



Clinical and molecular characteristics of *Chryseobacterium indologenes* isolates at a teaching hospital in Shanghai, China

Yixin Zhang^{1,2}, Dan Li^{1,2}, Yang Yang^{1,2}, Jiachun Su^{1,2}, Xiaogang Xu^{1,2}, Minggui Wang^{1,2}, Yijian Chen^{1,2}, Ying Li^{1,2}

¹Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai, China; ²Key Laboratory of Clinical Pharmacology of Antibiotics, National Health Commission, Shanghai, China

Contributions: (I) Conception and design: Y Chen, Y Li; (II) Administrative support: M Wang, X Xu; (III) Provision of study materials or patients: Y Zhang, J Su, Y Yang; (IV) Collection and assembly of data: Y Zhang; (V) Data analysis and interpretation: Y Zhang, D Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Yijian Chen; Ying Li. Institute of Antibiotics, Huashan Hospital, Fudan University, No. 12 Middle Wulumuqi Road, Shanghai 200040, China. Email: chenijian@fudan.edu.cn; ying_lihs@fudan.edu.cn.

Background: *Chryseobacterium indologenes* (*C. indologenes*) has recently emerged as a cause of life-threatening nosocomial infections in humans. This study aims to investigate the clinical characteristics, homology, and antimicrobial patterns of *C. indologenes* clinical isolates at a teaching hospital in Shanghai, China.

Methods: A total of 135 consecutive non-replicate clinical *C. indologenes* isolates from January 2010 to December 2018 were collected at a tertiary care university hospital in Shanghai, China. Genetic relatedness of the isolates was performed by pulsed-field gel electrophoresis (PFGE). The antimicrobial susceptibility of these isolates was measured by the microdilution broth method. The prevalence of β -lactamase genes was investigated by polymerase chain reaction (PCR), while the quinolone resistance-determining regions (QRDRs) were sequenced.

Results: All 135 *C. indologenes* isolates were collected from hospitalized patients with an average age of 55 years. Most of these clinical isolates were derived from ascites (59.3%) or urine (23.7%) specimens. Eighty (80/135) of the strains were classified as clone D by PFGE. *In vitro* drug susceptibility tests showed that minocycline and trimethoprim-sulfamethoxazole had sound antibacterial effects. However, more than 86% of the tested strains were resistant to cephalosporins (ceftazidime, cefotaxime), β -lactamase/ β -lactamase inhibitors (cefoperazone-sulbactam), and carbapenems (meropenem, imipenem). Metallo- β -lactamase *bla*_{IND} and type A broad-spectrum β -lactamase genes *bla*_{CIA} were present in 135 and 103 isolates, respectively. The clinical strains in our hospital mainly carried *bla*_{IND-2} (89.6%, 121/135). Compared with previous studies, these strains had a high rate of resistance to quinolones. The resistance rates to levofloxacin, ciprofloxacin, norfloxacin, gatifloxacin, and nemonoxacin were as high as 83.7–94.8%. The mutations at Ser83Val, Ser83Tyr, and Asp87Gly in the QRDRs of GyrA were significantly related to the resistance of *C. indologenes* to levofloxacin. All but one quinolone-resistant strain contained at least one significant mutation.

Conclusions: This study showed a clonal dissemination of *C. indologenes* isolates in infections at a tertiary care university hospital in Shanghai, China. Minocycline and trimethoprim-sulfamethoxazole had favorable *in vitro* antibacterial effects. However, the high resistance rate to β -lactams and quinolones was due to carrying β -lactamase (*bla*_{IND}, *bla*_{CIA}), and mutations in the QRDRs of GyrA.

Keywords: *Chryseobacterium indologenes* (*C. indologenes*); nosocomial infection; drug resistance; quinolone-resistance determining region

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Introduction

Chryseobacterium indologenes (*C. indologenes*), is a non-lactose fermenting gram-negative bacillus formally classified under the *Flavobacterium* CDC group IIb (1). Since its first identification in 1993 from the tracheal aspirate of a patient with ventilator-associated pneumonia (VAP) as an opportunistic pathogen (2), *C. indologenes* has been notorious for causing nosocomial infections due to its presence in fluid-associated devices which serve as a potential reservoir of infection (3). *C. indologenes* causes many kinds of infections such as catheter-related bacteremia, urinary tract infection, biliary tract infection, peritonitis, surgical wound infection, and hospital-associated pneumonia (HAP), especially in immunocompromised patients (4–7). Nosocomial infections attributable to *C. indologenes* have been increasingly reported in numerous countries and have caused significant morbidity and mortality. A study conducted in Taiwan found that 98% (212/215) of *C. indologenes* infections were nosocomial, and the mortality rates of patients with bacteremia or pneumonia was 35.4% (40/113) (8). More recently, Cantero *et al.* reported an outbreak of *C. indologenes* infections in an intensive care unit of a Spanish hospital where mortality reached 25% (3).

