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

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Research article

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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 every year. This study is an epidemiological analysis of the clinical and molecular characteristics of S . maltophilia infections in a Chinese teaching hospital. The goal is to obtain 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: A total of 93 isolates from the Renji Hospital were included, in which 81 isolates were cultured from sputum samples, and 86 patients had underlying diseases. All of the patients had received antibiotic therapeutics. The multilocus sequence typing (MLST) analysis indicated that 61 different sequence types (STs) were found (including 45 novel STs), and the MLST did not show significantly dominant STs. The resistant rates to Trimethoprim/sulfamethoxazole (TMP/SMX) and levofloxacin were 9.7% and 4.3%. All of 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%, respectively. The positive rates of the three biofilm genes rmlA , spgM, and rpfF were 82.8%, 92.5%, and 64.5%, respectively. The mean biofilm forming level of OD 492 was 0.54 ± 0.49, and there was no significant difference between different genders and age groups. Data from patients in the intensive care unit (ICU) and the control group were analyzed, and the independent risk factors of those who were infected in the ICU included immunosuppression and the increased of the use of antibiotics.

Conclusions : Most of the patients had prior medical usage histories 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 was an independent risk factor for S . maltophilia infection, which should receive more attention. No obvious clonal transmission was found in the same department.

Background

S. Maltophilia, called *Xanthomonas maltophilia* previously, is a non-fermentative, gram-negative aerobic bacilli. It is a cosmopolitan bacteria, which were originally plant pathogens, being ubiquitous in natural environments like water, soils and plants[1]. It can also be found in medical settings. *S. maltophilia* has been found to be a contaminant on numerous hospital devices, including dialysis devices, blood pressure monitors, faucets, sphygmomanometers, disinfectants, and ventilators, and has the ability to transmit between patients or from patients to healthy persons[2]. Clinical evidence has shown that *S. maltophilia* can cause nosocomial infections in immunocompromised hosts, such as respiratory system infections, joint infections, and skin infections[3]. Wu et al.[4] also found that the dominant flora in keratitis infection was *S. maltophilia.* In several 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[5].

With the abuse of different types of antibiotics, chemotherapy drugs, and immunosuppressants, and the widespread use of invasive exploration equipment, the isolation and infection rates of *S. maltophilia* in hospitals are continuing to grow. Brooke[2] reported that particular attention was required for inpatients with immunosuppression. *S. maltophilia* is intrinsically resistant to several kinds of antibiotics due to various resistance mechanisms. It can produce a penicillinase (L1) and a cephalosporinase (L2), which makes it easily resistant to β-lactam antibiotics, specifically carbapenems[6]. It can also produce aminoglycoside modifying enzymes and have a certain degree of resistance to aminoglycoside drugs, and the efflux pump system makes it resistant to a variety of antimicrobial agents[7]. Hence the continuous emergence of multidrug-resistant strains of *S. maltophilia*has have brought great challenges for the treatment of serious *S. maltophilia* infections[5].

There have been few studies that have investigated the comprehensive clinical and molecular characteristics of the *S. maltophilia* system in Shanghai. Therefore, in this study, multilocus sequence typing (MLST) is used to analyze the molecular epidemiological characteristics of *S. maltophilia* isolated from the Renji Hospital affiliated with the Shanghai Jiaotong University School of Medicine. The risk factors for acquiring *S. maltophilia* infections from ICU patients are also analyzed. The clinical information of the related patients are collected and, at the same time, the virulence genes and biofilm genes of *S. maltophilia* are detected. The aim of this study is to develop an intuitive description of the epidemic situation of the strain in clinic. This will provide the necessary groundwork for basic and mechanical studies and provide support for the prevention and treatment of *S. maltophilia* infections.

Results

Patients and bacteria strains

Patient clinical information 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 the ICU, and the rest were from a range of hospital departments (Figure 1). The rest included 13 strains from neurosurgery, 9 strains from emergency internal medicine, 8 strains from cadre health care, 6 strains from cardiovascular surgery, 5 strains from hematology, 4 strains from liver surgery, 3 strains from oncology, 3 strains from nephrology, 2 strains from emergency medicine, 2 strains from neurology, 2 strains from cholangio-pancreatic surgery, 1 from thoracic surgery, 1 from gastrointestinal surgery, 1 from respiratory medicine, 1 from digestive medicine, 1 from general surgery, and 1 from urology.

