Molecular epidemiology and risk factors of *Stenotrophomonas maltophilia* infections in a Chinese teaching hospital

Zhongliang Duan[1,#], Juanxiu Qin[2,#], Yao Liu[2], Cui Li[1], Chunmei Ying[1,*]

[1]Department of Clinical Laboratory, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China

[2]Department of Clinical Laboratory, Renji Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China

[#]These authors have contributed equally to this work

***Correspondence:**

Dr. Chunmei Ying, Obstetrics & Gynecology Hospital of Fudan University, 419 Fangxie Road, Shanghai 200011, China.

E-mail: ycmzh2012@163.com

Abstract

Background: *Stenotrophomonas maltophilia* (*S. maltophilia*) is an important opportunistic pathogen obtained in hospitals. With the abuse of broad spectrum antibiotics and invasive surgical devices, the rate of *S. maltophilia* infections is increasing year by year. This study is aimed at epidemiological analysis of the clinical and molecular characteristics of *S. maltophilia* infections in a Chinese teaching hospital. We wish to have a comprehensive understanding of the status of *S. maltophilia* infections, in order to provide strong epidemiological data for the prevention and treatment of *S. maltophilia* infections.

Results: 93 isolates from the Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine were included, in which 62 strains were from male patients and 81 isolates were cultured from sputum samples. 86 patients had

underlying diseases. All patients have received antibiotic therapeutics. MLST analysis indicated that 61 different sequence types (STs) were found (including 45 novel STs), and MLST did not show significantly adominant STs. PFGE showed weak genetic linkage between strains. The resistant rates of Trimethoprim/sulfamethoxazole (TMP/SMX) and levofloxacin were 9.7% and 4.3%. All the strains were susceptible to minocycline. The positive rates of the four virulence genes *Stmpr1*, *Stmpr2*, *Smf-1* and *Smlt3773 locus* were 79.6%, 91.4%, 94.6% and 52.7%. The positive rates of the three biofilm genes *rmlA*, *spgM* and *rpfF* were 82.8%, 92.5% and 64.5%. The mean biofilm forming level of OD_{492} was 0.54 ± 0.49, and there was no significant difference between genders and among different age groups. The data from patients with ICU and the control group were analyzed retrospectively, and the risk factors infected in ICU included the hypoimmunity and the increase of the use of antibiotics were independent risk factors.

Conclusion: Most of the patients had prior medical usage history and baseline diseases. The carrying rate of virulence genes was high, the drug resistance rate of *S. maltophilia* was low, and the biofilm formation ability was strong. The increased use of antibiotics is an independent risk factor for *S. maltophilia* infection, which should be payed more attention. No obvious clonal transmission was found in the same department.

Keywords: *Stenotrophomonas maltophilia*; MLST; biofilm; epidemiology; PFGE

INTRODUCTION

S. maltophilia is a non-fermentative, gram-negative aerobic bacilli, being cosmopolitan and ubiquitous in natural environment like water, soil and plant . *S. maltophilia* has been found as a contaminant of numerous hospital devices including dialysis devices, blood pressure monitors, faucets, sphygmomanometers, disinfectants, ventilators and has the ability to transmit between patients or patients and the healthy

persons(Brooke, 2012). Clinical evidence has shown that *S. maltophilia* can cause nosocomial infections in immunocompromised hosts such as respiratory system infection, joint infection and skin infection. Wu et al.(Wu *et al.*, 2016) also found that the dominant flora of *S. maltophilia* in the infection of keratitis. In many China Antimicrobial Surveillance Network (CHINET) reports, *S. maltophilia* was the third largest fungus among non-fermentative Gram-negative bacilli, just after *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and the number of clinical isolates showed an upward trend(Hu *et al.*, 2018).

With the abuse of different kinds of antibiotics, chemotherapy drugs and immunosuppressants, and the widespread use of invasive exploration equipments, the isolation and infection rate of *S. maltophilia* in hospitals are increasingly growing. Brooke(Brooke, 2012) reported that particular attention needed to be paid to inpatients with hypoimmunity. *S. maltophilia* contains a metal enzyme, β-lactamase, which offers this bacterium varying degrees of natural resistance to many β-lactam drugs(Okazaki & Avison, 2008). It can also produce aminoglycoside modifying enzymes and have a certain degree of resistance to aminoglycoside drugs, and the expression of efflux pump system makes it resistant to a variety of antimicrobial agents(Samonis *et al.*, 2012). However, the continuous emergence of multidrug-resistant strains of *S. maltophilia*has brought great challenges to the treatment of serious *S. maltophilia* infections(Hu *et al.*, 2018).

