



Differences in Clinical Manifestations, Antimicrobial Susceptibility Patterns, and Mutations of Fluoroquinolone Target Genes between *Chryseobacterium gleum* and *Chryseobacterium indologenes*

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ABSTRACT *Chryseobacterium* infections are uncommon, and previous studies have revealed that *Chryseobacterium gleum* is frequently misidentified as *Chryseobacterium indologenes*. We aimed to explore the differences in clinical manifestations and antimicrobial susceptibility patterns between *C. gleum* and *C. indologenes*. The database of a clinical microbiology laboratory was searched to identify patients with *Chryseobacterium* infections between 2005 and 2017. Species were reidentified using 16S rRNA gene sequencing, and patients with *C. gleum* and *C. indologenes* infections were included in the study. A total of 42 *C. gleum* and 84 *C. indologenes* isolates were collected from consecutive patients. A significant increase in *C. indologenes* incidence was observed. *C. gleum* was significantly more associated with bacteremia than *C. indologenes*. Patients with *C. gleum* infections had more comorbidities of malignancy and liver cirrhosis than those with *C. indologenes* infections. The overall case fatality rate was 19.8%. Independent risk factors for mortality were female sex and *C. indologenes* infection. These isolates were most susceptible to minocycline (73%), followed by trimethoprim-sulfamethoxazole (47.6%), tigecycline (34.1%), and levofloxacin (32.5%). *C. gleum* exhibited a significantly higher rate of susceptibility than *C. indologenes* to piperacillin, piperacillin-tazobactam, ceftazidime, tigecycline, and levofloxacin. Alterations in DNA gyrase subunit A were identified to be associated with fluoroquinolone resistance in *C. indologenes*. No nonsynonymous substitutions were observed in the quinolone resistance-determining regions (QRDRs) of *C. gleum*. Differences in epidemiology, clinical manifestations, and antimicrobial susceptibility patterns exist between *C. gleum* and *C. indologenes*. Additional investigations are needed to explore the significance of these differences.

KEYWORDS *Chryseobacterium gleum*, *Chryseobacterium indologenes*, drug resistance, quinolone resistance-determining region

Chryseobacterium, which normally exists in soil, water, and plants, is a genus of aerobic, Gram-negative, non-spore-forming, nonmotile, and nonfermenting, rod-shaped bacteria (1). These microorganisms are common pathogens of plants and animals but infrequently cause human infections (1). There are currently more than 100 species in the genus *Chryseobacterium* (2), among which *Chryseobacterium indologenes* is the most common species associated with human infections, followed by *Chryseobacterium gleum* (3–12). Nonetheless, *C. gleum* infections are substantially less frequently reported than infections with *C. indologenes* (3–12).

Our previous studies demonstrated that *C. indologenes* could be accurately identi-

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fied using traditional biochemically based phenotyping methods and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (13, 14). In contrast, *C. gleum* was not reliably identified using commercial biochemically based phenotyping identification or Vitek MS systems (bioMérieux, Marcy l’Etoile, France) (13, 14). Moreover, these microbial identification systems usually misidentified *C. gleum* as *C. indologenes*. Overall, accurate identification of *Chryseobacterium* species relies on 16S rRNA gene sequencing or the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonics GmbH, Bremen, Germany) (14, 15). Therefore, previous studies describing the epidemiology, clinical manifestations, virulence, pathogenesis, and antimicrobial susceptibilities of *C. indologenes* might have substantial bias if the identification was based on inaccurate methods.

Moreover, because the species in previous reports might not have been correctly identified, the actual clinical and molecular manifestations of *C. gleum* and *C. indologenes* remain unknown. To explore the exact epidemiology, clinical manifestations, and antimicrobial susceptibility patterns of *C. gleum* and *C. indologenes*, we apply 16S rRNA gene sequencing in this study to identify *Chryseobacterium* species and then compare clinical characteristics and antimicrobial susceptibility patterns between *C. gleum* and *C. indologenes* isolates causing human infections. We also investigate the contributions of mutations in the quinolone resistance-determining regions (QRDRs) to fluoroquinolone resistance in clinical isolates of *C. gleum* and *C. indologenes*.

