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Identification, characterization, and biofilm formation of clinical *Chryseobacterium gleum* isolates

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ABSTRACT

Chryseobacterium gleum is not commonly isolated from clinical source(s). Using 16S rRNA gene sequencing, we identified 15 *C. gleum* isolates from the Central Region Hospital Alliance, Taiwan, which were all misidentified: 14 as *Chryseobacterium indologenes* and 1 as *Elizabethkingia meningoseptica* using the Vitek 2 GN card. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a rapid and clinically applicable method, was evaluated for the identification of *C. gleum*, and the rate of species or probable species level identification reached 13.3% and 86.6%, respectively. Using pulsed-field gel electrophoresis analysis, all *C. gleum* isolates from central Taiwan were found to be epidemiologically unrelated. The most prevalent sample was urine (35.7%, 5/14), followed by sputum (28.6%, 4/14), whereas 1 isolate was from an unknown source. All of the isolates were susceptible to minocycline, 93.3% susceptible to trimethoprim/sulfamethoxazole, but were completely or highly resistant to the other drugs examined. Biofilm-forming ability was observed in 40.0% (6/15) isolates using the Luria–Bertani broth. To the best of our knowledge, this is the first focusing on exploring clinical *C. gleum* isolates.

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1. Introduction

Chryseobacterium (formerly *Flavobacterium*) is a glucose non-fermenting bacterium distributed in the soil, plants, and water. The inherent chlorine resistance in members of the *Chryseobacterium* genus possibly facilitates their spread in the hospital environment to cause nosocomial infections (Hsueh et al., 1996). In general, the most frequently encountered clinical *Chryseobacterium* species were *Chryseobacterium meningosepticum* (now named *Elizabethkingia meningoseptica*) and *Chryseobacterium indologenes* (Chen et al., 2013; Lin et al., 2010a). *C. indologenes* and 1 closely related species, *C. gleum*, formerly belonged to the *Flavobacterium* CDC group IIb (Yabuuchi et al., 1990). Both produced a deep yellow pigment and were negative for DNase production and lactose oxidation. Several studies concerning the clinical relevance of *C. indologenes* have been described (Afshar et al., 2013; Chen et al., 2013). *C. gleum* has been isolated from clinical specimens (Holmes et al., 1984; Yabuuchi et al., 1990); nevertheless, not much information is available on the clinical aspects of this bacterium.

Little is known about virulence factor(s) of the members of *Chryseobacterium*. It is found that the biofilm formation plays a role in the pathogenesis of *E. meningoseptica* and *C. indologenes* (Kodama et al., 2013; Lin et al., 2010a). It will be meaningful to detect the biofilm-forming potential of *C. gleum*.

In this study, 16S rRNA gene sequencing, Vitek 2 GN card, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) were used for the identification of clinical *C. gleum* isolates from central Taiwan, respectively. Giving the lack of informative data about *C. gleum* and its infection, the demographic information of patients, antibiogram, pulsed-field gel electrophoresis (PFGE) analysis, and biofilm formation of *C. gleum* isolates was also explored to obtain more information concerning *C. gleum* infection.

2. Materials and methods

2.1. Bacterial isolates and patients

The Central Laboratory of the Central Region Hospital Alliance (primarily including Taichung Hospital, Fong-Yuan Hospital, Changhwa Hospital, and Nantou Hospital) collected 15 *C. gleum* isolates from November 2007 to January 2011. The baseline demographic and clinical characteristics of all patients were retrospectively examined using the medical records of patients.

2.2. Identification by 16S rRNA gene sequencing and MALDI-TOF MS

Vitek 2 GN card (bioMerieux, Marcy l'Etoile, France) was used in the primary identification. For confirmation, 16S rRNA gene sequencing using primer pair (5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-

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GGYTACCTTGTTACGACTT-3′) was performed as a standard method (Chang et al., 2013).

MALDI-TOF MS was accomplished using the Microflex LT system in conjunction with the Bruker Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany) and then interpreted according to the manufacturer's instructions. In brief, a score of 2.300–3.000 represents a highly probable species identification; a score of 2.000–2.299 indicates a secure genus identification, probable species identification; a score of 1.700–1.999 indicates an identification to the genus level; a score of less than 1.700 is regarded as no identification.

2.3. PFGE analysis

The bacterial plug was prepared and the genomic DNA was digested with *Spe*I as previously described (Chang et al., 2013). The chromosome of *Salmonella enterica* serovar Braenderup H9812 digested with *Xba*I was used as the molecular marker (Hunter et al., 2005). PFGE was performed using the CHEF-DR III system (Bio-Rad, Hercules, USA). The Dice similarity coefficients were calculated, and dendrogram analysis was performed by unweighted pair group mean association using the GelComparII software (GelCompar II; Applied Maths NV, St-Martens-Latem, Belgium).