There are rare reports on the comprehensive clinical and molecular characteristics of *S. maltophilia* system in Shanghai, so we used multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) to analyze the molecular epidemiological characteristics of *S. maltophilia* isolated from Renji Hospital affiliated to Shanghai Jiaotong University School of Medicine. And analyzed the risk factors for acquiring *S. maltophilia* infections from ICU patients. The clinical information of the related patients were collected and at the same time, the virulence genes and biofilm genes of *S. maltophilia* were detected . We hope to make an intuitive description of the epidemic situation of the strain in clinic, providing the

necessary groundwork for its basic and mechanical researches, and providing support for the prevention and treatment of *S. maltophilia* infections.

MATERIALS AND METHODS

Materials and reagents

A total of 93 non-repetitive strains of *S. maltophilia* isolated from outpatients and inpatients in Renji hospital in 2014 were collected plus the patients' clinical information. The quality control strains include *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. These strains were identified by the Vitek automated system and confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS).

Bacteria were generally grown in tryptic soy broth (TSB; Oxoid) at 37°C. The antibitics for disk diffusion testing were levofloxacin, trimethoprim-sulfamethoxazole, cefoperazone/sulbactam, minocycline and piperacillin/tazobactam (Oxoid, UK).

Disk diffusion testing

Disk diffusion was performed according to CLSI 2016 recommendations of levofloxacin, trimethoprim-sulfamethoxazole and minocycline. The drug susceptibility of cefoperazone/sulbactam was determined according to the CLSI criteria for cefoperazone. Piperacillin/tazobactam referred to the CLSI criteria for the determination of Acinetobacter.

Multi-locus sequence typing (MLST) analysis

Primer sequences targeting the conserved regions of seven housekeeping genes of *S. maltophilia* were used as shown in MLST site (http://pubmlst.org/smaltophilia/). MLST was performed as described by Kaiser et al.(Kaiser *et al.*, 2009).

Seven pairs of primers for housekeeping genes (*atpD*, *gapA*, *guaA*, *mutM*, *nuoD*, *ppsA* and *recA*) were synthesized (Supplementary Table 1).

PCR mixture (2 × PCR mix, primers, DNA template and double distilled water) was incubated at 94°C for 5 min; 35 cycles at 94°C for 10 s, 55°C for 30 s, and 72°C for 30s; with a final extension step at 72°C for 1 min. The sequence was analyzed by DNAstar software, and the obtained sequence was submitted to MLST database to acquire sequence type. Seven housekeeper genes were assembled using MEGA.4 software, and the Phylogenetic analysis was carried out.

Pulsed-field gel electrophoresis (PFGE) analysis

The epidemiological relatedness of the strains was studied by pulsed-field gel electrophoresis (PFGE), as previously described(Pompilio *et al.*, 2011). PFGE method and conditions reference(Tanimoto, 2013; Tenover *et al.*, 1995), and slightly modified according to the pre-test： restriction enzyme Xbal (Roche Diagnostics) digestion at 37 ℃ for 3 hours; PGFE electrophoresis conditions according to the manufacturer's protocol (Bio-Rad): 2000 mL 0.5 *TBE, voltage 6.0 V max, temperature 14 ℃, pulse angle 120, start pulse 5 s, end pulse 20 s, electric swimming time 19h； image input BioNumerics (version 4.0, Applied Maths,Inc.) software, and building clustering tree. Salmonella BraenderupH9812 is PFGE molecular weight Marker

