Candida haemulonii and Closely Related Species at 5 University Hospitals in Korea: Identification, Antifungal Susceptibility, and Clinical Features

Mi-Na Kim,[1] Jong Hee Shin,[4,6] Heungsup Sung,[1] Kyungwon Lee,[2] Eui-Chong Kim,[3] Namhee Ryoo,[7] Jin-Sol Lee,[4,6] Sook-In Jung,[5] Kyung Hwa Park,[5] Seung Jung Kee,[4,6] Soo Hyun Kim,[4,6] Myung Geun Shin,[4,6] Soon Pal Suh,[4,6] and Dong Wook Ryang[4,6]

[1]Department of Laboratory Medicine, Ulsan University College of Medicine, [2]Department of Laboratory Medicine, Yonsei University College of Medicine, and [3]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Departments of [4]Laboratory Medicine and [5]Internal Medicine, Chonnam National University Medical School, and [6]Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, Gwangju, and [7]Department of Laboratory Medicine, Keimyung University Medical School, Daegu, Republic of Korea

Background. *Candida haemulonii*, a yeast species that often exhibits antifungal resistance, rarely causes human infection. During 2004–2006, unusual yeast isolates with phenotypic similarity to *C. haemulonii* were recovered from 23 patients (8 patients with fungemia and 15 patients with chronic otitis media) in 5 hospitals in Korea.

Methods. Isolates were characterized using D1/D2 domain and ITS gene sequencing, and the susceptibility of the isolates to 6 antifungal agents was tested in vitro.

Results. Gene sequencing of the blood isolates confirmed *C. haemulonii* group I (in 1 patient) and *Candida pseudohaemulonii* (in 7 patients), whereas all isolates recovered from the ear were a novel species of which *C. haemulonii* is its closest relative. The minimum inhibitory concentration (MIC) ranges of amphotericin B, fluconazole, itraconazole, and voriconazole for all isolates were 0.5–32 μg/mL (MIC_{50}, 1 μg/mL), 2–128 μg/mL (MIC_{50}, 4 μg/mL), 0.125–4 μg/mL (MIC_{50}, 0.25 μg/mL), and 0.03–2 μg/mL (MIC_{50}, 0.06 μg/mL), respectively. All isolates were susceptible to caspofungin (MIC, 0.125–0.25 μg/mL) and micafungin (MIC, 0.03–0.06 μg/mL). All cases of fungemia occurred in patients with severe underlying diseases who had central venous catheters. Three patients developed breakthrough fungemia while receiving antifungal therapy, and amphotericin B therapeutic failure, which was associated with a high MIC of amphotericin B (32 μg/mL), was observed in 2 patients.

Conclusions. *Candida* species that are closely related to *C. haemulonii* are emerging sources of infection in Korea. These species show variable patterns of susceptibility to amphotericin B and azole antifungal agents.

Candida haemulonii is an infrequent cause of human infection [1–4]. Recently, Sugita et al. [5] isolated a strain that did not belong to—but that was phylogenetically close to—*C. haemulonii* from the blood of a patient; this strain was denoted *Candida pseudohaemulonii* sp. nov. In these previous studies, clinical isolates of *C. haemulonii* and *C. pseudohaemulonii* were resistant to both amphotericin B and fluconazole [3–5]. Because drug resistance is a major problem in the treatment of yeast infection, identification of clinical isolates of these species is very important. However, conventional yeast identification systems have failed to identify *C. haemulonii* or *C. pseudohaemulonii* isolates [3–5].

During a multiple-center surveillance study conducted in Korea, we identified unusual yeast isolates with phenotypic similarities to *C. haemulonii* in 5 of the 11 participating hospitals. During the period 2004–2006, a total of 27 isolates were recovered from 23 patients (8 had fungemia, and 15 had chronic otitis media). In this study, we characterized these isolates by sequencing the internal transcribed spacer region and the D1/D2 domain of the large-subunit rRNA gene, and we determined the in vitro susceptibility of the isolates to 6 antifungal agents. For the first time, we

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Reprints or correspondence: Dr. Jong Hee Shin, Dept. of Laboratory Medicine, Chonnam National University Medical School, 8 Hakdong Dongku, Gwangju 501–757, Republic of Korea (shinjh@chonnam.ac.kr).