Among the patients with *S. maltophilia* infections, 62 males (66.7%) and 31 females (33.3%) were included. A total of 73 patients were aged 60 years and above (78.5%). Before bacterial 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 but primarily had fractures and malnutrition, and 86 (92.5%) had one to five underlying diseases. Of these, 25 had malignant tumors, 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, and 12 had liver injury. All of the patients had a history of antibiotic use (1-6 types), with an average of three antibiotics per person prior to isolation of *S. maltophilia*, of which 69 had used three or more antibiotics. The antibiotics primarily included cephalosporins (65/93), carbapenem (53/93), enzyme inhibitors (51/93), quinolones (35/93), glycopeptide (22/93), aminoglycosides (13/93), and tetracyclines (9/93) (Table 1).

Table 1. The clinical characteristics 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 the clonal typing of *S. maltophilia* was relatively scattered. According to the different alleles, the strains were assigned to 61 sequence types. Among them, 45 types of the 60 strains were different from those published on the PubMLST database (recorded as STnew1-STnew45). The other 33 strains consisted 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), and ST99 (n=2) and ST4, ST8, ST13, ST36, ST77, ST98, ST102, and ST112. The eight *S. maltophilia* strains of ST23 were distributed in five different departments, and the 30 strains of *S. maltophilia* isolated from the ICU were classified into 24 sequence types. *S. maltophilia* strains of the exact same sequence types were not collected in other departments, indicating that there was no obvious clonal transmission of *S. maltophilia* infections in this study [1]. The detailed results are shown in Figure 2.

Virulence gene detection

The results of the virulence gene detection showed that the carriage rates of the 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* that carried all four of the genes.

Analysis of drug resistance

The resistance rates of *S. maltophilia* to levofloxacin and TMP/SMX were 4.3% and 9.7%, respectively. All of *S. maltophilia* strains were sensitive to minocycline. Among these strains, one strain, numbered ji82, was resistant to both TMP/SMX and levofloxacin.

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 the male and female patients were OD_{492} of 0.52 ± 0.51 and OD_{492} of 0.57 ± 0.47, respectively, and there was no significant difference between the two gender groups. There was no significant difference in the biofilm formation ability between people aged 60 and above and those under 60 years old, as shown in Figure 3. In addition, 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*, and *rpfF* were 82.8% (77/93), 92.5% (86/93), and 64.5% (60/93), respectively. The point mutations of the *spgM* gene in the strains with strong biofilm forming abilities were relatively consistent and significantly different from those with weak biofilm forming abilities. The detailed sequencing results of some strains are shown in Figure 4 (the bases of the two strains with different biofilm forming abilities were selected

as the representatives). However, the other two biofilm genes did not have obvious point mutations in the strains with different biofilm forming abilities.

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 the virulence genes

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

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

By using a univariate analysis, it was concluded that the changes in lymphocytes, albumin, and the use of antibiotics were infection risk factors in the ICU patients (Table 3). After the multivariate analysis, the type of antibiotic use and lymphocyte count were found to be independent risk factors of infection with *S. maltophilia*, and the lymphocyte count was used as a common index for a routine blood examination (Table 4). These findings 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 the ICU

Items	Patients (n=30)	Control (n=60)	P value	OR(95%CI)
male (sex)	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 the 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[2, 8]. It is also responsible for many other infectious diseases including bacteremia, endocarditis, and urethral infection, especially among the immunocompromised, as well as those undergoing aggressive treatments[1]. In patients with pneumonia infections, the mortality rate can be as high as 14% to 69%[9, 10]. In recent years, *S. maltophilia* has been ranked third among non-fermentative gram-negative bacteria according to the CHINET monitoring service and has been relatively stable, following *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, but its isolation rate has displayed an increasing trend[5]. According to the results of this study, patients aged 60 and above were more susceptible to *S. maltophilia* infections, which may be related to their immunosuppression. Most of the patients had underlying diseases, and 37.6% of the patients had previously invasive examinations or treatments. In addition, there were relatively more male patients. These findings remind clinicians 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 outbreaks of *S. maltophilia* infections have typically not occurred. The primary discovered types found using MLST in this study were not consistent with that reported in other countries. In addition, the consistency between the MLST and PFGE results was poor, and the same ST genotype had no similarity with the PFGE typing (the PFGE results are not shown). This indicated that *S. maltophilia* had genetic diversity, which was consistent with the results of foreign studies regarding the differences between the two detection methods[11-13].