C. indologenes exhibits resistance to the majority of antimicrobial agents such as carbapenems, cephalosporins, aminoglycosides, and chloramphenicol which are used to empirically treat infections caused by other gram-negative bacteria (1,9). Therefore, the infection caused by *C. indologenes* is challenging to manage and often results in unfavorable outcomes (10). In addition, according to the SENTRY Antimicrobial Surveillance Program, clinical *C. indologenes* isolates from Asia generally have higher resistance rates to cephalosporins and carbapenems than other continents (11). The resistance rate of the *Chryseobacterium* species to ceftazidime and imipenem from 1997–2001 in Latin America and North America was 40–42.9% and 73.3–85.7%. In contrast, the resistance rates to ceftazidime and imipenem in Asia manifested as 87.5% and 100% (11). It has been speculated that *C. indologenes* is intrinsically resistant to cephalosporins and carbapenems due to its production of molecular class A β -lactamase *bla*_{CIA} and class B carbapenem-hydrolyzing β -lactamase *bla*_{IND} (12,13).

Minocycline, trimethoprim-sulfamethoxazole, and quinolones have demonstrated favorable *in vitro* susceptibility test results. Among them, quinolones were the most active anti-infective agents with susceptibility rates of

>95% in the SENTRY program (11). Together with their broad antibacterial spectrum and high tissue concentration, quinolones were recommended for the treatment of *C. indologenes* infections (14). However, the emergence of a quinolones-resistant *C. indologenes* strain has raised concern. It has been recently reported that the susceptibility rates to quinolones of *C. indologenes* isolates obtained in Taiwan during 2005–2017 have dropped to 16.7–19% (15). The quinolone resistance in *C. indologenes* has been attributed to alterations in DNA gyrase.

However, clinical reports and the molecular characterization of *C. indologenes* collected in mainland China are limited, resulting in relatively insufficient clinical evidence for the treatment of infectious diseases caused by the bacteria. This study aimed to explore the clinical characteristics, homology, and antimicrobial patterns of *C. indologenes* clinical isolates from January 2010 to December 2018 at a teaching hospital in Shanghai, China. Moreover, the distribution of *bla*_{CIA}, *bla*_{IND}, and the mutation of quinolone resistance-determining regions (QRDRs) were also investigated. We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/atm-21-933>).

Methods

Bacterial isolates

A total of 135 consecutive non-replicate clinical *C. indologenes* isolates from January 2010 to December 2018 were collected at a tertiary care university hospital in Shanghai, China. A 16s-rRNA sequence analysis was performed for bacterial identification (type strain, DSM 16777T158; GenBank accession no. LN681561). All the clinical isolates were stored at –80 °C prior to use. These isolates were collected from different specimens including ascites, sputum, and urine. Medical records were anonymously collected to review clinical information, including age, sex, hospitalization department, and type of infection acquisition. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Huashan Hospital, Fudan University, China (approval number: KY2017-274). Individual consent for this retrospective analysis was waived. Nosocomial infection was defined as previously reported (16).

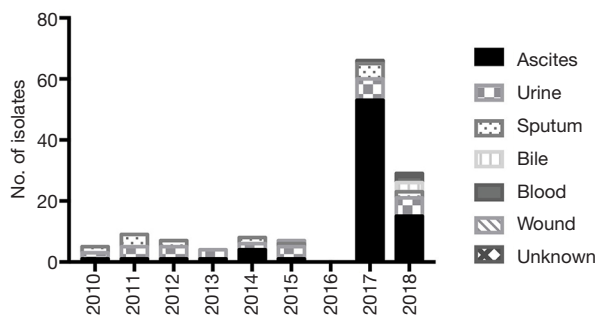


Figure 1 The distribution of 135 *C. indologenes* isolates according to the year and site of isolation. Unknown: missing information.

