.), TEF (*Fusarium* spp.), IGS1 (*Trichosporon* spp.), IGS (*C. neoformans* species complex [SC]).

Results: Overall, 318/416 (76.4%) fungi (154/216 yeasts and 164/200 moulds) produced a concordant ID compared to SQ at the species level. The following isolates were concordant within a SC not currently recognized by the Biotyper: one *C. duobushaemulonii* of the *C. haemulonii* SC, one *A. tubingensis* of the *A. niger* SC and four *A. sydowii* of the *A. versicolor* SC. Some species of yeasts had low strain representation in the library and gave a SV of <2 (*C. dubliniensis* 10/23 strains, *C. neoformans* 28/61, *R. mucilaginosa* 4/8). 16 yeasts had a SQ result not represented in the Bruker MS library (12 species including *C. fermentati*). 46 moulds had a SV of <2, including 26 giving an unreliable ID (SV ≤1.69). Only one mould had a discordant result compared to SQ; one *Scedosporium aurantiacum* gave a MS ID of *S. apiospermum*. 11 moulds (9 species) and two yeasts (*C. guilliermondii* and *C. neoformans*) repeatedly failed the manufacturer's extraction method (2.9%).

Conclusion: Use of the MS for ID of fungal isolates is an acceptable alternative to PCR-based methods due to its accuracy and speed. Manufacturer recommended methods may not be optimal for all strains. Strain representation in the MS Biotyper library is also a current limitation, but with the capability to build a custom library, we have been able to increase the strain representation of certain species and create a more robust MSP (Main SPectrum) library.

INTRODUCTION

Invasive fungal infections are a leading cause of morbidity and mortality in immunocompromised patients, and rapid and accurate identification (ID) of opportunistic fungi is critical for the management of antifungal therapy and the favorable prognosis of these patients. The ID of fungal pathogens is often troublesome and although clinical microbiology laboratories are able to provide an accurate ID for the more common yeast species, the emergence of less common fungal species causing opportunistic infections has challenged the identification systems that are based on phenotypic characteristics. However, uncommon *Candida* species and non-Candidal yeasts are increasingly recovered as causes of infection and methods based on the phenotypic characteristics of these organisms do not provide appropriate ID. Furthermore, the ID of filamentous fungi usually relies on the macroscopic and microscopic observation of colonies and adequate phenotypic ID of moulds requires highly skilled mycologists, who are found in a few reference laboratories, and prolonged testing intervals.

DNA sequencing is an accurate tool for fungal ID; however, few laboratories have the capability to perform such testing and while accurate, this methodology may add additional days to the turn-around time of results. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful diagnostic tool for accurate and rapid ID of bacteria and yeasts, and this method has recently been optimized for several filamentous fungi. In this study, we compared the accuracy of MALDI-TOF MS when compared to DNA sequencing based methods for 433 clinical fungal isolates submitted to a worldwide surveillance study during 2012.

MATERIALS AND METHODS

Organisms. Among the 2,190 viable fungal isolates received during the SENTRY Antimicrobial Surveillance Program in 2012, 443 moulds and uncommon yeast strains from 68 hospitals were identified by MALDI-TOF MS and DNA sequencing methods. Up to 40 fungal isolates per hospital were consecutively collected from bloodstream cultures or respiratory tract specimens of patients diagnosed with pneumonia caused by *Aspergillus* spp. or other moulds. Isolates were previously identified at participant institutions using methods routinely employed at the submitting laboratory. Purity of isolates was confirmed by subculturing the isolates on appropriate media followed by visual examination. Confirmatory ID and susceptibility testing were processed in a central monitory laboratory (JMI Laboratories, North Liberty, Iowa, USA).

DNA Sequencing Methods. DNA extractions were performed using QIAquick Extraction kit (Qiagen, Hilden, Germany; yeasts only) or UltraClean Microbial DNA Isolation kit (MO BIO Laboratories, Carlsbad, California, USA) and amplification and sequencing of the following genes: internal transcribed spacer (ITS) region, 28S ribosomal subunit, β-tubulin (*Aspergillus* only), translation elongation factor (TEF; *Fusarium* only), IGS (*Trichosporon* spp. or *Cryptococcus neoformans* species complex [SC]) were carried out as described previously. Nucleotide sequences were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared to available sequences through the internet using open access databases. TEF sequences were analyzed using *Fusarium*-ID database (http://www.isolate.fusariumdb.org/index.php) and the *Fusarium* multilocus sequence typing (MLST) database (http://www.chs.knaw.nl/fusarium/). Results were considered acceptable if homology was >99.5% with other entries in the databases used for comparison. Available sequences that were considerably different from the majority of entries for one species were considered outliers and discarded in the analysis. Additionally, if no match was found in the database, the ID was based on species complex, genus, family or order, according to the most current classifications systems.