RESULTS

Species identification. During the 13-year period of investigation, 156 nonduplicated *Chryseobacterium* isolates collected from consecutive patients were found in the database of the clinical microbiology laboratory. Seven isolates died after thawing, and 11 could not be identified to the species level using 16S rRNA gene sequencing. Ultimately, 138 isolates were identified to the species level, including 42 *C. gleum* isolates (type strain ATCC 35910^T; GenBank accession no. [GL379781](#)), 84 *C. indologenes* isolates (type strain DSM 16777^T; GenBank accession no. [LN681561](#)), 4 *C. arthrosphaerae* isolates (type strain CC-VM-7^T; GenBank accession no. [NR_116977](#)), 2 *C. culicis* isolates (type strain R4-1A^T; GenBank accession no. [FN554975](#)), 2 *C. cucumeris* isolates (type strain GSE06^T; GenBank accession no. [KX146463](#)), 2 *C. bernardetii* isolates (type strain CDC G229^T; GenBank accession no. [JX100816](#)), 1 *C. artocarp* isolate (type strain UTM-3^T; GenBank accession no. [KF751867](#)), and 1 *C. daecheongense* isolate (type strain CPW406^T; GenBank accession no. [AJ457206](#)).

Site of isolation. Among the 126 isolates of *C. gleum* and *C. indologenes*, 68 (54%) were collected from blood, 15 (11.9%) were from bile, 10 (7.9%) were from the tip of a central venous catheter, and 8 (6.3%) were from urine (Fig. 1A). *C. gleum* was significantly more prone to be isolated from blood than *C. indologenes* (66.7% for *C. gleum* versus 47.6% for *C. indologenes*; odds ratio [OR], 2.2; 95% confidence interval [CI], 1.02 to 4.76; $P = 0.043$). There was no statistically significant difference between the prevalences of these two species regarding the other sites of isolation.

Incidence of infection. The number of *Chryseobacterium* isolates and the incidence (number of cases per 100,000 admissions) distributed by the different years are shown in Fig. 1B. A significant increase in the incidence of *Chryseobacterium* infection was observed during the study period ($P = 0.043$). When the incidence of each species was calculated independently, a significant year-over-year increase was observed for *C. indologenes* ($P = 0.038$) but not for *C. gleum* ($P = 0.193$).

Clinical characteristics of *Chryseobacterium* infections. Of the 126 consecutive patients with *Chryseobacterium* infections, male predominance (68.3%) was observed (Table 1). The median age was 59.5 years (range, 13 to 91 years; mean \pm standard deviation, 59 \pm 19.2 years). The majority (81.7%) of patients were found to have at least one comorbidity, with the most common being cardiovascular diseases (46.8%), followed by diabetes mellitus (38.1%), malignancy (33.3%), and liver cirrhosis (15.9%). Health care-associated infection accounted for 99.2%. One-third of the patients presented with shock, and 35.7% of patients were admitted to intensive care units.