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describe the antifungal resistance and relevant clinical features of *Candida* species closely related to *C. haemulonii.*

METHODS

Microorganisms. This study analyzed 27 isolates (12 isolates recovered from blood samples from 8 patients and 15 isolates recovered from middle ear specimens from 15 patients) that were collected during the period May 2004–December 2006 at 5 university hospitals in Korea. All isolates were identified as *C. haemulonii* using the Vitek 2 YST system (bioMérieux) and were then reidentified using conventional identification methods, including API 20C (bioMérieux). In addition, clinical information was collected retrospectively and included patient demographic characteristics, clinical diagnosis, presence of a central venous catheter, receipt of antifungal therapy, and outcome of fungemia [6, 7]. Therapeutic failure was defined either as the persistence of *Candida* in the bloodstream despite receipt of 3 days of antifungal therapy or as the development of breakthrough candidemia while receiving antifungal agents for 3 days [8, 9].

Sequencing the internal transcribed spacer region and D1/D2 domain. The first isolates from each of the 23 patients (isolates 1–23) were analyzed. The internal transcribed spacer region (including the 5.8S rRNA gene) and D1/D2 domain of the large-subunit rRNA gene were amplified using the primer pairs pITS-F and pITS-R [10] and NL1 and NL4 [11], respectively. Sequence similarity searches were performed using BLAST at the NCBI database (http://www.ncbi.nlm.nih.gov/blast). The D1/D2 domain sequences of 8 blood and 15 ear isolates have been deposited in GenBank (accession numbers EU881946–EU881953 for isolates 1–8 and EU881954–EU881968 for isolates 9–23, respectively; the internal transcribed spacer–region sequences were given accession numbers EU881969–EU881976 for isolates 1–8 and EU884176–EU884190 for isolates 9–23, respectively). Five representative isolates (isolates 1–3, 9, and 10) were deposited in the Korea Collection for Type Culture (Daejeon, Korea) as strains KCTC 17806–17810.

Antifungal susceptibility testing. The in vitro susceptibilities of the isolates to amphotericin B were determined using the Etest (AB Biodisk) according to the manufacturer's instructions [12]. Antifungal susceptibilities to fluconazole, itraconazole, voriconazole, caspofungin, and micafungin were tested using the standard methods of the Clinical and Laboratory Standards Institute [13, 14]. The interpretive susceptibility criteria used for fluconazole and itraconazole were those specified by the Clinical and Laboratory Standards Institute [13]. For voriconazole, we used the tentative interpretative breakpoints recently established for use with *Candida* species [15].

RESULTS

Species identification. The identification and antifungal susceptibilities of 27 isolates recovered from 23 patients are shown in table 1. The D1/D2 domain and internal transcribed spacer sequences of isolate 1 (from patient 1) demonstrated 99%–100% identity with those of reference strains of *C. haemulonii* group I available in GenBank, and this isolate was confirmed to be *C. haemulonii* group I. The blood isolates recovered from patients 2–8 were identical and were 100% homologous to the sequence of *C. haemulonii* DMST 17134, which has recently been designated *C. pseudohaemulonii.* All ear isolates (from patients 9–23) constituted a novel species that is closely related to *C. haemulonii.* All isolates recovered from the 23 patients were identified as *C. haemulonii* using the Vitek 2 YST yeast card system. With use of the API 20C system, the blood isolates recovered from patients 1–8 were identified as *Kodamaea ohmeri,* whereas the ear isolates recovered from patients 9–23 were identified as *Rhodotorula glutinis.*

