inhibitory concentration (MIC) distribution of the isolates against imipenem varied according to the MBL type. The MIC range of imipenem in the 38 *bla*$_{\text{IMP-1}}$-carrying isolates was 16–64 μg/mL, whereas the MICs of imipenem in the *bla*$_{\text{VIM-2}}$-carrying isolates were $\geq$128 μg/mL. Moreover, resistance to piperacillin, cephalosporins, aminoglycosides and ciprofloxacin was significantly higher in MBL producers than in MBL non-producers ($P < 0.05$). These results suggest that infection by MBL-producing *P. aeruginosa* severely compromises the selection of appropriate antimicrobial agents and may cause significant morbidity and mortality of infected patients.

Class 1 integrons were characterised to determine whether the MBL genes were inserted as a gene cassette. The *intI1* gene was detected in all MBL producers. All *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{VIM-2}}$ genes were inserted in the gene cassettes of class 1 integrons, as demonstrated by PCR mapping. Southern hybridisation showed that the *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{VIM-2}}$ genes were located on plasmids. This finding suggests that *P. aeruginosa* carrying MBL genes possibly transfer their MBL genes to other bacteria through horizontal transfer of plasmids.

Pulsed-field gel electrophoresis was performed to determine the clonal relatedness of MBL producers. Forty-seven MBL producers were classified into 40 pulsotypes at a similarity value of 0.85 (Fig. 1). Nine *bla*$_{\text{VIM-2}}$-carrying *P. aeruginosa* isolates were sporadically distributed in the dendrogram, suggesting that they originated from different clones. Among the 38 *bla*$_{\text{IMP-1}}$-carrying *P. aeruginosa* isolates, 25 isolates were each distributed in a single pulsotype and 13 isolates belonged to 6 pulsotypes. These results suggest that the prevalence of MBL-producing *P. aeruginosa* isolates is mainly due to the acquisition of MBL genes in *P. aeruginosa* isolates originating from different ancestors.

The present study is the first to demonstrate dissemination of the *bla*$_{\text{IMP-1}}$ gene among genetically unrelated *P. aeruginosa* in a South Korean hospital. Since the *bla*$_{\text{IMP-1}}$ and *bla*$_{\text{VIM-2}}$ genes could be transferred horizontally by plasmids, active surveillance is needed to prevent nationwide spread of the *bla*$_{\text{IMP-1}}$ gene among *P. aeruginosa* strains.

Funding: This study was supported by a grant from the Korean Health 21 R & D Project, Ministry of Health and Welfare, Republic of Korea (03-PJ1-CH03-0002).

Competing interests: None declared.

Ethical approval: Not required.

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doi: 10.1016/j.ijantimicag.2008.02.005

Antimicrobial activity of *Inula helenium* L. essential oil against Gram-positive and Gram-negative bacteria and *Candida* spp.

Sir,

In this work we report results regarding the in vitro antimicrobial activity of *Inula helenium* L. dried root extracts obtained by stepwise supercritical fluid extraction (SFE) and by hydrodistillation (HD). *Inula helenium* L. (Compositae family) is a perennial plant widely occurring in Europe and East Asia. Its oil is one of the richest sources of sesquiterpenoid lactones, which have strong anthelminthic activity and have the potential to induce detoxifying enzymes [1,2]. *Inula helenium* L. roots (C-040705130905) were purchased from Minardi (Bagnacavallo-Ravenna, Italy). Supercritical CO_2 extraction was performed in a laboratory apparatus equipped with a 400 cm^3 extraction vessel operated in the single-pass mode of passing CO_2 through the fixed bed of charged vegetable particles. Extraction was carried out in a semibatch mode: batch charging of vegetable matter and continuous flow solvent. HD was performed for 4 h in a circulatory Clevenger-type apparatus up to exhaustion of the oil contained in the matrix, which was the same material as used in the SFE.