MALDI-TOF MS. All yeasts were grown on Sabouraud dextrose agar (Remel Lenexa, Kansas, USA) and extracted using the complete extraction procedure as recommended by the manufacturer. Moulds were grown on potato dextrose agar slants (Remel) until mature and transferred to Sabouraud dextrose broth (Becton Dickinson, Maryland, USA). Inoculated broth tubes were placed on a rotator and incubated for 24-48 hours at 35°C or room temperature, as appropriate. Fungal material was centrifuged, and the pellet was washed twice with sterile water. The pellet was then suspended in water and ethanol, centrifuged and decanted. 50 µL of 70% formic acid was added, mixed and allowed to incubate (>10 minutes). Acetonitrile (Sigma Aldrich, St. Louis, Missouri, USA) was then added and centrifuged and the supernatant was applied to the target followed by the overlay of α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) in 50% acetonitrile and 2.5% trifluoroacetic acid (Sigma Aldrich) matrix.

MALDI-TOF MS identification results with score values (LogScore) ≥2.0 were considered to the species level. A score value from 1.7 to 1.9 was classified as genus level and <1.7 was considered an unreliable result. MALDI-TOF MS results of "no peaks found" after being submitted to multiple extraction procedures was considered final.

RESULTS

- Among 443 fungal isolates (218 yeasts and 225 moulds), DNA sequencing methods were able to produce an ID to species level for 216 (99.1%) yeasts and 200 (88.8%) moulds. Only one mould (0.4%; 0.2% overall) could not be identified to a genus level using DNA sequencing of at least two targeted genes.
- MALDI-TOF MS identified 154 yeasts isolates to species level with high confidence (LogScores >2.0; **Table 1**), including all isolates of 13 species (*Candida lusitaniae* [29 isolates], *C. kefyr* [14], *Trichosporon asahii* [14] and *C. pelliculosa* [eight] and other less common species; **Table 2**).
- Another 18 yeast species had variable MALDI-TOF MS results (**Table 2**) with 74 isolates displaying high scores/species ID (**Table 3**), and another 38 had lower scores (1.9 to 1.7) that were considered "Genus level ID". There were 24 isolates displaying an unreliable ID (<1.7) or that failed to produce MS spectra (one *C. neoformans var. grubii* and one *C. guilliermondii*).
- Among 57 *C. neoformans var. grubii* and five *C. neoformans var. neoformans*, 33 isolates were identified by the MALDI-TOF MS as *C. neoformans*. These isolates, along with one isolate each of *C. inconspicua*/*Pichia cactophila* and *C. duobushaemulonii* (**Table 3**) were considered to have MALDI-TOF MS acceptable results, although DNA sequencing was more specific.
- Two yeast isolates were not identified to species level by DNA sequencing, one *Candida* (98.0% ITS homology with *C. blankii*) and one *Cryptococcus* (99.2% 28S identity with *C. dimennae*) using 28S and ITS (IGS was attempted for *Cryptococcus* spp.) and had unreliable MALDI-TOF MS results.
- Among 225 moulds, 169 isolates had species level ID with MALDI-TOF MS and 164 had concordant results with DNA sequencing methods (**Table 1**). Four isolates showed species level ID with MALDI-TOF MS and genus only by DNA sequencing (two *Alternaria alternata*, one *Fusarium proliferatum* and one *Curvularia pallescens*; **Table 4**).
- Concordant MALDI-TOF MS and DNA sequencing genus/species results were observed for all *Aspergillus fumigatus* (107 isolates) and five other species (**Table 2**).
- One *Scedosporium aurantiacum* was identified by MALDI-TOF MS as *S. apiospermum* with high confidence (LogScore 2.454).
- Genus level only ID was achieved for 19 (8.4% overall) moulds by MALDI-TOF MS and among those three also had only genus level ID by DNA sequencing methods.
- A total of 37 (16.4% overall) moulds had unreliable ID by MALDI-TOF MS and among those, 18 had only genus level ID using DNA sequencing methods. Furthermore, 11 moulds could not be extracted using the procedure recommended by the manufacturer (five unspeciated using DNA sequencing).

Table 1. Summary MALDI-TOF MS results for 443 fungal isolates tested during a worldwide surveillance and comparison to DNA sequencing results ("gold standard").