Antifungal susceptibilities. The MIC of amphotericin B ranged from 0.5 to 32 μg/mL (MIC_{50}, 1 μg/mL) for all 27 isolates. High-level amphotericin B resistance (MIC, 32 μg/mL) was observed for blood isolates of *C. pseudohaemulonii* recovered from 5 patients (patients 2, 3, and 6–8). The MICs of fluconazole, itraconazole, and voriconazole for all 27 isolates were 2–128 μg/mL (MIC_{50}, 4 μg/mL), 0.125–4 μg/mL (MIC_{50}, 0.25 μg/mL), and 0.03–2 μg/mL (MIC_{50}, 0.06 μg/mL), respectively. Blood isolates of *C. haemulonii* from patient 1 and ear isolates from the 7 other patients were resistant to both fluconazole and itraconazole and showed decreased susceptibility to voriconazole (MIC, 0.5–1 μg/mL). All 27 isolates were susceptible to caspofungin (MIC, 0.125–0.25 μg/mL) and micafungin (MIC, 0.03–0.06 μg/mL) (table 1).

Clinical characteristics of the patients. The clinical characteristics of all 8 patients with fungemia are summarized in figure 1. All patients had severe underlying diseases and central venous catheters. Two patients (patients 1 and 2) had neutropenia. Four patients (50%) had previously received antifungal therapy, and breakthrough fungemia developed in 3 patients while they were receiving itraconazole (patient 1), fluconazole (patient 2), or both fluconazole and amphotericin B (patient 7). Of the 3 patients (patients 2, 4, and 5) who received amphotericin B therapy for fungemia, 2 experienced amphotericin B therapeutic failure. Patient 4, who had severe immunodeficiency, died despite having received 4 days of amphotericin B therapy; his isolate had an MIC of amphotericin B of 0.5 μg/mL. Patient 2, whose isolates were found to have an MIC of amphotericin B of 32 μg/mL, experienced persistent candidemia despite having received 12 days of amphotericin B therapy and finally died. Patient 5, however, whose isolate demonstrated an MIC of amphotericin B of 0.5–1 μg/mL, recovered after

Table 1. Results of identification and antifungal susceptibility testing for 27 isolates recovered from 23 patients.

Patient, isolate	Hospital	Source	Identification by D1/D2 domain and ITS sequence analysis	Vitek 2 YST result[a]	API 20C data		MIC, μg/mL					
					Result[a]	Code no.	AMB	FLU	ITR	VOR	Casp	Mica
1												
1	A	Blood	*Candida haemulonii* group I	*C. haemulonii* (89)	*Kodamaea ohmeri* (75.3)	6002174	1	64	4	1	0.125	0.06
1a	A	Blood	NT	*C. haemulonii* (89)	*K. ohmeri* (75.3)	6002174	2	64	4	1	0.125	0.06
2: 2	A	Blood	*Candida pseudohaemulonii*	*C. haemulonii* (95)	*K. ohmeri* (98.6)	6142136	32	2	0.25	0.03	0.125	0.06
3: 3	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (92)	*K. ohmeri* (99.9)	6142176	32	4	0.125	0.06	0.125	0.03
4: 4	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (93)	*K. ohmeri* (99.9)	6142176	0.5	2	0.125	0.06	0.125	0.03
5												
5	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (92)	*K. ohmeri* (99.9)	6142176	0.5	2	0.25	0.06	0.125	0.03
5a	B	Blood	NT	*C. haemulonii* (88)	*K. ohmeri* (99.9)	6142176	0.5	2	0.25	0.06	0.125	0.03
5b	B	Blood	NT	*C. haemulonii* (92)	*K. ohmeri* (99.9)	6142176	1	4	0.25	0.06	0.125	0.03
5c	B	Blood	NT	*C. haemulonii* (92)	*K. ohmeri* (99.9)	6142176	0.75	4	0.25	0.06	0.125	0.03
6: 6	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (92)	*K. ohmeri* (99.9)	6142176	32	4	0.125	0.06	0.125	0.03
7: 7	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (95)	*K. ohmeri* (81.7)	6002136	32	2	0.125	0.06	0.125	0.03
8: 8	B	Blood	*C. pseudohaemulonii*	*C. haemulonii* (97)	*K. ohmeri* (99.2)	6106136	32	2	0.125	0.06	0.125	0.03
9: 9	C	Ear	*Candida* sp. nov.[b]	*C. haemulonii* (98)	*Rhodotorula glutinis* (99.9)	6102073	1	128	2	1	0.125	0.03
10: 10	C	Ear	*Candida* sp. nov.	*C. haemulonii* (94)	*R. glutinis* (99.9)	2102073	1.5	128	2	0.5	0.25	0.06
11: 11	C	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.9)	6102073	0.75	8	0.5	0.125	0.125	0.03
12: 12	C	Ear	*Candida* sp. nov.	*C. haemulonii* (91)	*R. glutinis* (99.9)	6102073	1.5	128	2	2	0.25	0.03
13: 13	C	Ear	*Candida* sp. nov.	*C. haemulonii* (89)	*R. glutinis* (99.9)	6102073	0.5	128	2	1	0.25	0.03
14: 14	C	Ear	*Candida* sp. nov.	*C. haemulonii* (95)	*R. glutinis* (99.9)	6102073	0.38	64	1	0.5	0.125	0.03
15: 15	C	Ear	*Candida* sp. nov.	*C. haemulonii* (89)	*R. glutinis* (99.9)	6102073	0.38	64	2	1	0.25	0.03
16: 16	C	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.9)	6102073	0.75	32	1	0.25	0.25	0.03
17: 17	D	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.5)	2102073	1.5	4	0.125	0.03	0.125	0.03
18: 18	D	Ear	*Candida* sp. nov.	*C. haemulonii* (95)	*R. glutinis* (99.9)	6102073	1	4	0.125	0.03	0.125	0.03
19: 19	D	Ear	*Candida* sp. nov.	*C. haemulonii* (91)	*R. glutinis* (99.9)	2102173	1	2	0.25	0.03	0.125	0.03
20: 20	D	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.9)	6102073	1.5	4	0.125	0.03	0.125	0.03
21: 21	E	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.9)	2102173	1	2	0.125	0.03	0.25	0.03
22: 22	E	Ear	*Candida* sp. nov.	*C. haemulonii* (98)	*R. glutinis* (99.9)	6102073	0.75	4	0.125	0.06	0.25	0.03
23: 23	E	Ear	*Candida* sp. nov.	*C. haemulonii* (99)	*R. glutinis* (99.9)	6102073	1.5	64	4	2	0.25	0.03