Homology analysis

The homology analysis of clinical isolates was performed by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared in agarose blocks and digested with *Xho*I. DNA fragments were separated by PFGE on a CHEF Mapper XA (Bio-Rad, Hercules, CA, USA) for 19 h at 14 °C, at 6 V/cm, a pulse angle of 120, and pulse times ranging from 0.5 to 13.6 s. We constructed dendrograms using the Dice coefficient and unweighted pair-group method using average linkage clustering. Isolates were regarded as a joint pulsed-field group if the Dice similarity coefficient >85% in this study. The result was analyzed by BioNumerisc software version 7.6 (Applied-Maths, Belgium)

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of 16 antimicrobial agents were measured by the microdilution broth method according to the Clinical and Laboratory Standards Institute 29th edition (CLSI 29th ed.), including cefotaxime, ceftazidime, piperacillin-tazobactam, cefoperazone-sulbactam, imipenem, meropenem, norfloxacin, levofloxacin, gatifloxacin, ciprofloxacin, moxifloxacin, nemonoxacin, amikacin, minocycline, trimethoprim-sulfamethoxazole (TMP-SMZ), and rifampicin (17). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, and *Enterococcus faecalis* ATCC29212 were used as quality control reference strains. The results were interpreted according to the criteria of “other non-*Enterobacteriaceae*” in CLSI 29th ed., except that the MICs of rifampin were interpreted according to the *Enterococcus* susceptibility breakpoints.

Detection and sequencing of β -lactamase genes and QRDRs

The presence of crucial β -lactamase [*bla*_{CIA}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} (group1, group2, group9), *bla*_{KPC}, *bla*_{OXA48}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}] (12,18,19) and QRDRs (GyrA, GyrB, ParC, and ParE) (15) were amplified and sequenced by polymerase chain reaction (PCR) with primers and PCR conditions as previously described. The primers and PCR conditions for the amplification of *bla*_{IND} are listed in Table S1.

Statistical analysis

Statistical analysis was conducted by a two-tailed Student's *t*-test or chi-square test with SPSS Statistics 25 software (IBM, <https://www.ibm.com>), and a P value less than 0.05 was considered statistically significant.

Results

Bacterial isolates and site of isolation

Among 135 clinical *C. indologenes* isolates, 39 strains were isolated from 2010 to 2016, accounting for 28.9%. Since then, there has been a continual growth in the number of isolates. In 2017 and 2018, 66 strains and 30 strains were collected, accounting for 48.9% and 22.2%, respectively (Figure 1). The isolates were mainly collected from ascites (77/135, 57.0%) and urine (32/135, 23.7%). Other sites of isolation included sputum (18/135, 13.3%), bile (3/135, 2.2%), blood (2/135, 1.5%), wound secretions (1/135, 0.7%), and two isolates were missing information.

Demographic data and clinical characteristics

The demographic data and clinical characteristics of the 135 *C. indologenes* infections are summarized in Table 1. Male patients predominated (97/135, 71.9%), and the mean age was 55 years (range, 5–98 years), with 36 patients over 65 years. Most of these patients came from the general surgery department (79/135, 58.5%), followed by the intensive care unit (ICU) (8/135, 5.9%), the neurosurgery department (8/135, 5.9%), and the gerontology department (7/135, 5.2%). Remaining patients came from other departments including the infectious disease, nephrology, hematology, neurology and pneumology departments. Nosocomial infection accounted for the vast majority (84.4%, 114/135), three infections were identified as

Table 1 Demographic data and clinical characteristics of patients with *Chryseobacterium indologenes* infection

Variables	Total (n=135)
Sex, n (%)	
Male	97 (71.9)
Female	38 (28.1)
Age	
Range, years	5–98
Median, years	55
Age ≥65, n (%)	36 (26.7)
Department, n (%)	
General surgery	79 (58.5)
Intensive care unit	8 (5.9)
Neurosurgery	8 (5.9)
Gerontology	7 (5.2)
Infectious disease	5 (3.7)
Nephrology	4 (3.0)
Hematology	3 (2.2)
Neurology	2 (1.5)
Pneumology	1 (0.7)
Others*	18 (13.3)
Type of infection acquisition, n (%)	
Community-acquired	3 (2.2)
Healthcare-associated	114 (84.4)
Unclear*	18 (13.3)

*, information was hard to ascertain due to missing data.

community-acquired, while others remained difficult to ascertain due to lack of information.