Biofilm formation assay

The overnight cultured *S. maltophilia* was diluted in TSB to D_{600} of 0.01. 200 µl of the solution for each well was cultured at 37 ℃ for 24 hours in 96-well plate (Corning, USA). The biofilm formation ability was determined by dye crystal violet staining. After incubation, the plate was fixed at 60 ℃ for 1 hour. The non-adherent bacteria was removed and washed with sterile PBS for 4 times, then 50 µL crystal violet dye was added to each well and kept at room temperature for 5 minutes, followed by rinsing under running tap water. The plate was dried at room temperature and 250µL 33% glacial acetic acid was added to each well to dissolve the staining for 15min. To measure the absorbance optical density was read at 492 nm. A low cut-off (ODc) was defined by 3× standard deviation (SD) above the mean OD of control wells, and the

ability of biofilm formation was classified as follows: no biofilm producer (OD≤ODc), weak biofilm producing (ODc<OD≤2×ODc), moderate biofilm producing (2×ODc<OD≤4×ODc), and strong biofilm producing (4×ODc<OD) .

Detection of biofilm and virulence genes

Three biofilm genes *rmlA*, *spgM* and *rpfF* and four virulence genes *Stmpr1*，*Stmpr2*，*smf-1*and *Smlt3773* were amplified by PCR. PCR was performed as previously described in MLST part, and these genes were showed in Supplementary Table 1.

Risk factor analysis

The clinical information of 30 patients with *S. maltophilia* infection from ICU was collected. Each *S. maltophilia*-infected patient was matched for 2 patients without *S. maltophilia* infection from the same department, the same period and the age difference of less than 3 years. Meanwhile, the routine of blood infection and the results of biochemical tests were analyzed. At the same time, clinical diseases, operations, treatments and other items for each patient were combined to analyze the risk factors of *S. maltophilia* infections in patients from ICU.

Statistical analysis

SPSS 22 and GraphPad Prism 8 software were used for data processing. The normality was analyzed by Shapiro-Wilk test, Mean ± SD and one-way ANOVA were used for continuous variables, and chi-square test was used for categorical data. Percentage(%) was used for positive rate. Values of $p < 0.05$ were considered to be statistically significant. The correlation between clonal typing and drug resistance rate, and the correlation between biofilm and drug resistance rate were compared by Pearson correlation coefficient.

RESULTS

Patients and bacteria strains

The clinical information of patients was collected and a total of 93 strains of non-repetitive *S. maltophilia* were isolated. Among them, 30 strains of *S. maltophilia* were isolated from ICU and the rest were from a range of hospital departments (Figure 1). The rest included 13 strains of neurosurgery, 9 strains of emergency internal medicine, 8 strains of cadre health care, 6 strains of cardiovascular surgery, 5 strains of hematology, 4 strains of liver surgery, 3 strains of oncology, 3 strains of nephrology, 2 strains of emergency medicine, 2 strains of neurology, There were 2 strains in cholangiopancreatic surgery, 1 in thoracic surgery, 1 in gastrointestinal surgery, 1 in respiratory medicine, 1 in digestive medicine, 1 in general surgery and 1 in urology.

Among the patients with *S. maltophilia* infections, 62 males (66.7%) and 31 females (33.3%) were included. 73 patients were aged 60 years and above (78.5%). Before bacteria isolation, 35 patients (37.6%) were subjected to invasive examinations or treatments. Among all the *S. maltophilia* strains, there were 81 strains (87.1%) isolated from sputum, 7 strains (7.5%) from drains, 2 strains from pleural effusion, 1 strain from ascites, 1 strain from urine and 1 strain from blood.

Of the 93 patients, 7(7.5%) had no basic diseases, mainly with fracture and malnutrition, and 86 (92.5%) had one to five underlying diseases: 25 had malignant tumor, 24 had hypertension, 12 had coronary heart disease, 10 had renal insufficiency, 5 had leukemia, 14 had head trauma, 20 had chronic bronchitis or pneumonia, 12 had liver injury. All patients had a history of antibiotic use (1-6 kinds), with an average of 3 antibiotics per person before isolation of *S. maltophilia*, of which 69 had used 3 or more antibiotics. The antibiotics mainly include cephalosporins (65/93), carbapenem (53/93), enzyme inhibitors (51/93), quinolones (35/93), glycopeptide (22/93), Aminoglycosides (13) and tetracyclines (9)(Table 1).