All of the patients had prior antibiotic usage (1 to 6 types) prior to the isolation of *S. maltophilia*, of which, 69 of the patients had used antibiotics three or more times. The majority of the used antibiotics included cephalosporins, carbapenem, and enzyme inhibitors. However, the use of cephalosporins and carbapenem can easily cause *S. maltophilia* to be selected as the dominant flora. According to the epidemic characteristics of *S. maltophilia*, it is believed that *S. maltophilia* infection is an endogenous infection under the interaction between drug selection and its own environment, rather than an interpersonal infection in a ward[2].

S. maltophilia exhibits complicated resistance to a broad array of antibiotics, limiting available therapeutic options. Because *maltophilia* is intrinsically resistant to a variety of antibiotics, a drug resistance analysis to other drugs was not performed in this study. However, three commonly used antimicrobial agents against *S. maltophilia* infections in clinic were analyzed. The results of the antibiotic susceptibility test showed that the *S. maltophilia* strains isolated in this study were sensitive to three targeted antimicrobial agents in clinic, and the drug resistance rate was low. However, one strain was still resistant to two of the tested antibiotics. Due to the existence of complicated multidrug-resistant

mechanisms in *S. maltophilia* and the worsened co-infection phenomenon, it is suggested that diligence to this infection be still needed, especially in monitoring of the multidrug-resistant *S. maltophilia*. In the analysis of risk factors, in addition to the analysis of common factors, indicators related to immunosuppression, such as leukocytes, neutrophils, and immunoglobulins, were specifically examined. However, leukocytes and neutrophils are greatly affected by the outside influences, the number of which will rise once there is a bacterial infection. It was speculated 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 method and basis for guiding clinically *S. maltophilia* monitoring, prevention, and control. In the group infected with *S. maltophilia*, the concentration of albumin was higher than that of the control group. However, whether this was caused by compensation or other factors needs to be deeply studied. In this study, the time of carbapenem usage and endotracheal intubation, which are likely to be risk factors, were not accurately recorded. This was a major defect of this study, and a supplement study to investigate these two indicators will be performed in the future. There are also some other inadequacies in this study. It was found that mutations in the *spgM* gene in the strains with strong biofilm forming abilities were significantly different from those with weak biofilm forming abilities. However, further exploration was not performed to investigate the exact mechanisms. This aspect requires further attention in a future study.

Conclusions

The genotyping of the strains showed high diversity, indicating the distant correlation of these strains and the low-occurring clonal transmission of *S. maltophilia* in the same department. This suggested that clinical *S. maltophilia* infections are perhaps endogenous infections under antibiotic selection. This conclusion is a reminder that antibiotics should be reasonably used so as to reduce the incidence of infection. The mutations in the *spgM* gene are associated with biofilm forming abilities, which is worthy of future research. Due to the strong biofilm forming ability and high virulence gene carrying rate, high risk patient groups should receive more attention to avoid potential risk of *S. maltophilia* infection.

Methods

Materials and reagents

A total of 93 non-repetitive strains of *S. maltophilia* isolated from outpatients and inpatients from Renji hospital in 2014 were collected, in addition to the patients' clinical information. These strains were identified using the VITEK-2 Compact System (bioMérieux, France) and confirmed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany). The samples were then stored at 80°C.

The bacteria were then grown in tryptic soy broth (TSB; Oxoid) at 37°C overnight. The antibiotics used for disk diffusion testing were levofloxacin, trimethoprim-sulfamethoxazole, and minocycline (Oxoid, UK). The quality control strains included *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853.