Homology analysis

The homology analysis of 135 *C. indologenes* isolates was determined by PFGE. With a similarity coefficient of 85% as a cutoff value, eight significant clusters were found (Figure 2). Among them, the D type was the most prevalent, accounting for 59.3% (80/135), followed by the H and E types which accounted for 23.0% (31/135) and 8.9% (12/135), respectively.

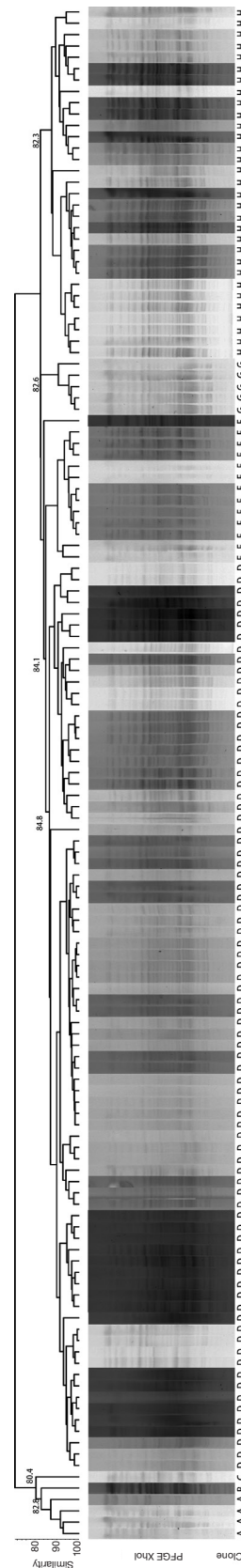


Figure 2 Dendrogram generated from the analysis of 135 *C. indologenes* isolates.

Table 2 Antimicrobial susceptibility of 135 *Chryseobacterium indologenes* isolates ($\mu\text{g/mL}$)

Antimicrobial agent	S (%)	I (%)	R (%)	MIC ₅₀	MIC ₉₀	Susceptibility breakpoint	MIC range
Cefotaxime	0.0	5.9	94.1	>128	>128	≤ 8	1–128
Ceftazidime	6.7	5.9	87.4	>128	>128	≤ 8	1–128
Piperacillin-tazobactam	37.0	9.6	53.3	>128/4	>128/4	$\leq 16/4$	1/4–128/4
Cefoperazone-sulbactam	10.4	3.0	86.7	>128/64	>128/64	$\leq 8/4$	1/0.5–128/64
Imipenem	0.7	1.5	97.8	>64	>64	≤ 4	0.5–64
Meropenem	0.0	2.2	97.8	>64	>64	≤ 4	0.5–64
Norfloxacin	13.3	3.0	83.7	>32	>32	≤ 2	0.25–32
Levofloxacin	14.8	0.7	84.4	32	>32	≤ 2	0.25–32
Gatifloxacin	14.1	1.5	84.4	>32	>32	≤ 2	0.25–32
Ciprofloxacin	12.6	0.7	86.7	>32	>32	≤ 1	0.25–32
Moxifloxacin	23.7	20.0	56.3	8	>32	≤ 2	0.25–32
Nemonoxacin	5.2	0.0	94.8	>32	>32	≤ 2	0.25–32
Amikacin	0.7	1.5	97.8	>128	>128	≤ 16	1–128
Minocycline	98.5	0.7	0.7	2	4	≤ 4	0.5–64
TMP-SMZ	97.8	0.0	2.2	0.5/9.5	1/19	$\leq 2/38$	0.125/2.375–16/304
Rifampicin	72.6	3.7	23.7	0.5	16	≤ 1	0.25–32

S, susceptible; I, intermediate; R, resistant; MIC, minimal inhibitory concentration; MIC₅₀, minimal inhibitory concentration for 50% isolates; MIC₉₀, minimal inhibitory concentration for 90% isolates; TMP-SMZ, trimethoprim-sulfamethoxazole.