Table 1 The Clinical characters of adult and pediatric patients

	Adult (n=93)
Demographics	
Age(year, average,range)	66.3 (16-99)

Gender: male	62 (66.7%)
Baseline diseases, n(%)	
Hypertension	24 (25.8)
Heart Disease	12 (12.9)
Malignancy	25 (26.9)
Pulmonary Disease	20 (21.5)
Liver Disease	12 (12.9)
Leukemia	5 (5.4)
Head trauma	14 (15.1)
Strain isolation n(%)	
ICU	30 (32.3)
Sputum	81 (87.1)
Invasive operation n(%)	35 (37.6)
Previous antibiotics usage n(%)	
The number of antibiotics ≥3	69 (74.2)
Cephalosporins	65 (69.9)
Carbapenems	53 (57.0)
Enzyme Inhibitors	51 (54.8)
Quinolones	35 (37.6)
Glycopeptides	22 (23.7)
Aminoglycosides	13 (14.0)

MLST analysis

The distribution of clonal typing of *S. maltophilia* is relatively scattered. According to the different alleles, strains were assigned to 61 sequence types. Among them, 45 types of 60 strains were different from those published on PubMLST database (recorded as STnew1-STnew45). The other 33 strains were of existing types in the database, of which a relatively larger number was ST23 (n=8). There were also strains of ST5 (n=3), ST15 (n=3), ST24 (n=3), ST3 (n=2), ST84 (n=2), ST89 (n=2), ST99 (n=2) and ST4, ST8, ST13, ST36, ST77, ST98, ST102, and ST112. The 8 *S. maltophilia* strains of ST23 were distributed in 5 different departments, and the 30 strains of *S. maltophilia* isolated from the ICU could be classified into 24 sequence

types. In other departments, we also did not acquire *S. maltophilia* strains of the exact same sequence types, indicating that there was no obvious clonal transmission of *S. maltophilia* infections in this study [1]. The detailed results are shown in Figure 2.

PFGE typing results

According to the diagnostic criteria of PFGE typing, for different banding types, if the similarity reaches 95%, it can be classified into the same subtype(Popovic *et al.*, 2001), PFGE profiles can be classified into a group or cluster if there are no more than 3 bands, indicating that there may be correlation between strains. Through the analysis of 93 SMA strains, the results showed that the clustering situation was scattered and could be divided into 73 clusters, of which 13 strains could be divided into the same cluster (from 8 departments, not from the same department). Another five and two strains were divided into the same cluster, and the others were quite different. These results suggest that these strains do not have an outbreak in the department, and the detailed results are shown in Supplementary Figure1.

Virulence gene detection

The results of virulence gene detection showed that the carriage rates of four virulence genes were 79.6% (74/93) for *Stmpr1*, 91.4% (85/93) for *Stmpr2*, 94.6% (88/93) for *Smf-1* and 52.7% (49/93) for *Smlt3773*. There were 31 strains of *S. maltophilia* carrying all of the four genes.

Analysis of drug resistance

The resistance rates of *S. maltophilia* to levofloxacin, TMP/SMX and piperacillin/tazobactam were 4.3%, 9.7% and 8.6%, respectively. The resistance rate to cefoperazone/sulbactam was 4.3% and all of *S. maltophilia* strains were sensitive to minocycline. Two strains of them (ji20 and ji55) were resistant to both piperacillin/tazobactam and cefoperazone/sulbactam, and one strain ji73 was resistant to TMP/SMX, piperacillin/tazobactam and cefoperazone/sulbactam. One strain ji82 was resistant to four tested drugs except minocycline.

Biofilm forming ability

The average biofilm forming ability of *S. maltophilia* was OD_{492}=0.54 ± 0.49 (0.044 - 2.34). The OD values of *S. maltophilia* isolated from male and female patients were OD_{492} of 0.52 ± 0.51 and OD_{492} of 0.57 ± 0.47, and there was no significant difference between the two gender groups. There was no significant difference in biofilm formation ability between people aged 60 and above and those under 60 years old, as shown in Figure 3. At the same time, the drug resistance and biofilm forming ability of the strains were analyzed, and there was no obvious correlation between the drug-resistant phenotype and the biofilm forming ability, as shown in Table 2. The carrying rates of the three biofilm genes *rmlA*, *spgM*, *rpfF* were 82.8% (77/93), 92.5% (86/93) and 64.5% (60/93), respectively. The point mutations of *spgM* gene in the strains with strong biofilm forming ability were relatively consistent and significantly different from those with weak biofilm forming ability. The detailed sequencing results of some strains are shown in Figure 4 (the bases of two strains with different biofilm forming ability are selected as the representative). while the other two biofilm genes don't have obvious point mutations in strains with different biofilm forming ability.