NOTE. Antifungal MICs were determined using Etest (AB Biodisk) for amphotericin B (AMB) and the Clinical and Laboratory Standards Institute broth microdilution method for the remaining antifungals. Vitek 2 YST and API 20C were manufactured by bioMérieux. Casp, caspofungin; FLU, fluconazole; ITR, itraconazole; ITS, internal transcribed spacer region; Mica, micafungin; NT, not tested; VOR, voriconazole.

[a] Numbers in parenthesis are the probability of correct identification (as a percentage).

[b] All of the ear isolates (isolates 9–23) constituted a novel species that is closely related to *C. haemulonii*.

fluconazole therapy was switched to amphotericin B therapy. Therapeutic failure was observed in 6 patients (patients 1–5 and 7), and 3 patients (patients 2, 4, and 7) died of uncontrolled septicemia.

A novel species closely related to *C. haemulonii* was isolated from the ears of 15 patients (12 adult and 3 pediatric patients) who had chronic otitis media. All of these patients had a history of antibiotic use, and 7 had undergone surgery, including tympanoplasty, mastoidectomy, or ventilation tube insertion. Eight patients had a single positive culture result, and 5 of these cultures also grew *Candida parapsilosis* or *Staphylococcus aureus*. Persistent positive culture results occurred for 7 patients for periods lasting from 48 to 353 days. Direct microscopic examination also revealed the presence of yeasts and neutrophils in the ear specimens, although no histopathologic evidence of fungal infection was obtained in any of these patients. Of these 7 patients, 3 received antifungal therapy. The ear symptoms in 1 patient (patient 11) disappeared after 60 days of amphotericin B therapy (total dose, 3 g), whereas 2 patients (patients 9 and 10) who received fluconazole (for 3 days) or itraconazole (for 7 days) required follow-up as outpatients, because their ear symptoms did not improve.