A Hewlett-Packard (Palo Alto, CA) 5890 series II gas chromatograph was employed for gas chromatography–mass spectrometry (GC–MS) analysis. Multiresistant bacterial strains were isolated at the Department of Biomedical Science (Sassari University, Italy) from swab specimens of wounds, catheter tips and blood or urine culture. American Type Culture Collection (ATCC) strains were also tested. Bacteria were maintained and tested on nutrient agar and nutrient broth, and yeasts were maintained on Sabouraud dextrose agar and Sabouraud dextrose broth, all purchased from Difco Laboratories (Detroit, MI). Susceptibility testing (disk diffusion tests on Müller–Hinton agar) was performed on all bacterial strains with BBL™ Sensi-Discs™ (Becton Dickinson, Franklin Lakes, NJ). Determination of the minimum inhibitory concentration (MIC) of *I. helenium* essential oil was carried out using the broth microdilution method [3]. Dimethyl sulfoxide (DMSO) was used as a solvent control, whilst streptomycin and bifonazole were used as standard antibacterial and antifungal drugs. The essential oil of the roots of *I. helenium* L. consisted mainly of alantolactone, isoalantolactone and β-elemene. The total essential oil yield extracted using the supercritical technique was 1.7% by weight with respect to the material charged in the extractor. The oil isolated by HD gave a yield of ca. 1.0%. Detailed identification of and the area percentages of the compounds found in the hydrodistilled oil are reported in Table 1. The antimicrobial activities of *I. helenium* extracts are shown in Table 2. The MIC values range from 0.009 mg/mL to >14 mg/mL. HD and SFE oil extracts showed clear activity against *Bacillus cereus*, *Staphylococcus aureus* ATCC 29213 and an *Enterococcus faecium* clini-

Table 1
Retention time (T_R), Kovats index (I_K) and chromatographic area percentages of compounds found in *Inula helenium* L. oil extracted by hydrodistillation (HD) and using CO$_2$ under supercritical fluid extraction (SFE)

T_R	I_K	Chromatographic area %		Compound
		HD	SFE	
6.74	1004	–	0.1	1,8-Cineole
21.02	1362	1.5	1.9	β-Elemene
22.18	1387	0.2	0.3	(E)-Caryophyllene
23.47	1417	0.1	0.2	Epi-β-santalene
24.33	1438	0.2	0.3	Drima-7,9(11)-diene
24.54	1443	0.4	0.5	γ-Gurjunene
24.85	1450	0.2	0.3	γ-Himachalene
25.09	1456	1.0	1.5	Valencene
25.41	1463	0.4	0.7	α-Selinene
26.16	1481	–	0.2	β-Bisabolene
26.57	1490	–	0.1	Calamenene
28.68	1539	0.2	0.3	Germacrene B
28.87	1543	0.2	0.3	Ledol
29.87	1566	–	0.6	(Z)-Sesquilavandulol
30.85	1587	–	0.2	(E)-Sesquilavandulol
31.49	1601	0.5	0.6	β-Eudesmol
31.91	1613	0.4	0.4	Selin-11-en-4-α-ol
32.55	1632	–	0.4	Cadalene
32.96	1644	0.4	0.7	Acorenone
40.99	1867	51.3	42.3	Alantolactone
42.32	1904	36.9	35.4	Isoalantolactone

Table 2
Antimicrobial activity of *Inula helenium* L. oil extracted by hydrodistillation (HD) and using CO$_2$ under supercritical fluid extraction (SFE)

Strain	MIC (mg/mL)			
	HD	SFE	Streptomycin	Bifonazole
Enterococcus faecium[a]	0.12	0.12	0.01	N.D.
Enterococcus faecalis ATCC 24912	2.9	2.9	N.D.	N.D.
Staphylococcus aureus ATCC 29213	0.6	3.7	0.05	N.D.
Staphylococcus epidermidis[a]	3.7	14.8	0.06	N.D.
Bacillus cereus[a]	0.3	N.D.	0.06	N.D.
Escherichia coli ATCC 25922	14.8	14.8	0.02	N.D.
Acinetobacter baumannii[a]	0.017	N.D.	0.02	N.D.
Serratia marcescens[a]	14.8	N.D.	0.06	N.D.
Salmonella Typhimurium ATCC 14028	14.8	14.8	0.02	N.D.
Aeromonas sobria[a]	7.4	14.8	0.01	N.D.
Pseudomonas aeruginosa[a]	14.8	14.8	0.06	N.D.
Candida albicans ATCC 2091	0.07	0.07	N.D.	N.D.
C. albicans[a]	0.009	0.12	N.D.	0.02
C. albicans[a]	0.017	0.12	N.D.	0.02
C. albicans[a]	0.07	N.D.	N.D.	0.02
Candida glabrata[a]	0.12	0.12	N.D.	0.03
C. glabrata[a]	0.07	0.07	N.D.	0.03
C. glabrata[a]	0.017	0.07	N.D.	0.02
C. glabrata[a]	0.07	N.D.	N.D.	0.05
Candida parapsilosis[a]	0.12	0.12	N.D.	0.02
C. parapsilosis[a]	0.07	N.D.	N.D.	0.05
C. parapsilosis[a]	0.07	N.D.	N.D.	0.05
Candida tropicalis[a]	0.06	0.12	N.D.	0.05
C. tropicalis[a]	0.6	N.D.	N.D.	N.D.
C. tropicalis[a]	0.3	N.D.	N.D.	0.05