Organism group (no. of isolates with DNA sequencing to species level [overall isolates tested])	MALDI-TOF MS results compared to DNA sequencing to species level (no. of isolates overall [%]; n=416[a])			Overall MALDI-TOF MS results (no. of isolates [%]; n=443)		
	Species level	Genus level	Unreliable ID or failed extraction for MALDI-TOF MS	Species level	Genus level	Unreliable ID or failed extraction
Yeasts (216 [218])	154 (71.3)	38 (17.6)	24 (11.1)	154 (72.6)	38 (17.4)	26 (11.9)
Moulds (200 [225])	164 (82.0)	17 (8.5)	19 (9.5)	169 (75.1)	19 (8.4)	37 (16.4)
Overall (416 [443])	318 (76.4)	55 (13.3)	43 (10.3)	323 (72.9)	61 (13.8)	59 (13.3)

a. Isolates that could not be identified to species level by DNA sequencing methods were not included in this analysis.

Table 2. Fungal species displaying all MALDI-TOF MS IDs in agreement with DNA sequencing methods.

Organism group (no. of isolates)	Organism ID by DNA sequencing and MALDI-TOF MS	No. of isolates (%) identified by MALDI-TOF MS to species level (LogScore, ≥2.0)
Yeasts (80)	*Candida lusitaniae*	29 (100.0)
	Candida kefyr	14 (100.0)
	Trichosporon asahii	14 (100.0)
	Candida pelliculosa	8 (100.0)
	Candida orthopsilosis	3 (100.0)
	Magnusiomyces capitatus	3 (100.0)
	Candida lipolytica	2 (100.0)
	Arthrographis kalrae	1 (100.0)
	Candida catenulata	1 (100.0)
	Lodderomyces elongisporus	1 (100.0)
	Malassezia pachydermatis	1 (100.0)
	Pichia manshurica	1 (100.0)
	Rhodotorula minuta	1 (100.0)
	Trichosporon mycotoxinivorans	1 (100.0)
Moulds (118)	*Aspergillus fumigatus*	107 (100.0)
	Aspergillus terreus	5 (100.0)
	Scedosporium apiospermum	3 (100.0)
	Penicillium citrinum	1 (100.0)
	Rhizopus oryzae	1 (100.0)
	Scedosporium prolificans	1 (100.0)

Table 3. Comparative results for DNA sequencing methods and MALDI-TOF MS for uncommon yeasts that displayed discordant results for these two methodologies.

Molecular ID (no. of isolates)[a]	MALDI-TOF MS ID (no. of isolates)	MALDI-TOF MS LogScore (no. of isolates [%]): ≥2.0 (Species level)	1.9 - 1.7 (Genus level)	≤1.69 (Unreliable ID)	No Peaks Found[b]
Cryptococcus neoformans var. grubii (57)	*Cryptococcus neoformans* (32), *Cryptococcus* spp. (19), unreliable ID/failed extraction (6)	32 (56.1)	19 (33.3)	5 (8.8)	1 (1.8)
Candida dubliniensis (23)	*Candida dubliniensis* (13), *Candida* spp. (10)	13 (56.5)	10 (43.5)	0	0
Candida guilliermondii (13)	*Candida guilliermondii* (12), failed extraction (1)	12 (92.3)	0	0	1 (7.7)
Saccharomyces cerevisiae (10)	*Saccharomyces cerevisiae* (9), *Saccharomyces* spp. (1)	9 (90.0)	1 (10.0)	0	0
Rhodotorula mucilaginosa (8)	*Rhodotorula mucilaginosa* (4), *Rhodotorula* spp. (4)	4 (50.0)	4 (50.0)	0	0
Cryptococcus neoformans var. neoformans (5)	*Cryptococcus neoformans* (1), *Cryptococcus* spp. (1), unreliable ID (3)	1 (20.0)	1 (20.0)	3 (60.0)	0
Candida fermentati (3)	Unreliable ID (3)	0	0	3 (100.0)	0
Candida fabianii (2)	Unreliable ID (2)	0	0	2 (100.0)	0
Candida inconspicua/*Pichia cactophila* (2)[c]	*Pichia cactophila* (2)	2 (100.0)	0	0	0
Cryptococcus gattii (2)	*Cryptococcus* spp. (2)	0	2 (100.0)	0	0
Geotrichum clavatum (2)	Unreliable ID (2)	0	0	2 (100.0)	0
Candida bracarensis (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Candida duobushaemulonii (1)[d]	*Candida haemulonii* (1)	1 (100.0)	0	0	0
Candida fluviatilis (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Candida rugosa (1)	*Candida* spp. (1)	0	1 (100.0)	0	0
Candida thasaenensis (1)	*Candida* spp. (1)	0	0	1 (100.0)	0
Candida thermophila (1)	*Candida* spp. (1)	0	0	1 (100.0)	0
Cryptococcus laurentii (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Cryptococcus cyanovorans (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Sporobolomyces nylandii (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Candida spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Cryptococcus spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0