Antimicrobial susceptibility testing

The MICs results of 16 antimicrobial agents are shown in Table 2. Minocycline and trimethoprim/sulfamethoxazole appeared to be the most potent antimicrobial agents against *C. indologenes*, with a low resistance rate of 0.7% (1/135) and 2.2% (3/135), respectively. Rifampicin also showed a good antibacterial effect (resistance rate 23.7%). More than 86% of the tested strains were resistant to cephalosporins (cefotaxime and ceftazidime), β -lactamase/ β -lactamase inhibitors (cefoperazone-sulbactam), carbapenems (meropenem and imipenem), and aminoglycosides (amikacin). Furthermore, most *C. indologenes* clinical isolates were resistant to quinolones, and the resistance rates to norfloxacin, levofloxacin, gatifloxacin, and ciprofloxacin were all over 83%, followed by moxifloxacin (resistance rate 56.3%). We also tested the new fluorine-free quinolone

nemonoxacin. However, the result was unsatisfactory with a drug resistance rate of 94.8%. At the same time when the high level of resistance to quinolones was detected, the MIC₅₀ values of norfloxacin, levofloxacin, gatifloxacin, ciprofloxacin, and nemonoxacin were >32 $\mu\text{g/mL}$.

Detection of β -lactamase genes

All *C. indologenes* isolates carried the metallo- β -lactamase *bla*_{IND}, and the type A broad-spectrum β -lactamase *bla*_{CIA} was present in 103 isolates. According to the sequence results, *bla*_{IND-2} was found in 121 (89.6%) isolates, which accounted for the majority, followed by *bla*_{IND-7} (n=4), *bla*_{IND-3} (n=3), *bla*_{IND-14} (n=3), *bla*_{IND-5} (n=2), *bla*_{IND-8} (n=1), and *bla*_{IND-11} (n=1). None of these isolates had a combination of more than two types of *bla*_{IND}. No *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M} (group 1, group 2, group 9), *bla*_{KPC}, *bla*_{OXA48}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM} were detected.

Table 3 Amino acid alterations in the quinolone resistance-determining regions of *Chryseobacterium indologenes* isolates

Amino acid	Susceptibility of levofloxacin, n (%)		P value
	Susceptible (n=20)	Non-susceptible (n=115)	
Position 83 of GyrA			
Serine	20 (100.0)	2 (1.7)	<0.001
Valine	0 (0.0)	50 (43.5)	<0.001
Tyrosine	0 (0.0)	60 (52.2)	<0.001
Phenylalanine	0 (0.0)	3 (2.6)	>0.999
Position 87 of GyrA			
Aspartic acid	19 (95.0)	53 (46.1)	<0.001
Glycine	1 (5.0)	62 (53.9)	
Position 425 of GyrB			
Leucine	2 (10.0)	2 (1.7)	0.1044
Isoleucine	18 (90.0)	113 (98.3)	
Position 473 of GyrB			
Lysine	2 (10.0)	2 (1.7)	0.1044
Arginine	18 (90.0)	113 (98.3)	
Position 539 of ParE			
Valine	20 (100.0)	105 (91.3)	0.3575
Isoleucine	0 (0.0)	10 (8.7)	

Mutations in the QRDRs

The mutations of amino acids in the QRDRs of *C. indologenes* are shown in Table 3. QRDR mutations of GyrA, GyrB, and ParE were detected. In the QRDR of GyrA, among the 115 levofloxacin non-susceptible isolates, 60 isolates carried mutations at Ser83Tyr, 50 isolates carried mutations at Ser83Val, 3 isolates carried mutations at Ser83Phe, and 62 isolates carried the mutation at Asp87Gly in GyrA. Compared with susceptible isolates, mutations at Ser83Tyr, Ser83Val, and Asp87Gly were significantly correlated with levofloxacin non-susceptibility ($P < 0.001$). Amino acid mutations were detected in GyrB at codons 425 and 473, and in ParE at codon 539 but these mutations were not significantly related to the susceptibility of levofloxacin ($P = 0.081$, $P = 0.081$, $P = 0.362$). No amino acid mutation was found in ParC. It is noteworthy that all but one resistant strain contained at least one significant mutation.

Analysis of quinolones-resistant level

The connection between the level of quinolones resistance and significant amino acid mutations at codons 83 and 87 of GyrA were analyzed. As shown in Table 4, the strain with a single amino acid substitution in GyrA at Ser83Val ($n = 50$) and Ser83Tyr ($n = 1$) was moderately resistant to levofloxacin ($MIC_{50} = 8 \mu\text{g/mL}$). The isolates had two mutations in GyrA at Ser83Tyr and Asp87Gly ($n = 59$) and showed a high level of levofloxacin resistance ($MIC_{50} > 32 \mu\text{g/mL}$). We also found three strains carrying substitutions in GyrA at Phe83Ser and Asp87Gly. Although Phe83Ser was not related to levofloxacin resistance, the strains containing these two mutations also showed high levels of resistance ($MIC_{50} > 32 \mu\text{g/mL}$). Strains carrying the GyrA subunit double-site mutations showed a 4–32 times higher MIC value for quinolones than the single-point mutation strains. The same trend was observed in gatifloxacin, ciprofloxacin, and moxifloxacin.