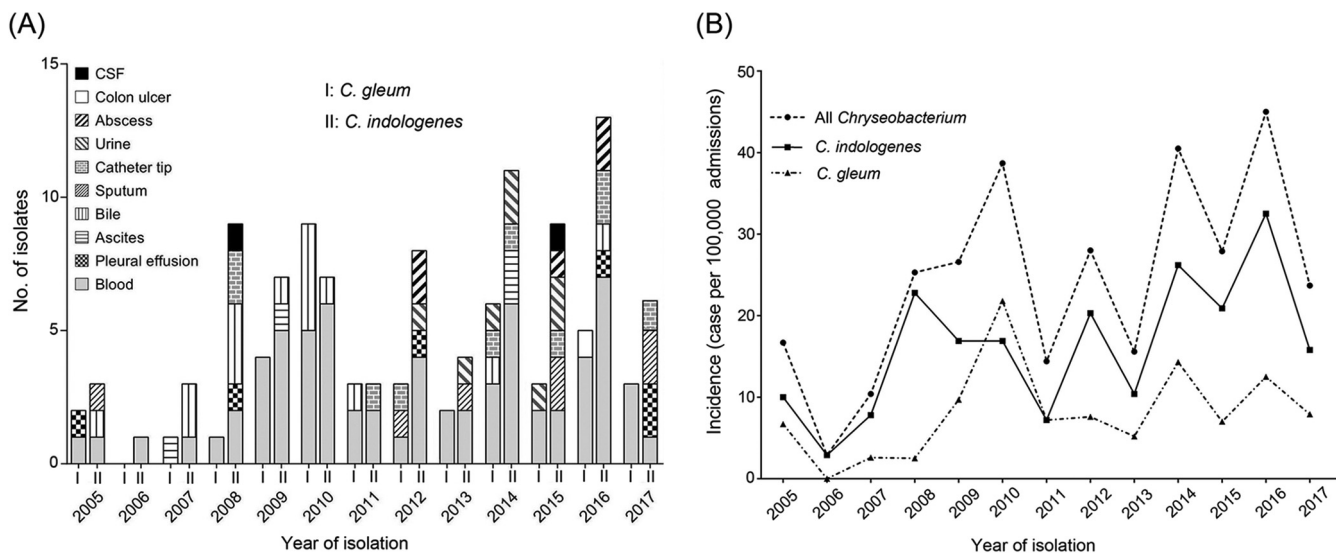


FIG 1 (A) Isolation sites of *C. gleum* and *C. indologenes* from 2005 to 2017. Blood was the most common site of isolation, followed by bile, the tip of a central venous catheter, and urine. (B) The incidence of *Chryseobacterium* from 2005 to 2017. A significant increase in the incidence of all *Chryseobacterium* isolates (chi-square for trend; $\chi^2 = 4.1$; $P = 0.043$) and *C. indologenes* ($\chi^2 = 4.32$; $P = 0.038$) was recognized. The incidence of *C. gleum* did not significantly increase during the study period ($\chi^2 = 1.7$; $P = 0.193$).

Empirical antibiotics administered included β -lactams (52.4%), β -lactam/lactamase inhibitors (46%), fluoroquinolones (50%), carbapenems (11.1%), aminoglycosides (14.3%), tigecycline (9.5%), and trimethoprim-sulfamethoxazole (7.1%), either singly or in combination. Inappropriate empirical antimicrobial therapy was recognized in 88.9% of patients. The overall mortality rate of those with *Chryseobacterium* infections was 19.8%.

Comparison of *C. gleum* and *C. indologenes* infections. When comparing patients with *C. gleum* and *C. indologenes* infections, we found no significant differences in the age of onset, sex, or presence of diabetes mellitus, cardiovascular disease, end-stage renal disease, and chronic obstructive pulmonary disease. However, the prevalence of malignancy (OR, 3; 95% CI, 1.37 to 6.55; $P = 0.005$) and of liver cirrhosis (OR, 4.93; 95% CI, 1.79 to 13.58; $P = 0.001$) was significantly higher in patients with *C. gleum* infections than in those with *C. indologenes* infections. There was no significant difference in inappropriate empirical antimicrobial therapies between these two groups. Patients with *C. gleum* infections had a significantly lower risk of shock (OR, 0.22; 95% CI, 0.09 to 0.58; $P = 0.001$), rate of admission to an intensive care unit (OR, 0.15; 95% CI, 0.05 to 0.42; $P < 0.001$), and case fatality rate (OR, 0.32; 95% CI, 0.1 to 0.99; $P = 0.04$) than those with *C. indologenes* infections (Table 1).

Factors associated with mortality. Univariate analysis showed that female patients generally and all patients with *C. indologenes* infection had higher mortality rates. Moreover, multivariate logistic regression analysis revealed that female sex (adjusted OR, 5.34; 95% CI, 2.04 to 13.97; $P = 0.001$) and *C. indologenes* infection (adjusted OR, 4.01; 95% CI, 1.2 to 13.38; $P = 0.024$) were independent risk factors for mortality in these *Chryseobacterium*-infected patients (Table 2).

Antimicrobial susceptibility testing. The MICs and susceptibilities of *Chryseobacterium* bacteria are presented in Table 3. The MICs of β -lactams, β -lactam/ β -lactamase inhibitors, carbapenems, and aminoglycosides were generally high. Furthermore, almost all *Chryseobacterium* bacteria showed little susceptibility to aminoglycosides, carbapenems, aztreonam, and tetracycline. These isolates were most susceptible to minocycline (73%), followed by susceptibility to trimethoprim-sulfamethoxazole (47.6%), tigecycline (34.1%), levofloxacin (32.5%), piperacillin-tazobactam (19.8%), and piperacillin (19%).

Analysis of the MICs and susceptibilities of each species revealed that the MIC₅₀ values of minocycline, tigecycline, ciprofloxacin, levofloxacin, and trimethoprim-

TABLE 1 Comparison of demographic characteristics, clinical information, and outcomes of patients with *C. gleum* and *C. indologenes* infections