a. Isolates underlined did not have a species level ID by DNA sequencing methods using 28S and ITS (IGS was attempted to *Cryptococcus* spp.).
b. The isolates did not produce a MS spectra after multiple extraction attempts (failed extraction).
c. DNA sequencing methods (ITS and 28S [D1/D2]) were not able to differentiate *Candida inconspicua* and *Pichia cactophila*.
d. *C. duobushaemulonii* and *C. haemulonii* are both part of the *C. haemulonii* species complex and very similar, thus although it was listed here, it was not considered a discordant result. Furthermore, *C. duobushaemulonii* was not included in the MALDI-TOF spectrum library.

Table 4. Comparative results for DNA sequencing methods and MALDI-TOF MS for moulds that displayed discordant results for these two methodologies.

Molecular ID (no. of isolates)[a]	MALDI-TOF MS ID (no. of isolates)	MALDI-TOF MS LogScore (no. of isolates [%]): ≥2.0 (Species level)	1.9 - 1.7 (Genus level)	≤1.69 (Unreliable ID)	No Peaks Found[b]
Aspergillus flavus SC (18)[c]	*Aspergillus flavus*/*oryzae* (14), *Aspergillus* spp. (3), failed extraction (1)	14 (77.8)	3 (16.7)	0	1 (5.5)
Aspergillus niger SC (13)	*Aspergillus niger* (12), *Aspergillus* spp. (1)	12 (92.3)	1 (7.7)	0	0
Aspergillus nidulans (6)	*Aspergillus nidulans* (4), unreliable ID/failed extraction (2)	4 (66.7)	0	1 (16.7)	1 (16.7)
Aspergillus terreus SC (5)	*Aspergillus terreus* (3), unreliable ID (2)	3 (60.0)	0	2 (40.0)	0
Aspergillus sydowii (5)[d]	*Aspergillus versicolor* (4), *Aspergillus* spp. (1)	4 (80.0)	1 (20.0)	0	0
Gibberella fujikuroi SC (4)	*Fusarium moniliforme* (1), *Fusarium* spp. (3)	2 (50.0)	2 (50.0)	0	0
Penicillium subgenus terverticillata (4)	*Penicillium chrysogenum* (1), failed extraction (3)	1 (25)	0	0	3 (75.0)
Rhizopus microsporus group (4)	*Rhizopus microsporus* (2), *Rhizopus* spp. (2),	2 (50.0)	2 (50.0)	0	0
Fusarium solani SC (3)	*Fusarium* spp. (1), unreliable ID (2)	0	1 (33.3)	2 (66.6)	0
Paecilomyces lilacinus (2)	*Paecilomyces lilacinus* (1), *Paecilomyces* spp. (1)	1 (50.0)	1 (50.0)	0	0
Aspergillus candidus (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Aspergillus clavatus (1)	*Aspergillus* spp. (1)	0	1 (100.0)	0	0
Aspergillus nomius (1)	*Aspergillus* spp. (1)	0	1 (100.0)	0	0
Aspergillus tubingensis (1)[e]	*Aspergillus niger* (1)	1 (100.0)	0	0	0
Aspergillus udagawae (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Cadophora malorum (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Epicoccum nigrum (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Fusarium incarnatum-*equiseti* SC (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Fusarium oxysporum SC (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Leptosphaerulina chartarum (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Lichtheimia ramosa (1)	*Lichtheimia* spp. (1)	0	1 (100.0)	0	0
Mucor circinelloides (1)	*Mucor ramosissimus*/*circinelloides* (1)	1 (100.0)	0	0	0
Penicillium subgenus aspergilloides (1)	*Penicillium glabrum* (1)	1 (100.0)	0	0	0
Penicillium subgenus biverticillata (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Sarocladium (*Acremonium*) *kiliense* (1)	*Acremonium* spp. (1)	0	1 (100.0)	0	0
Scopulariopsis brevicaulis (1)	Failed extraction (1)	0	0	0	1 (100.0)
Talaromyces (*Penicillium*) *verruculosus* (1)	*Penicillium* spp. (1)	0	1 (100.0)	0	0
Scedosporium aurantiacum (1)	*Scedosporium apiospermum* (1)[f]	1 (100.0)	0	0	0
Curvularia/*Cochliobolus* spp. (7)	***Curvularia pallescens* (1)[g]**, *Curvularia* spp. (2) unreliable ID/failed extraction (4)	1 (14.3)	2 (28.6)	2 (28.6)	2 (28.6)
Alternaria spp. (3)	***Alternaria alternata* (2)[g]**, failed extraction (1)	2 (66.6)	0	0	1 (33.3)
Corpinellus spp. (2)	Unreliable ID (2)	0	0	2 (100.0)	0
Penicillium spp. (2)	Unreliable ID (2)	0	0	2 (100.0)	0
Acremonium spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	
Cladosporium spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Cunninghamella spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Exophiala spp. (1)	Failed extraction (1)	0	0	0	1 (100.0)
Fonsecaea spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Fusarium spp. (1)	***Fusarium proliferatum* (1)[g]**	1 (100.0)	0	0	0
Hamigera spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Microascus spp. (1)	Unreliable ID (1)	0	0	1 (100.0)	0
Ochroconis spp. (1)	Failed extraction (1)	0	0	0	1 (100.0)
Phoma spp. (1)	*Phoma* spp. (1)	0	1 (100.0)	0	0
Unspeciated mold (1)	Unreliable ID (1)	0	0	1 (100.0)	0