DISCUSSION

To date, very little is known regarding the clinical characteristics and antifungal susceptibility profiles of clinical isolates of *Candida* species closely related to *C. haemulonii*. Through the characterization of 23 patient isolates recovered from 5 Korean hospitals during 2004–2006, we demonstrated the emergence of *C. haemulonii* (1 patient), *C. pseudohaemulonii* (7 patients), and a novel species closely related to *C. haemulonii* (15 patients)

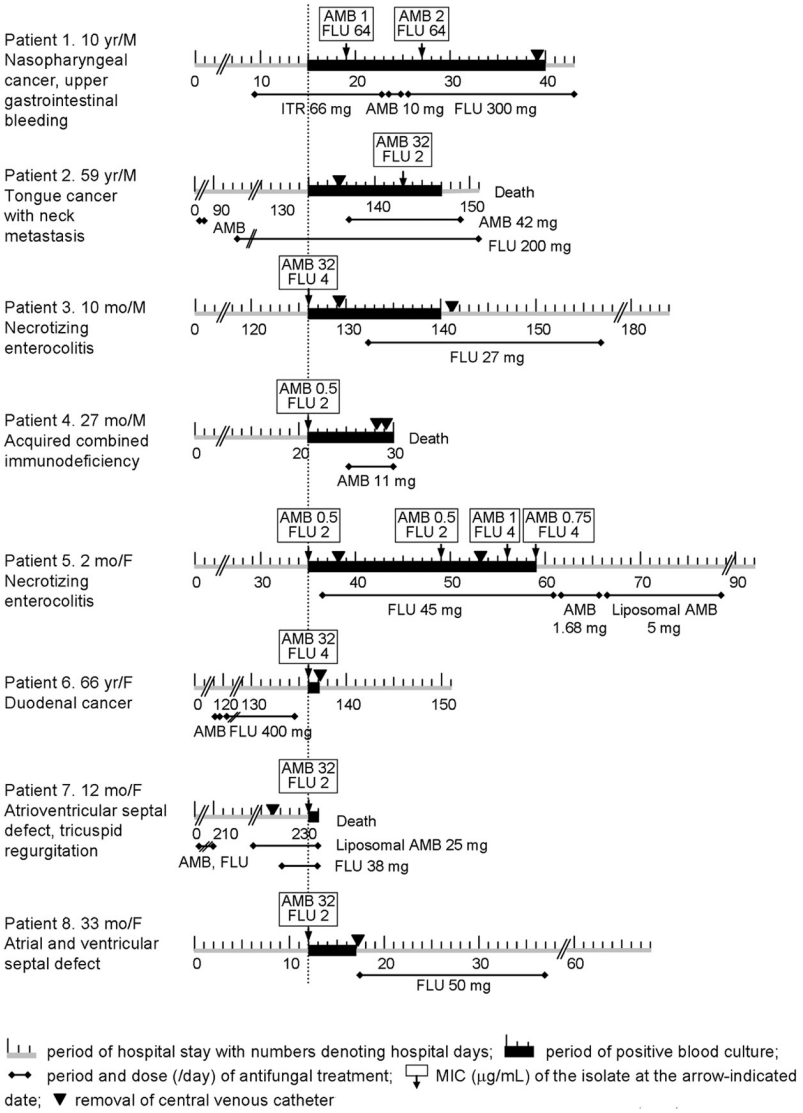

Figure 1. Treatment regimens, MICs for *Candida* isolates, and outcomes for 8 patients with fungemia due to *Candida haemulonii* (patient 1) or *Candida pseudohaemulonii* (patients 2–8). All patients had central venous catheters in place when the first positive culture results were obtained, and cultures of the catheter tips removed from 6 patients (patients 1, 3–6, and 8) yielded >15 colonies of the same *Candida* species. Therapeutic failure, which was defined either as persistence of *Candida* species in the bloodstream despite receipt of 3 days of antifungal therapy or as development of breakthrough candidemia during receipt of a 3-day course of antifungal therapy, was observed in 6 patients (patients 1–5 and 7). Of the 5 patients (patients 2, 3, 6, 7, and 8) whose isolates demonstrated high MICs of amphotericin B (AMB; 32 μg/mL), 2 patients (patients 2 and 7) received AMB therapy, and both patients experienced AMB therapeutic failure. F, female; FLU, fluconazole; ITR, itraconazole; M, male, mo, months; yr, years.

in Korea. During the study period, these species accounted for ~0.5% of 1601 cases of candidemia and 0.1% of 13,869 otitis media cases in Korean hospitals. These species are noteworthy for their variable antifungal susceptibility patterns.