2.4. Biofilm analysis

The bacteria were subcultured several times on brain–heart infusion agar plates before performing the experiment. After washing, the bacteria were re-suspended in 3 different media (Luria–Bertani [LB], Tryptic soy broth [TSB], and M9 minimal medium [M9]) to McFarland 0.5, then diluted 100-fold with respective media. Subsequently, 125-μL aliquots were pipetted to a 96-well microtiter plate, incubated at 37 °C for 18 h, washed 3 times with phosphate buffer solution, and then stained using 0.25% crystal violet solution. All reactions were performed in triplicate. Any isolate whose absorption at OD_{570} was greater than 2-fold of blank was regarded as a biofilm former (Stepanovic et al., 2000). Two clinical *C. indologenes* strains, strain 64 and 39, in our collection were used as positive and negative control, respectively. For biofilm observation, plate assay (Harvey et al., 2007) was performed and viewed using scanning electron microscope (S-3000N; Hitachi High-Technologies Corporation, Tokyo, Japan).

2.5. Drug susceptibility testing

The MICs toward gentamicin, amikacin, ceftazidime, ceftriaxone, cefepime, chloramphenicol, ciprofloxacin, colistin, imipenem, piperacillin, piperacillin/tazobactam, minocycline, and trimethoprim/sulfamethoxazole were examined using Vitek 2 AST card (bioMérieux) for gram-negative bacilli. The CLSI criteria for other non-Enterobacteriaceae was used to interpret the data from drug susceptibility assays (CLSI, 2011).

3. Results

3.1. Identification of C. gleum

In this study, 16S rRNA gene sequencing was used as a standard method to confirm 15 *C. gleum* isolates, which were previously misidentified as 14 *C. indologenes* and 1 *E. meningoseptica* using the Vitek 2 GN card, with at least 97.4% probability (data not shown), by the clinical microbiology laboratory. According to our Vitek 2 database of *C. gleum*, *E. meningoseptica*, and *C. indologenes* strains confirmed by 16S rRNA gene sequencing (Supplementary Table 1), not a single reaction possesses the discriminatory power to differentiate *C. gleum* from *C. indologenes* and *E. meningoseptica*.

However, owing to the labor-intensive and time-consuming nature of 16S rRNA gene sequencing, there is an urgent need for an alternate molecular methodology. MALDI-TOF MS, which has been applied by clinical microbiology laboratories for the diagnosis of a variety of bacteria (Jacquier et al., 2011; Saffert et al., 2011), has not been evaluated for its feasibility to identify clinical *C. gleum* isolates. Using this method, the rate for *C. gleum* (score ≥2.300) and probable *C. gleum* (score between 2.000 and 2.299) identification was 13.3% (2/15) and 86.7% (13/15), respectively (Table 1).

Table 1
Comparison of Bruker Biotyper with Vitek 2 GN card for the identification of 15 *C. gleum* isolates.

Bruker Biotyper		Vitek 2	
Identification	Score	*C. indologenes*	*E. meningoseptica*
		Acceptable[a] (n = 14)	Acceptable (n = 1)
C. gleum	≥2.300	2	0
	2.000–2.299	12	1

[a] ≥85% identity.

3.2. Clinical features

The average age of patients was 61.2 ± 19.1 years (excluding 1 patient less than 1 year old and 1 patient age unknown) with 6 patients aged over 70 years. There were 6 patients with known underlying diseases (1 rectal cancer and cerebrovascular disease, 1 malignant spinal cord tumor, 1 lung cancer, 1 diabetes mellitus and cerebrovascular disease, 1 cerebrovascular disease, and 1 diabetes mellitus and chronic tracheitis) (data not shown), but it was difficult to gather the complete medical records of the remaining patients. Three patients had polymicrobial infection (1 patient with group B *Streptococcus*, 1 patient with *Stenotrophomonas maltophilia*, and 1 patient with *Acinetobacter baumannii* and *Staphylococcus aureus*) isolated from urine, wound, and ascitic fluid, respectively (data not shown). The primary sample sources were urine, sputum, and wound at a frequency of 35.7% (5/14), 28.6% (4/14), and 14.3% (2/14), respectively, whereas 1 isolate was from an unknown source (Table 2).

3.3. PFGE pattern

Based on the criteria of Tenover et al. (1995), the PFGE pattern of *C. gleum* isolates in this study demonstrated genetic diversity (Fig. 1). No

Table 2
Demographic and clinical characteristics of 15 patients with *C. gleum* infections.

Patient	Isolate	Hospital[a]	Age	Gender	Source	Clinical diagnosis
1	14	O	<1	M	Urine	Urinary tract infection
2	32	A	73	M	Sputum	Pneumonia
3	36	B	84	F	Ascitic fluid	Peritonitis
4	38	O	-[b]	-	-	-
5	44	B	41	M	Urine	Urinary tract infection
6	53	A	81	F	Urine	Urinary tract infection
7	61	A	50	F	Wound	Wound infection
8	63	A	79	F	CVC	Septicemia, Head trauma
9	65	O	23	-	Sputum	Pneumonia
10	69	A	83	M	Urine	Urinary tract infection
11	87	C	44	F	Wound	Bedsore
12	92	A	49	M	Urine	Urinary-tract infection
13	93	A	56	F	Sputum	Pneumonia
14	101	C	63	M	Blood	Septicemia
15	102	O	70	M	Sputum	Pneumonia

M = male; F = female; CVC = central venous catheter.
[a] A, B, and C: 3 different hospitals of the Central Region Hospital Alliance; O: samples from some central Taiwan clinics.
[b] Unknown.

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