Table 4 The correlation between amino acid mutations at different sites of GyrA and MIC₅₀ (µg/mL) of quinolones

No. amino acid mutation	No. isolates (n)	Amino acid of GyrA		MIC range (MIC ₅₀)			
		83	87	Levofloxacin	Ciprofloxacin	Gatifloxacin	Moxifloxacin
Wild	21	–	–	≤0.25–32 (0.5)	≤0.25–>32 (0.5)	≤0.25–16 [1]	≤0.25–8 (≤0.25)
1	1	–	Asp87Gly	1	1	1	0.5
1	50	Ser83Val	–	8–16 [8]	16–>32 [32]	4–>32 [8]	2–32 [4]
1	1	Ser83Tyr	–	8	32	32	4
2	3	Ser83Phe	Asp87Gly	32–>32 (>32)	>32	16–>32 (>32)	8–>32 (>32)
2	59	Ser83Tyr	Asp87Gly	8–>32 (>32)	>32	8–>32 [32]	4–>32 [16]

MIC₅₀, minimal inhibitory concentration for 50% isolates.

Discussion

C. indologenes is widely distributed in plants, soil and water, and in the past was rarely considered a human pathogen (9). Based on a PUBMED literature search using the keywords ‘*Chryseobacterium indologenes* infections’, we identified 44 studies published in the period 2010–2019 which are summarized in Table S2. Analysis of the scientific literature suggests that the highest frequency of *C. indologenes* infections occurs in Asia, several clinical cases were occurred in North America, South America, and Europe. *C. indologenes* infections were mainly sporadic, sometimes outbreaks could also be a threat in hospital. Susceptible populations are mainly infants (especially premature babies), the elderly, long-term hospitalized patients, patients with serious underlying diseases or immunocompromised. *C. indologene* can cause multiple types of infection, the most frequently reported infections were pneumonia and bacteraemia. The long-term indwelling devices especially tracheal tubes and intravascular catheters increased the risk of infection. It is worth noting that *C. indologenes* infections have also been gradually appearing in patients with normal immunity and no catheter implantation. In this study, we conducted a 9-year retrospective study and described a clonal dissemination of hospital-acquired infection caused by *C. indologenes*. Our findings are in accord with previous literature indicating that most cases of *C. indologenes* are due to nosocomial infection. It should be stressed that *C. indologenes* can be colonized and spread in the hospital environment through contaminated and humid medical equipment (20). Furthermore, with the development of medical technology and the increased use of various invasive procedures and broad-spectrum antibacterial drugs, there has been a phenomenal growth of nosocomial infection

caused by *C. indologenes* (9). The clonal dissemination of multidrug-resistant bacteria in hospitals has become a public health problem that jeopardizes patients and increases medical burden. This is the first report to describe a clonal dissemination of *C. indologenes* in mainland China. According to our results, this clonal dissemination was related to an intra-abdominal infection after liver transplantation in the general surgery department, which highlights the need for active surveillance and implementation of infection control measures.

The susceptibilities of *C. indologenes* vary dramatically from one region to another, making it difficult to choose an effective drug for empirical treatment (11). Previous studies have demonstrated that *Chryseobacterium* sepsis is nearly uniformly resistant to extended-spectrum penicillin, aztreonam, cephalosporins, imipenem, meropenem, chloramphenicol, and aminoglycosides. Quinolones, minocycline, rifampicin, and trimethoprim-sulfamethoxazole are, however, usually effective (7,8,10,21). Quinolones used to be considered an appropriate antimicrobial agent to treat *C. indologenes* infections (22). However, according to a recent study, the resistance rate to quinolones is rising, especially for those strains isolated in Asia. In our study, *in vitro* drug susceptibility tests showed that minocycline and trimethoprim-sulfamethoxazole had sound antibacterial effects on *C. indologenes*, with resistance rates of 0.7% and 2.2%, respectively. Cephalosporins, β-lactam/β-lactamase inhibitors, carbapenems, and aminoglycosides showed insufficient activity against these pathogens, and our study showed a much higher resistance rate to these antimicrobial agents than previous reports. Moreover, the high resistance rate to quinolones suggested that they were no longer an appropriate choice. It is noteworthy that our study showed the lowest susceptibility

to ciprofloxacin compared with other Asian studies (12.6% vs. 14.7–68%) (10,15,22,23).