In this study, all 23 patient isolates were misidentified by the API 20C system, but all were identified correctly at the species level via ITS2 and D1/D2 domain sequence analysis. The Vitek 2 YST system, which was introduced in Korea in 2006, identified all isolates as *C. haemulonii*. Therefore, in clinical microbiology laboratories, biochemical investigations using the Vitek YST system can be routinely applied to identify presumptive isolates of *C. haemulonii* and closely related species, although molecular diagnostic tools for accurate identification at the species level should also be employed.

In previous reports, all isolates of *C. haemulonii* and *C. pseudohaemulonii* were resistant to both amphotericin B and fluconazole [3–5]. However, we found that our isolates, which we recovered from 23 patients, demonstrated variable patterns of susceptibility to amphotericin B (MIC, 0.5–32 μg/mL) and fluconazole (MIC, 2–128 μg/mL). The fluconazole-resistant strains

recovered from 8 patients (35%) appeared to demonstrate azole cross-resistance (MIC of itraconazole, 1–4 μg/mL; MIC of voriconazole, 0.5–2 μg/mL). Although no antifungal resistance evolved in serial cultures of specimens obtained from the same patient, the genotyping results revealed that all ear isolates, for which the MIC of fluconazole was 2–128 μg/mL, had the same or similar genotypes (data not shown). These findings combined suggest that these species are innately less susceptible to fluconazole (MIC, ⩾2 μg/mL) than are most other species of *Candida* and that they may develop high-level resistance to fluconazole, similar to *Candida glabrata* [6].

Both *C. haemulonii* and *C. pseudohaemulonii* are reported to be susceptible to caspofungin and micafungin [4, 5]. In this study, for all 27 isolates, the MIC ranges for caspofungin and micafungin were 0.125–0.25 μg/mL and 0.03–0.06 μg/mL, respectively, suggesting that these 2 echinocandins are active against not only *C. haemulonii* isolates, but also against isolates of the 2 *Candida* species that are closely related to *C. haemulonii*.

Fungemia due to *C. pseudohaemulonii* is extremely rare; to our knowledge, there has been only 1 documented case [5]. In this article, we report 7 cases of *C. pseudohaemulonii* fungemia and 1 case of *C. haemulonii* fungemia identified at 2 hospitals in Korea. The most common underlying condition for the patients was central venous catheter use. Breakthrough fungemia developed in 3 patients, and therapeutic failure was observed in 6 patients. Therapeutic success was not observed when the patient was administered an antifungal drug to which the isolate was highly resistant. Of the 5 patients whose isolates had an MIC of amphotericin B of 32 μg/mL, 2 received amphotericin B therapy, and both experienced amphotericin B therapeutic failure: one patient (patient 7) developed breakthrough fungemia during amphotericin B therapy, and the other (patient 2) had persistent candidemia despite having received 12 days of amphotericin B therapy. This demonstrates that a high MIC of amphotericin B is associated with therapeutic failure.

It is difficult to judge the clinical significance of *Candida* isolates recovered from ear specimens, and confirmation generally requires histopathologic examination. Although the ear isolates were repeatedly detected in specimens from 7 patients, histopathologic proof of fungal infection was not obtained for any of these patients. Instead, 8 patients had only a single positive culture result, and some had mixed culture results, suggesting that this new species is a commensal in the middle ear. Although the specific reasons for ear involvement of the new species are not fully understood, we found that isolation of this species was often accompanied by frequent manipulation of the ear canal and the use of antibiotics. In addition, genotyping results for all 15 isolates from 3 hospitals suggest intra- and interhospital transmission of a clonal strain in Korea. Additional studies are needed to assess the role for this species in the development or maintenance of chronic otitis media.

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Potential conflicts of interest. All authors: no conflicts.

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