MIC, minimum inhibitory concentration; N.D., not determined.

[a] Clinical isolate.

cal strain resistant to ampicillin, erythromycin, penicillin and tetracycline (MIC > 0.03 mg/mL); *Staphylococcus epidermidis* was susceptible to 3.7 mg/mL of HD-extracted oil and to 14.8 mg/mL of SFE-extracted oil; the strain was isolated from a nasal swab and was resistant to ampicillin, cefalothin, erythromycin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole. *Aeromonas sobria* was resistant to ampicillin (0.192 mg/mL) and gentamicin (0.125 mg/mL). *Pseudomonas aeruginosa* was the least susceptible to *I. helenium* oil, with a MIC of 14.8 mg/mL. *Candida* strains were the most susceptible to *I. helenium* oil, with MIC values ranging from 0.009 mg/mL to 0.12 mg/mL. A comparison between the antimicrobial activity of extracts obtained with CO_2 in a supercritical state and HD suggests that the second technique is preferable to the first. HD extracts appear to be more active against *S. aureus* ATCC 29213, *S. epidermidis* and *A. sobria* as well as against different *Candida* spp. The HD oil exerted a much stronger bacteriostatic effect against yeasts, with MICs ranging from 0.6 mg/mL to 0.009 mg/mL. Comparing the in vitro antifungal activity of different essential oils, in particular that of tea tree and bergamot oil, *I. helenium* oil appears to have a stronger activity against *Candida* spp. [4]. Considering the different groups of chemical compounds present in *Inula helenium* oil, it is most likely that the antimicrobial activity is attributable to a synergism between components. Nevertheless, the best antimicrobial activity of essential oil extracted by HD could be due to the presence of the main constituents alantolactone and isoalantolactone. These two compounds have been reported to have significant activity against *Mycobacterium tuberculosis* [5]. Dorn et al. [6] reported that *I. helenium* oil has remarkable antineoplastic activity towards different tumour cell lines; however, few publications have documented its antimicrobial activity. In summary, our results suggest that *I. helenium* HD-extracted oil is more active against microorganisms than SFE-extracted oil. HD oil was more active against Gram-positive than Gram-negative bacteria. Furthermore, the most interesting activity of HD oil was against C*andida* spp., thus the oil may be useful in the clinical management of fungal infections. However, further studies on various fractions as well as clinical trials are required for preparing a possible phytoformulation to combat candidiasis.

Funding: Italian MIUR 40%.

Competing interests: None declared.

Ethical approval: Not required.

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doi: 10.1016/j.ijantimicag.2008.02.006

Diffuse cutaneous dissemination of visceral leishmaniasis during human immunodeficiency virus (HIV) infection, despite negligible immunodeficiency: repeated failure of liposomal amphotericin B administration, followed by successful long-term pentamidine and paromomycin administration

Sir,

An atypical episode of human immunodeficiency virus (HIV)-associated visceral leishmaniasis complicated by a prolonged course and a diffuse and non-specific cutaneous involvement was characterised by the absence of epidemiological clues and a lack of efficacy of repeated attack/maintenance cycles of liposomal amphotericin B despite a satisfactory cell-mediated immune response maintained throughout the entire case history, thanks to a concurrent, effective combination antiretroviral treatment. Only a very prolonged administration of the older pentamidine isethionate, associated with seven consecutive weeks of oral paromomycin, led to a very slow, but complete, cure both of visceral leishmaniasis and its related, long-lasting cutaneous dissemination, in the absence of untoward events and recurrences.

An acquired immune deficiency syndrome (AIDS) patient from Northern Italy with no history of travel through endemic