Parameter	Value for the parameter			OR (95% CI) ^a	P value
	Total group (n = 126)	<i>C. gleum</i> (n = 42)	<i>C. indologenes</i> (n = 84)		
Sex (no. of patients [%])					
Male	86 (68.3)	27 (64.3)	59 (70.2)	0.76 (0.35–1.67)	0.499
Female	40 (31.7)	15 (35.7)	25 (29.8)	1.31 (0.6–2.88)	0.499
Age (yr)					
Range	13–91	20–91	13–90		
Median	59.5	58.5	62		
Mean ± standard deviation	59 ± 19.2	58.8 ± 16.5	59.1 ± 20.4		0.913
Comorbidity (no. of patients [%])					
Diabetes mellitus	103 (81.7)	36 (85.7)	67 (79.8)	1.52 (0.55–4.2)	0.415
Cardiovascular disease	48 (38.1)	13 (31)	35 (41.7)	0.63 (0.29–1.38)	0.243
Cardiovascular disease	59 (46.8)	17 (40.5)	42 (50)	0.68 (0.32–1.44)	0.313
End-stage renal disease (no. of patients [%])	5 (4)	1 (2.4)	4 (4.8)	0.49 (0.05–4.51)	0.664
Malignancy	42 (33.3)	21 (50)	21 (25)	3 (1.37–6.55)	0.005
Liver cirrhosis	20 (15.9)	13 (31)	7 (8.3)	4.93 (1.79–13.58)	0.001
Chronic obstructive pulmonary disease	10 (7.9)	2 (4.8)	8 (9.5)	0.48 (0.1–2.34)	0.494
Type of infection acquisition (no. of patients [%])					
Community-acquired infection	1 (0.8)	0	1 (1.2)		0.999
Health care-associated infection	125 (99.2)	42 (100)	83 (98.8)	1.51 (1.33–1.71)	0.999
Laboratory data					
White blood cell count (cells/mm ³)	14,039 ± 39,343	9,578 ± 5,697	16,270 ± 47,959		0.37
Hemoglobin (g/dl)	10.4 ± 2.3	10.8 ± 2.1	10.2 ± 2.4		0.187
Platelet count (×10 ³ cells/mm ³)	198,629 ± 120,124	212,905 ± 108,507	191,492 ± 125,542		0.348
Serum creatinine (mg/dl)	1.7 ± 1.5	1.6 ± 1.7	1.8 ± 1.4		0.487
Shock (no. of patients [%])	42 (33.3)	6 (14.3)	36 (42.9)	0.22 (0.09–0.58)	0.001
Admission to intensive care unit (no. of patients [%])	45 (35.7)	5 (11.9)	40 (47.6)	0.15 (0.05–0.42)	<0.001
Inappropriate empirical antimicrobial therapy (no. of patients [%])	112 (88.9)	38 (90.5)	74 (88.1)	1.28 (0.38–4.37)	0.688
Outcome					
Case fatality (no. [%])	25 (19.8)	4 (9.5)	21 (25)	0.32 (0.1–0.99)	0.04

^aOR, odds ratio; CI, confidence interval.

sulfamethoxazole for *C. gleum* were generally lower than those for *C. indologenes*. *C. gleum* was most susceptible to minocycline (83.3%), levofloxacin (59.5%), trimethoprim-sulfamethoxazole (57.1%), and tigecycline (52.4%). *C. indologenes* was also most susceptible to minocycline (67.9%), followed by susceptibility to trimethoprim-sulfamethoxazole (42.9%), tigecycline (25%), and levofloxacin (19%). *C. gleum* expressed a significantly higher susceptibility rate than *C. indologenes* to piperacillin (OR, 3.7; 95% CI, 1.47 to 9.29; $P = 0.004$), piperacillin-tazobactam (OR, 3.32; 95% CI, 1.35 to 8.18; $P = 0.007$), ceftazidime (OR, 3.44; 95% CI, 1.2 to 9.83; $P = 0.017$), tigecycline (OR, 3.3; 95% CI, 1.51 to 7.21; $P = 0.002$), and levofloxacin (OR, 6.25; 95% CI, 2.75 to 14.22; $P < 0.001$).

Mutations in the QRDRs. No nonsynonymous substitutions were observed in the QRDRs of GyrA, GyrB, ParC, or ParE in any isolate of *C. gleum*. However, amino acid alternations were recognized at positions 83 and 87 in GyrA and at position 66 in ParC of *C. indologenes* (Table 4). Four different amino acids were identified at position 83 in GyrA of *C. indologenes*, namely, serine ($n = 24$), phenylalanine ($n = 4$), valine ($n = 12$), and tyrosine ($n = 44$). Isolates of *C. indologenes* with serine at position 83 in GyrA were significantly more susceptible to fluoroquinolones ($P < 0.001$); in contrast, isolates with tyrosine at position 83 in GyrA were less susceptible to fluoroquinolones ($P < 0.001$). There were also four different amino acids at position 87 in GyrA of *C. indologenes*. Isolates with aspartate at position 87 in GyrA were significantly associated with levo-