Disk diffusion testing

Disk diffusion was performed according to the Clinical and Laboratory Standards Institute (CLSI) 2019 recommendations for levofloxacin, trimethoprim-sulfamethoxazole (TMP/SMX), and minocycline.

Multi-locus sequence typing (MLST) analysis

Primer sequences targeting the conserved regions of seven housekeeping genes of *S. maltophilia* were used, as shown on the MLST site (http://pubmlst.org/smaltophilia/). The MLST was performed as described by Kaiser et al.[14].

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

The PCR mixture (2ÍPCR 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 using DNAstar software, and the obtained sequence was submitted to the MLST database to acquire the sequence type. Seven housekeeper genes were assembled using MEGA.4 software, and a phylogenetic analysis was conducted.

Biofilm formation assay

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

Risk factor analysis

Clinical information of 30 patients with *S. maltophilia* infections from the ICU was collected. Each *S. maltophilia*-infected patient was matched with two patients without *S. maltophilia* infection from the same department during the same period and with age differences of less than three years. In addition, the blood infection routines and the results of their biochemical tests were analyzed. Additionally, clinical diseases, operations, treatments, and other items for each patient were combined to analyze the risk factors of *S. maltophilia* infection in patients from the ICU.

Statistical analysis

SPSS 22 (IBM SPSS Statistics for Windows 22.0, IBM Corp. Armonk, NY, USA) and GraphPad Prism 8 (GraphPad software Inc.; San Diego, CA, USA) were used for the data processing. The normality was analyzed using the Shapiro-Wilk test. The median and range (or mean ± SD) and a one-way ANOVA were used for the continuous variables, and a chi-square test was used for the categorical data. Percentages (%) were used for the positive rates, such as the drugs resistant rate, biofilm genes positive rate, and virulence genes positive rate. Values of $p < 0.05$ were considered to be statistically significant. The correlation between the clonal typing and drug resistance rate, and the correlation between the biofilm and drug resistance rate were compared using the Pearson correlation coefficients.

Declarations

Competing interests

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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Figures