a. Isolates underlined did not have a species level ID by DNA sequencing methods using two or more of the following genes: 28S, ITS, TEF, β-tubulin.
b. The isolates did not produce a MS spectra after multiple extraction attempts (failed extraction).
c. *A. flavus and A.oryzae* could not be differentiated by molecular or MALDI-TOF MS methodologies.
d. *A. sydowii* is part of the *A. versicolor* species complex.
e. *A. tubingensis* is part of the *A. niger* species complex.
f. Discordant compared with DNA sequencing based ID, but high confidence score for MALDI-TOF MS.
g. **Red/bolded** isolates had MALDI-TOF MS ID results to species level, but DNA sequencing methods produced only genus level ID with >99.5% confidence.

CONCLUSIONS

- Overall, MALDI-TOF MS was able to correctly identify 323 (72.7%; 72.6% yeasts, 75.1% moulds) fungal clinical isolates to species level and an additional 61 (13.8%; 17.4 and 8.4% for yeasts and moulds, respectively) to genus level only.
- In 13.3% (11.9 and 16.4% for yeasts and moulds, respectively) of the isolates MALDI-TOF MS failed to produce a result, and those strains were usually uncommon species/genus. In only one instance when the MALDI-TOF MS result gave an acceptable score value, was the species different (same genus) from that determined by DNA sequencing.
- ID of uncommon moulds was challenging and 25 isolates could not be identified with confidence to species level or at all (only one isolate) by DNA sequencing of two or more genes. The majority of these strains belonged to very uncommon species/genus.
- Strain representation with these uncommon fungal species is one limitation of the MALDI-TOF MS, but with the capability to create your own library and the efforts by the manufacturer to expand the current libraries predictably will reduce this problem in the future.

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REFERENCES

1. Buchan BW, Ledeboer NA (2013). Advances in identification of clinical yeast isolates by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 51: 1359-1366.
2. Cassagne C, Cella AL, Suchon P, Normand AC, Ranque S, Piarroux R (2013). Evaluation of four pretreatment procedures for MALDI-TOF MS yeast identification in the routine clinical laboratory. *Med Mycol* 51: 371-377.
3. Cassagne C, Ranque S, Normand AC, Fourquet P, Thiebault S, Planard C, Hendrickx M, Piarroux R (2011). Mould routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *PLoS One* 6: e28425.
4. Clinical and Laboratory Standards Institute (2008). *MM18-A. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline*. Wayne, PA: CLSI.
5. Pfaller MA, Woosley LN, Messer SA, Jones RN, Castanheira M (2012). Significance of molecular identification and antifungal susceptibility of clinically significant yeasts and moulds in a global antifungal surveillance program. *Mycopathologia* 174: 259-271.
6. Van Herendael BH, Bruynseels P, Bensaid M, Boekhout T, De Baere T, Surmont I, Mertens AH (2012). Validation of a modified algorithm for the identification of yeast isolates using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). *Eur J Clin Microbiol Infect Dis* 31: 841-848.