TABLE 2 Factors associated with mortality in patients with *Chryseobacterium* infections

Factor	Outcome (no. of patients [%])		Univariate analysis		Multivariate analysis	
	Death (n = 25)	Survival (n = 101)	OR (95% CI) ^a	P value	OR (95% CI)	P value
Isolate						
<i>C. gleum</i>	4 (16)	38 (37.6)	0.32 (0.1–0.99)	0.04	0.25 (0.08–0.83)	0.024
<i>C. indologenes</i>	21 (84)	63 (62.4)	3.17 (1.01–9.93)	0.04	4.01 (1.2–13.38)	0.024
Age of ≥65 yr	13 (52)	39 (38.6)	1.72 (0.71–4.16)	0.224	1.17 (0.38–3.66)	0.785
Gender						
Male	10 (40)	76 (75.2)	0.22 (0.09–0.55)	0.001	0.19 (0.07–0.49)	0.001
Female	15 (60)	25 (24.8)	4.56 (1.82–11.43)	0.001	5.34 (2.04–13.97)	0.001
Underlying disease						
Diabetes mellitus	11 (44)	37 (36.6)	1.36 (0.56–3.3)	0.497	0.66 (0.23–1.93)	0.446
Cardiovascular disease	14 (56)	45 (44.6)	1.58 (0.66–3.83)	0.305	0.86 (0.28–2.61)	0.784
End-stage renal disease	2 (8)	3 (3)	2.84 (0.45–17.99)	0.258	1.44 (0.17–12.21)	0.74
Malignancy	5 (20)	37 (36.6)	0.43 (0.15–1.25)	0.114	0.85 (0.25–2.85)	0.787
Liver cirrhosis	2 (8)	18 (17.8)	0.4 (0.09–1.86)	0.36	0.87 (0.15–5)	0.876
Chronic obstructive pulmonary disease	3 (12)	7 (6.9)	1.83 (0.44–7.65)	0.414	2.13 (0.43–10.48)	0.352
Inappropriate empirical antimicrobial therapy	21 (84)	91 (90.1)	0.58 (0.17–2.02)	0.385	0.53 (0.13–2.1)	0.364

^aOR, odds ratio; CI, confidence interval.

floxacin ($P < 0.001$) and ciprofloxacin ($P = 0.001$) susceptibility, but those with tyrosine at position 87 in GyrA were significantly less susceptible to levofloxacin ($P < 0.01$) and ciprofloxacin ($P = 0.018$). Despite the amino acid alteration at position 66 in ParC of *C. indologenes*, no significant difference in the fluoroquinolone susceptibility was observed.

DISCUSSION

As stated above, *Chryseobacterium* infections in humans, particularly *C. gleum* infections, are rarely reported. By using 16S rRNA gene sequencing as a reference method, we have identified 42 *C. gleum* and 84 *C. indologenes* isolates as well as 12 isolates of six uncommon *Chryseobacterium* species over the past 13 years. We reported the clinical features of six rarely observed *Chryseobacterium* species in our previous study (14). It is noteworthy that *C. gleum* accounted for approximately one-third of all *Chryseobacterium* bacteria isolated from clinical specimens, suggesting that the incidence of *C. gleum* infections in humans is substantially underestimated.

According to previous studies, *Chryseobacterium* commonly causes bacteremia, catheter-related infection, and pneumonia, and these infections are usually hospital acquired (3–11). Indeed, a previous study found that 47% (17/36) of *C. indologenes*-infected patients developed bacteremia (7), and in a study of 215 *Chryseobacterium* isolates (4), 64.2% and 18.1% were obtained from sputum and blood, respectively. Our current study also showed that patients infected with *Chryseobacterium*, particularly *C. gleum*, are prone to bacteremia.

Patients with *Chryseobacterium* infections usually have comorbidities, such as cardiovascular disease, malignancy, chronic kidney disease, diabetes mellitus, and chronic obstructive pulmonary disease (3–11). In our study, one or more comorbidities were recognized in 81.7% of *Chryseobacterium*-infected patients. Furthermore, patients infected with *C. gleum* had a significantly higher prevalence of malignancy and liver cirrhosis than those with *C. indologenes* infections. The host-pathogen interaction between humans and *Chryseobacterium* requires further investigation.