Our study revealed that *C. indologenes* displayed an increased rate of resistance to previously potent antibiotics. Hence, we investigated the resistant mechanism of β -lactams and fluoroquinolones. Usually, *C. indologenes* shows intrinsic resistance to carbapenems and cephalosporins as a result of molecular class A β -lactamase *bla*_{CIA} and class B carbapenem-hydrolyzing β -lactamase *bla*_{IND}. These two β -lactamases are peculiar to the *Chryseobacterium* species. Lin *et al.* detected *bla*_{IND1-4} in clinical isolates of *C. indologenes* during 2004 to 2006 in Hefei, China, and reported that *bla*_{IND1} (32.1%, 9/28) and *bla*_{IND2} (35.7%, 10/28) were the most prevalent (23). To date, twelve new *bla*_{IND} alleles have been registered in GenBank, but as yet no investigation of the prevalence of all 16 *bla*_{IND} alleles and *bla*_{CIA} in clinical isolates has been conducted. In our study, we found that all isolates carried *bla*_{IND}, and 76.2% (103/135) of isolates carried *bla*_{CIA}. The dominant genotype was *bla*_{IND-2}, accounting for 89.6% (121/135).

The mechanism of resistance to quinolones is mainly caused by mutations in QRDRs (GyrA, GyrB, ParC, and ParE subunits), efflux pumps (QepA and OqxAB), DNA topoisomerase protection protein Qnr, and quinolone acetyltransferase Aac (6')-Ib-cr (24–27). The gene mutations in QRDRs resulting in changes of the enzyme's affinity for quinolone is the primary resistant mechanism of quinolones (28). Quinolone resistance in *C. indologenes* was previously identified to be associated with the alterations in DNA gyrase. A study reported that mutations of Ser83Tyr and Asp87Tyr in the GyrA subunit lead to the non-susceptibility of *C. indologenes* ($P < 0.001$, $P = 0.018$) (15). In this study, we found that both the mutations at codons 83 and 87 of the GyrA were associated with quinolones resistance ($P < 0.001$), and the mutation types were Ser83Val, Ser83Tyr, and Asp87Gly. When the hydroxyl-containing serine at position 83 of the GyrA mutates into valine or tyrosine, it will affect the formation of hydrogen bonds and the replacement of aliphatic chains. After replacing aspartic acid at position 87 with glycine, the negatively charged amino acids are replaced by uncharged amino acids, and the charge of the peptide chain changes accordingly. These changes in amino acids verify that the formation of hydrogen bonds and the presence of negatively charged amino acids play a vital role in the interaction of quinolones with gyrase-DNA complexes (26). This is the first study to report that Ser83Val and Asp87Gly are associated with quinolones resistance of *C. indologenes*. Among the

Enterobacteriaceae, the most frequently identified mutations in GyrA were at codons 83 and 87 (29). Notably, double mutations in GyrA are related to high levels of resistance to fluoroquinolones in *Escherichia coli* isolates (30). We detected a similar phenomenon in *C. indologenes* insofar as double amino acid alterations in the QRDRs of GyrA were significantly associated with high levels of quinolones resistance.

The present results were limited by small sample size and retrospective single-center study design. A prospective multi-center study should be conducted to monitor antimicrobial agent resistance pattern and mechanisms of *C. indologenes*. In conclusion, this study reveals the clinical characteristics and antimicrobial susceptibility patterns of *C. indologenes*. A clonal dissemination of *C. indologenes* in Huashan Hospital has been observed. Minocycline and TMP-SMZ were the most suitable antimicrobial agents to treat infections caused by this pathogen. An increased resistance rate to β -lactams and quinolones was exhibited compared with previous studies. The resistance mechanism of β -lactams in *C. indologenes* is mediated by *bla*_{CIA} and *bla*_{IND}. The resistance mechanism of quinolones is related to amino acid mutations in the quinolone resistance-determining region of the GyrA subunit.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at <http://dx.doi.org/10.21037/atm-21-933>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm-21-933>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of Huashan Hospital, Fudan University, China (approval number: KY2017-274). Individual consent for this retrospective analysis was waived.

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