Table 2 Drug-resistant rates and relationship between the drug resistance and biofilm formation

Antibiotics	Resistant rate	Pearson's correlation
levofloxacin	4.3%	0.02
sulfamethoxazole	9.7%	0.04
piperacillin / tazobactam	8.6%	0.1
cefoperazone / sulbactam	4.3%	0.08
minocycline	0%	NA

The carriage of virulence genes

The carrigae of four virulence genes *Stmpr1*，*Stmpr2*，*smf-1*and *Smlt3773locus* were 79.6%, 91.4%, 94.6% and 52.7%.

Analysis of risk factors of ICU patients infected with the *S. maltophilia*

By univariate analysis, we concluded that the changes of lymphocytes, albumin and the use of antibiotics are the influencing factors (Table 3). After multivariate analysis, the type of antibiotic use and lymphocyte count were the independent risk factors of infection with *S. maltophilia*, and the lymphocyte count was used as a common index of blood routine examination (Table 4). The observation of it may be used as a new reference index for clinical sensitivity and control of *S. maltophilia*.

Table 3 Univariate analysis of risk factors of *S. maltophilia* infections in ICU

Items	Patients (n=30)	Control (n=60)	P value	OR(95%CI)
male gender	23（76.7%）	38（63.3%）	0.263	0.565（0.208-1.534）
age(years)	64.8 ± 19.1	65.5 ± 16.9	0.873	
leukocyte	11.5 ± 5.4	10.9 ± 4.1	0.777	
neutrophil	9.4 ± 4.9	9.4 ± 3.9	0.767	
lymphocyte	1.3 ± 0.9	0.9 ± 0.4	0.012	
monocyte	0.7 ± 0.5	0.5 ± 0.4	0.536	
albumin	30.6 ± 4.2	28.3 ± 5.7	0.033	
globulin	29.0 ± 6.4	28.2 ± 7.0	0.286	
prealbumin	129.0 ± 52.3	124.8 ± 49.9	1.000	
surgeries	14（46.7%）	27（45.0%）	0.496	0.724（0.286-1.835）
organ transplantation	5（16.7%）	9（15.0%）	0.987	0.990（0.296-3.310）
malignant tumor	8（26.7%）	15（25.0%）	0.894	0.933（0.337-2.585）
hypertension	7（23.3%）	15（25.0%）	0.923	0.949

				（0.328-2.748）
diabetes	3（10.0%）	9（15.0%）	0.397	0.547（0.135-2.213）
pulmonary infection	9（30.0%）	16（26.7%）	0.990	1.007（0.374-2.712）
cardiopathy	4（13.3%）	9（15.0%）	0.841	0.875（0.238-3.213）
liver injury	4（13.3%）	7（11.7%）	0.972	1.024（0.272-3.856）
trachea intubation	12（40.0%）	21（35.0%）	0.941	1.036（0.406-2.640）
Chemotherapy	2（6.7%）	3（5.0%）	0.843	1.205（0.189-7.681）
immunosuppressor	9（30.0%）	17（28.3%）	0.867	0.920（0.343-2.464）
number of antibiotics	3.6 ± 1.2	3.0 ± 1.1	0.029	
carbapenems	21（70.0%）	40（66.7%）	0.731	1.187（0.445-3.167）
Cephalosporins	20（66.7%）	45（75.0%）	0.604	0.771（0.289-2.059）
quinolones	16（53.3%）	30（50.0%）	1.000	1.000（0.396-2.523）

Table 4 Multivariate logistic regression analysis associated with *S. maltophilia* infections in ICU

Risk factors	B value	Wals	P value	OR value	95% CI	
					lower limit	upper limit
lymphocyte	1.077	4.208	0.04	2.937	1.049	8.222
albumin	0.099	3.05	0.081	1.104	0.988	1.234
antibiotics	0.596	5.956	0.015	1.814	1.124	2.927