Chryseobacterium infection had a high mortality rate, with a reported overall rate of 13.9% to 40.7% (3–8). Nevertheless, research to date has not employed an accurate microbial identification method to assess the mortality of patients with *C. gleum* and *C. indologenes* infections. In our study, the overall case fatality rate of patients with *Chryseobacterium* infections was 19.8%, with the rate of *C. gleum* infection being much

TABLE 3 The MIC and susceptibility of *Chryseobacterium* in this study

Drug	All <i>Chryseobacterium</i> isolates (n = 126)												P value ^c
	<i>C. gleum</i> (n = 42)				<i>C. indologenes</i> (n = 84)				OR (95% CI) ^b	S (no. [%])			
	MIC ₅₀	MIC ₉₀	S (no. [%]) ^a	MIC ₅₀	MIC ₉₀	S (no. [%])	MIC ₅₀	MIC ₉₀			S (no. [%])		
Piperacillin	64	>64	24 (19)	64	>64	14 (33.3)	>64	>64	10 (11.9)	3.7 (1.47–9.29)	0.004		
Piperacillin-tazobactam	128/4	>128/4	25 (19.8)	64/4	128/4	14 (33.3)	128/4	>128/4	11 (13.1)	3.32 (1.35–8.18)	0.007		
Ticarcillin-clavulanic acid	>64/2	>64/2	0	>64/2	>64/2	0	>64/2	>64/2	0				
Ceftazidime	>16	>16	17 (13.5)	>16	>16	10 (23.8)	>16	>16	7 (8.3)	3.44 (1.2–9.83)	0.017		
Cefepime	>32	>32	22 (17.5)	32	>32	8 (19)	>32	>32	14 (16.7)	1.18 (0.45–3.07)	0.74		
Ceftriaxone	>32	>32	0	>32	>32	0	>32	>32	0				
Aztreonam	>16	>16	0	>16	>16	0	>16	>16	0				
Imipenem	>8	>8	0	>8	>8	1 (2.4)	>8	>8	0		0.333		
Meropenem	>8	>8	0	>8	>8	0	>8	>8	0				
Gentamicin	>8	>8	0	>8	>8	0	>8	>8	0				
Tobramycin	>8	>8	0	>8	>8	0	>8	>8	0				
Amikacin	>32	>32	2 (1.6)	>32	>32	0	>32	>32	2 (2.4)		0.552		
Tetracycline	>8	>8	0	>8	>8	0	>8	>8	0				
Minocycline	2	8	92 (73)	2	8	35 (83.3)	4	8	57 (67.9)	2.41 (0.95–6.13)	0.065		
Tigecycline	4	>8	43 (34.1)	2	8	22 (52.4)	4	>8	21 (25)	3.3 (1.51–7.21)	0.002		
Ciprofloxacin	>2	>2	23 (18.3)	2	>2	9 (21.4)	>2	>2	14 (16.7)	1.36 (0.54–3.47)	0.514		
Levofloxacin	8	>8	41 (32.5)	<1	8	25 (59.5)	>8	>8	16 (19)	6.25 (2.75–14.22)	<0.001		
Trimethoprim-sulfamethoxazole	4/76	>4/76	60 (47.6)	<2/38	>4/76	24 (57.1)	4/76	>4/76	36 (42.9)	1.78 (0.84–3.76)	0.13		

^aS, susceptible.

^bOR, odd ratio; CI, confidence interval.

^cComparison of the susceptibility rates between *C. gleum* and *C. indologenes*.

TABLE 4 Amino acid alterations in the quinolone resistance-determining regions of *C. indologenes*

Amino acid residue	Levofloxacin resistance				Ciprofloxacin resistance				P value
	No. (%) of susceptible isolates (n = 16)	No. (%) of nonsusceptible isolates (n = 68)	OR (95% CI) ^a	P value	No. (%) of susceptible isolates (n = 14)	No. (%) of nonsusceptible isolates (n = 70)	OR (95% CI)	P value	
Position 83 of GyrA									
Serine	13 (81.3)	11 (16.2)	22.46 (5.47–92.12)	<0.001	13 (92.9)	11 (15.7)	69.73 (8.26–588.73)	<0.001	
Phenylalanine	0	4 (5.9)		0.999	0	4 (5.7)		0.999	
Valine	1 (6.3)	11 (16.2)	0.345 (0.04–2.89)	0.447	0	12 (17.1)		0.203	
Tyrosine	2 (12.5)	42 (61.8)	0.09 (0.2–0.42)	<0.001	1 (7.1)	43 (61.4)	0.05 (0.01–0.39)	<0.001	
Position 87 of GyrA									
Aspartate	15 (93.8)	29 (42.6)	20.17 (2.52–161.55)	<0.001	13 (92.9)	31 (44.3)	16.36 (2.03–131.95)	0.001	
Glycine	1 (6.3)	18 (26.5)	0.19 (0.02–1.5)	0.104	1 (7.1)	18 (25.7)	0.22 (0.03–1.82)	0.174	
Histidine	0	1 (1.5)		0.999	0	1 (1.4)		0.999	
Tyrosine	0	20 (29.4)		0.01	0	20 (28.6)		0.018	
Position 66 of ParC									
Aspartate	1 (6.3)	3 (4.4)	1.44 (0.14–14.87)	0.578	1 (7.1)	3 (4.3)	1.72 (0.17–17.83)	0.525	
Valine	15 (93.8)	65 (95.6)	0.69 (0.07–7.13)	0.578	13 (92.9)	67 (95.7)	0.58 (0.06–6.04)	0.525	

^aOR, odds ratio; CI, confidence interval.

lower than that of *C. indologenes* infection (9.5% versus 25%). After adjusting for confounding variables in the multivariate analysis model, we found *C. indologenes* infection to be an independent risk factor for mortality. However, the reason for the different mortality rates of patients infected with *C. gleum* and *C. indologenes* is unknown. Further studies to explore the possible factors, such as virulence or pathogen-host interaction, are warranted.

Information about the susceptibilities of *Chryseobacterium* to antimicrobial agents is limited. Previous studies have demonstrated that *Chryseobacterium* is usually resistant to ceftriaxone, aztreonam, carbapenems, and aminoglycosides but variably susceptible to piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, minocycline, tigecycline, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole (4–12). The study by Hsueh et al. (7) reported that 75% (27/36) of *C. indologenes* isolates obtained in 1992 to 1995 were susceptible to minocycline, 63.9% were susceptible to piperacillin, 41.7% were susceptible to ceftazidime, and 30.6% were susceptible to trimethoprim. Kirby et al. (12) reported that >85% of 20 *C. indologenes* isolates collected during 1997 to 2001 were susceptible to piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole. In addition, Chen et al. (4) evaluated the antimicrobial susceptibilities of 113 *C. indologenes* isolates obtained in 2004 to 2011 and found that 87.6% were susceptible to trimethoprim-sulfamethoxazole, 41.8% were susceptible to tigecycline, 34.4% were susceptible to levofloxacin, 31.6% were susceptible to ciprofloxacin, and 29.4% were susceptible to piperacillin-tazobactam. Regardless, the species of *C. indologenes* in these above-mentioned studies was identified using inaccurate methods. Consequently, these susceptibility results likely do not represent the genuine antimicrobial susceptibility patterns of *C. indologenes*.

In our study, all *Chryseobacterium* strains examined showed the highest susceptibilities to minocycline, trimethoprim-sulfamethoxazole, tigecycline, and levofloxacin. Interestingly, a significant difference in the susceptibility rates was found between *C. gleum* and *C. indologenes*, whereby *C. gleum* was significantly more susceptible to piperacillin, piperacillin-tazobactam, ceftazidime, tigecycline, and levofloxacin. Only a few studies to date have examined the antimicrobial susceptibility of *Chryseobacterium* using accurate identification methods (10, 11). For example, Lo and Chang (10) and Chang et al. (11) used 16S rRNA gene sequencing to identify *Chryseobacterium* species and reported the susceptibilities of 15 *C. gleum* and 34 *C. indologenes* strains as follows (in respective order): minocycline, 100% and 100%; trimethoprim-sulfamethoxazole, 93.3% and 52%; ciprofloxacin, 40% and 14.7%; piperacillin, 0 and 2.9%; and piperacillin-tazobactam, 0 and 2.9%. In their reports, the susceptibility rates of *C. gleum* to trimethoprim-sulfamethoxazole and ciprofloxacin were also much higher than those of *C. indologenes*.

Fluoroquinolones are a suggested empirical antimicrobial therapy for *Chryseobacterium* infection (6, 12). However, our study demonstrated that a large number of *Chryseobacterium* isolates were not susceptible to fluoroquinolones. Moreover, *C. indologenes* isolates were significantly less susceptible to fluoroquinolones than *C. gleum* isolates. The mechanisms for fluoroquinolone resistance include gene alterations in drug target enzymes (GyrA, GyrB, ParC, and ParE), alterations in drug permeation (efflux pumps), and the plasmid-encoded quinolone resistance protein Qnr (16). Gene mutations in drug target enzymes are the major mechanism for fluoroquinolone resistance in many microorganisms (16). However, there is no information concerning the mechanisms of fluoroquinolone resistance in *Chryseobacterium*. Our study revealed that several *C. indologenes* isolates possessed amino acid alternations in the QRDR of GyrA, and these nonsynonymous substitutions were significantly associated with fluoroquinolone resistance. But no nonsynonymous amino acid substitution was identified in the QRDRs of *C. gleum*. Other mechanisms, such as efflux pumps and Qnr, could be responsible for fluoroquinolone resistance in *C. gleum*.