DISCUSSION

S. maltophilia is an environmental global emerging gram-negative multiple-drug-resistant organism that is most commonly associated with respiratory infections in human beings(Brooke, 2012). It is also responsible for many other infectious diseases including bacteremia, endocarditis and urethral infection etc, especially among the immunocompromised as well as those with aggressive treatments(Adegoke *et al.*, 2017). In patients with pneumonia infection, the mortality rate can be as high as 14% to 69%(Victor *et al.*, 1994; Jang *et al.*, 1992). In recent years, *S. maltophilia* has been ranked third among non-fermentative gram-negative bacilli according to CHINET monitoring and has been relatively stable, just after *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, but its isolation rate showed an increasing trend(Hu *et al.*, 2018). From our study, we found that the patients aged 60 and above were more susceptible to *S. maltophilia* infections, which may be related to their hypoimmunity. Most of the patients had underlying diseases, and 37.6% of the patients have had previously invasive examinations or treatments, and there were relatively more male patients. The findings remind us to do a good job in the prevention of *S. maltophilia* infections for certain population groups.

The sequence types of 93 strains of *S. maltophilia* were quite different, which indicated that these isolates had loose associations and thus the outbreak of *S. maltophilia* infections has hardly occurred. The main discovered types of MLST by our study were not consistent with that reported in other countries. At the same time, the consistency between MLST and PFGE was poor, and the same ST genotype had no similar with PFGE typing, indicating that SMA had genetic diversity, which was consistent with the foreign reports on the differences between the two detection methods(Tanimoto, 2013; Nicoletti *et al.*, 2011).

All patients had prior antibiotic usage (1 to 6 kinds) before our isolation of *S. maltophilia*, of which 69 had been used for 3 or more times. The majority of the used antibiotics included cephalosporins, carbapenem and enzyme inhibitors. On the other hand, the use of cephalosporins and carbapenem is easily able to cause *S. maltophilia*

to be selected as the dominant flora. According to the epidemic characteristics of *S. maltophilia*, we believe that *S. maltophilia* infection should be endogenous infection under the interaction between drug selection and its own environment, rather than interpersonal infection in ward(Brooke, 2012).

S. *maltophilia* exhibits complicated resistance to a broad array of antibiotics, limiting the available therapeutic options. Because *S. maltophilia* is intrinsically resistant to a variety of antibiotics, we did not do drug resistance analysis to other drugs, but the five commonly used antimicrobial agents against *S. maltophilia* infections in clinic. The results of antibiotic sensitivity test showed that *S. maltophilia* strains isolated in this study were sensitive to five targeted antimicrobial agents in clinic, and the drug resistance rate was low. However, a strain was still resistant to four of the tested antibiotics. Considering the existence of the complicated multidrug-resistant mechanisms of *S. maltophilia* and the worsened co-infection phenomenon, it is suggested that we should not relax on its infection, especially the monitoring of multidrug-resistant *S. maltophilia*. In the analysis of risk factors, in addition to the analysis of common factors, we specially examined the indicators related to hypoimmunity, such as leukocytes, neutrophils and immunoglobulins. However, leukocytes and neutrophils are greatly affected by the outside influence, the number of which will rise once there is a bacterial infection. We speculate that lymphocytes which are less affected by stress and microbial factors, and immunoglobulin, albumin and prealbumin affected by nutritional factors may be used as observable indicators. The results also showed that lymphocytes and albumin were indeed single factors of *S. maltophilia* infection, and lymphocytes could be used as independent influencing factors to provide an important way and basis for guiding clinically *S. maltophilia* monitoring, prevention and control. In the group infected with *S. maltophilia*, the concentration of albumin is higher than that of the control group, however, whether it is caused by compensation or other factors needs to be deeply studied. In our study, the time of carbapenem usage and endotracheal intubation which were likely to be risk factors, were not accurately recorded. This is a

major defect in our study thus we will make a supplement to these two indicators lately.

There are also some inadequacies in this study. We found the mutations of *spgM* gene in the strains with strong biofilm forming ability were significantly different from those with weak biofilm forming ability. But we have not done further explore to evidence the exact mechanisms in this study, which needs to be payed further attention in our next study.