Conclusions. This study reveals the different epidemiologies, clinical manifestations, and antimicrobial susceptibility patterns between *C. gleum* and *C. indologenes*. In particular, *C. gleum* demonstrates a significantly lower case fatality rate than *C. indologenes* and a higher susceptibility rate to several antimicrobial agents. To better understand these life-threatening pathogens, additional studies are necessary to investigate the significance of these differences.

MATERIALS AND METHODS

Ethics. This study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. This study was approved by the Institutional Review Board of E-Da Hospital (EMRP-106-105). The need for patient informed consent was waived by the Institutional Review Board because the retrospective analysis of routine cultures posed no more than minimal risk of harm to the subjects.

Study setting and design. This study was performed at a 1,000-bed university-affiliated medical center in Taiwan. Microbial isolates were routinely collected according to the clinical requirements of patients. The computer database of the clinical microbial laboratory was searched for cultures that yielded *Chryseobacterium* species between January 2005 and December 2017. The microbial laboratory used an API/ID32 phenotyping kit (bioMérieux) (2005 to 2013) and Vitek MS System (2014 to 2017) to identify clinical isolates. All isolates were stored as glycerol stocks at -80°C until use. The species of *Chryseobacterium* was reidentified using 16S rRNA gene sequencing, and patients infected with *C. gleum* and *C. indologenes* were included. Demographic information, underlying illnesses, clinical manifestations, and outcomes were collected from medical records.

Definition. Empirical antimicrobial therapy was defined as inappropriate if the isolates were not susceptible to the prescribed antibiotics. Shock was defined as systolic pressure of <90 mm Hg, a reduction of 40 mm Hg in the systolic blood pressure from baseline, or a condition requiring inotropic agents to maintain blood pressure.

Species identification using 16S rRNA gene sequencing. The frozen bacteria were thawed and subcultured on tryptic soy agar with 5% sheep blood (Becton, Dickinson, Sparks, MD, USA). The colony was then inoculated onto fresh tryptic soy agar with 5% sheep blood and grown overnight. Total DNA from freshly collected colonies was prepared using a Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). The primers and protocols for amplification and sequencing of the 16S rRNA gene are listed in Table S1 in the supplemental material (17). The assembled sequences of 16S rRNA genes were submitted to the National Center for Biotechnology Information website for comparison with GenBank sequence databases using the Basic Local Alignment Search Tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). To determine similarity, 16S rRNA sequences among isolates were compared to those of type strains in the GenBank sequence databases. An isolate was considered to be a *Chryseobacterium* species match if it shared $\geq 99.5\%$ 16S rRNA sequence identity with the respective type strain in GenBank (18).

Antimicrobial susceptibility testing. The MICs of antibiotics were examined using the broth microdilution method of the Sensititre 96-well panels according to the manufacturer's instructions (Thermo Fisher Scientific/Trek Diagnostics Systems, Oakwood Village, OH, USA). Susceptibility was interpreted according to the MIC criteria for "other non-*Enterobacteriaceae*" in the Clinical and Laboratory Standards Institute (CLSI) guidelines (19). Because there are no interpretive susceptibility criteria for "other non-*Enterobacteriaceae*" to tigecycline in the CLSI guidelines, the tigecycline MIC was interpreted according to the *Enterobacteriaceae* susceptibility breakpoints of the U.S. Food and Drug Administration (susceptible MIC, ≤ 2 mg/liter; intermediate MIC, 4 mg/liter; resistant MIC, ≥ 8 mg/liter) (20).

Identification of mutations in the QRDRs. The primers and PCR conditions for the amplification of the QRDRs in *gyrA*, *gyrB*, *parC*, and *parE* are listed in Table S1.

Data analysis. SPSS software (version 24.0; IBM, Armonk, NY, USA) was used to analyze the data. Categorical variables were calculated using a chi-square test or Fisher's exact test, as appropriate. All factors associated with mortality were computed via univariate analyses and with the logistic regression model of multivariate analysis using backward stepwise methods by the likelihood ratio. The significance of variables was calculated by the odds ratio (OR), 95% confidence interval (CI), and two-tailed *P* value. A factor was considered statistically significant at a *P* value of <0.05 .

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.02256-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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We report that we have no conflicts of interest.

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