In a word, the infection-related strains of *S. maltophilia* had strong biofilm formation ability and high virulence gene carrying rate. Before the isolation, most of the patients had basic diseases and a history of the usage of antibiotics. Some of them have had invasive operations. The genotyping of the strains showed high diversity, indicating the distant correlation of these strains and hardly-occurred clonal transmission of *S. maltophilia* in the same department, which suggested that clinical *S. maltophilia* infections is perhaps, an endogenous infection under antibiotics selection. Aggressive testing and treatment are supposed to be rationally applied to reduce the potential risk of *S. maltophilia* infections.

CONFLICT OF INTRERST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

Zhongliang Duan and Juanxiu Qin operated all the experiments and this manuscript. Yao Liu assisted in writing manuscript. Cui Li assisted in data analysis. Chunmei Ying conceived and supervised this study. All authors approve this manuscript.

ETHICS STATEMENT

This study was approved by Institutional Review Board of the Renji Hospital. As a retrospective study, informed consent was granted exemption.

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References:

Adegoke, A. A., Stenstrom, T. A. & Okoh, A. I. (2017). Stenotrophomonas maltophilia as an Emerging Ubiquitous Pathogen: Looking Beyond Contemporary Antibiotic Therapy. *FRONT MICROBIOL* **8**, 2276.

Brooke, J. S. (2012). Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. *CLIN MICROBIOL REV* **25**, 2-41.

Hu, L. F., Xu, X. H., Li, H. R., Gao, L. P., Chen, X., Sun, N., Liu, Y. Y., Ying, H. F. & Li, J. B. (2018). Surveillance of antimicrobial susceptibility patterns among Stenotrophomonas maltophilia isolated in China during the 10-year period of 2005-2014. *J Chemother* **30**, 25-30.

Jang, T. N., Wang, F. D., Wang, L. S., Liu, C. Y. & Liu, I. M. (1992). Xanthomonas maltophilia bacteremia: an analysis of 32 cases. *J FORMOS MED ASSOC* **91**, 1170-1176.

Kaiser, S., Biehler, K. & Jonas, D. (2009). A Stenotrophomonas maltophilia multilocus sequence typing scheme for inferring population structure. *J BACTERIOL* **191**, 2934-2943.

Nicoletti, M., Iacobino, A. & Prosseda, G. & other authors (2011). Stenotrophomonas maltophilia strains from cystic fibrosis patients: genomic variability and molecular characterization of some virulence determinants. *INT J MED MICROBIOL* **301**, 34-43.

Okazaki, A. & Avison, M. B. (2008). Induction of L1 and L2 beta-lactamase production in Stenotrophomonas maltophilia is dependent on an AmpR-type regulator. *Antimicrob Agents Chemother* **52**, 1525-1528.

Pompilio, A., Pomponio, S. & Crocetta, V. & other authors (2011). Phenotypic and genotypic characterization of Stenotrophomonas maltophilia isolates from patients with cystic fibrosis: genome diversity, biofilm formation, and virulence. *BMC MICROBIOL* **11**, 159.

Popovic, T., Schmink, S. & Rosenstein, N. A. & other authors (2001). Evaluation of pulsed-field gel electrophoresis in epidemiological investigations of meningococcal disease outbreaks caused by Neisseria meningitidis serogroup C. *J CLIN MICROBIOL* **39**, 75-85.

Samonis, G., Karageorgopoulos, D. E., Maraki, S., Levis, P., Dimopoulou, D., Spernovasilis, N. A., Kofteridis, D. P. & Falagas, M. E. (2012). Stenotrophomonas maltophilia infections in a general hospital: patient characteristics, antimicrobial susceptibility, and treatment outcome. *PLOS ONE* **7**, e37375.

Tanimoto, K. (2013). Stenotrophomonas maltophilia strains isolated from a university hospital in Japan: genomic variability and antibiotic resistance. *J MED MICROBIOL* **62**, 565-570.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J CLIN MICROBIOL* **33**, 2233-2239.

Victor, M. A., Arpi, M., Bruun, B., Jonsson, V. & Hansen, M. M. (1994). Xanthomonas maltophilia bacteremia in immunocompromised hematological patients. *Scand J Infect Dis* **26**, 163-170.

Wu, A. L., Yeh, L. K. & Ma, D. H. & other authors (2016). Clinical Characteristics of Stenotrophomonas maltophilia Keratitis. *CORNEA* **35**, 795-800.