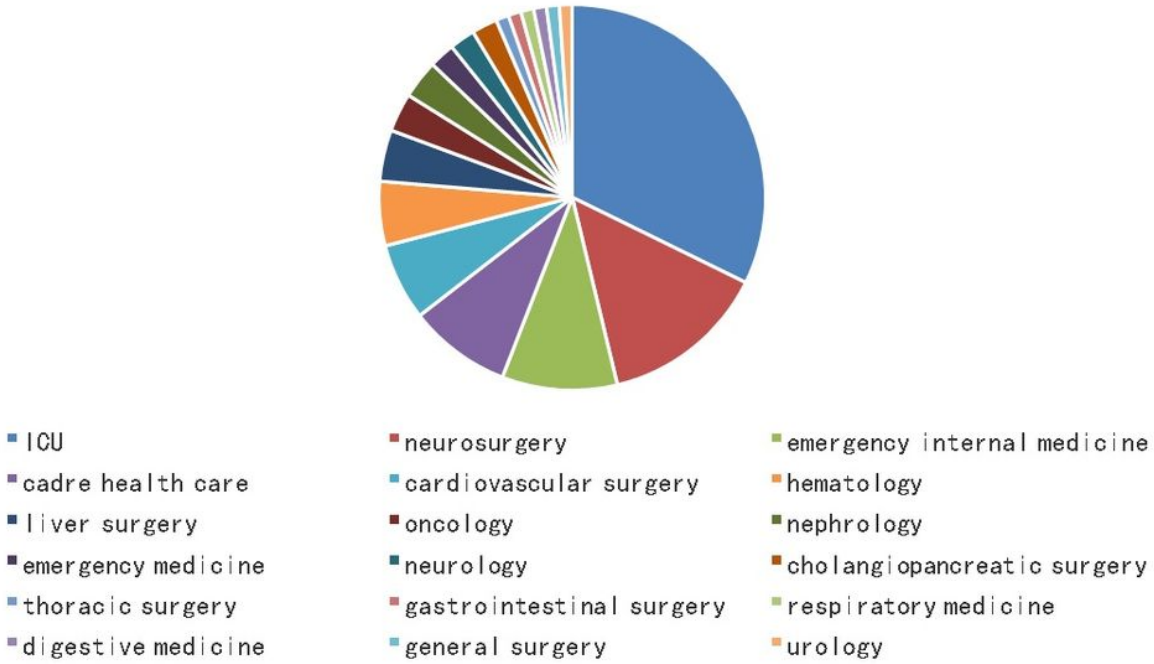

Figure 1

The distribution of S. maltophilia in the different departments The pie graph shows the distribution of the wards. among the top three wards, Blue, red, and yellow represent the ICU, neurosurgery, and emergency internal medicine ward, respectively.

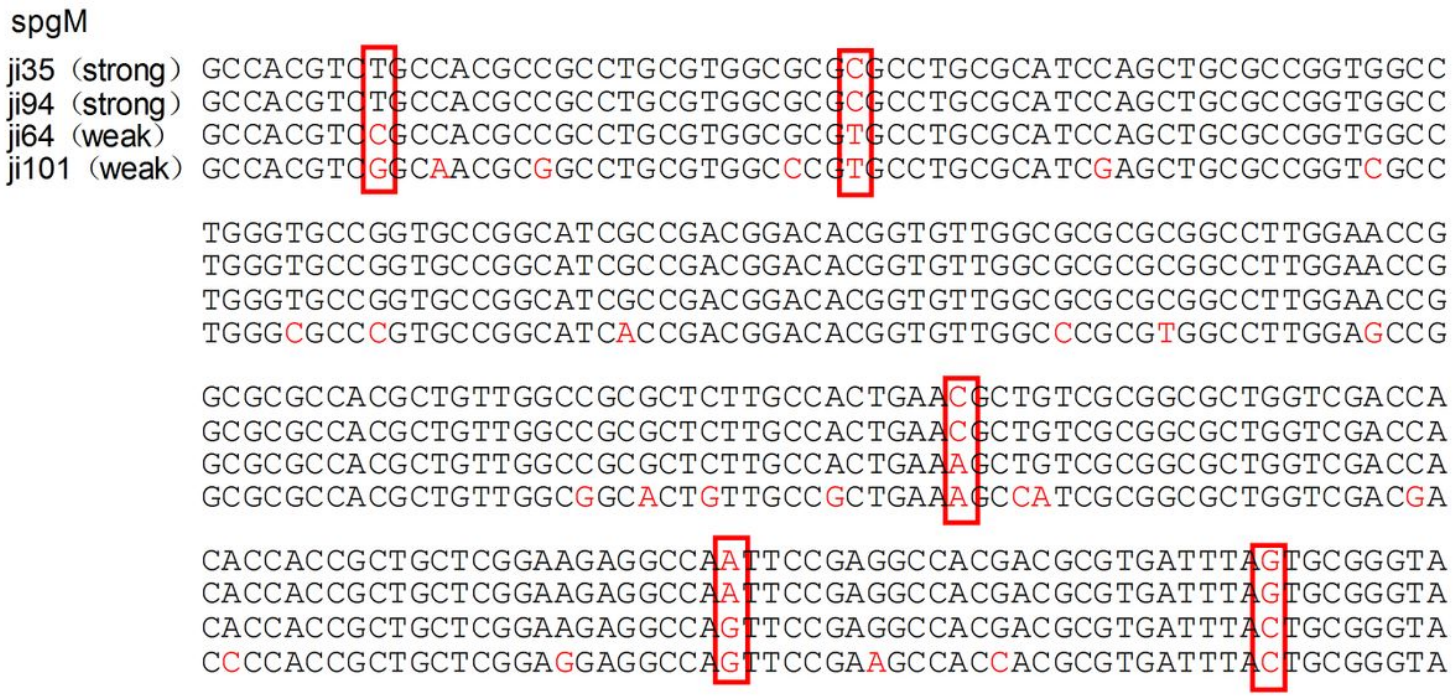

Figure 2

The MLST results of 93 S. maltophilia isolates This is a neighbor-joining tree analysis for the concatenated data for all seven loci of the 93 strains. The tree was rooted with the corresponding concatenate.

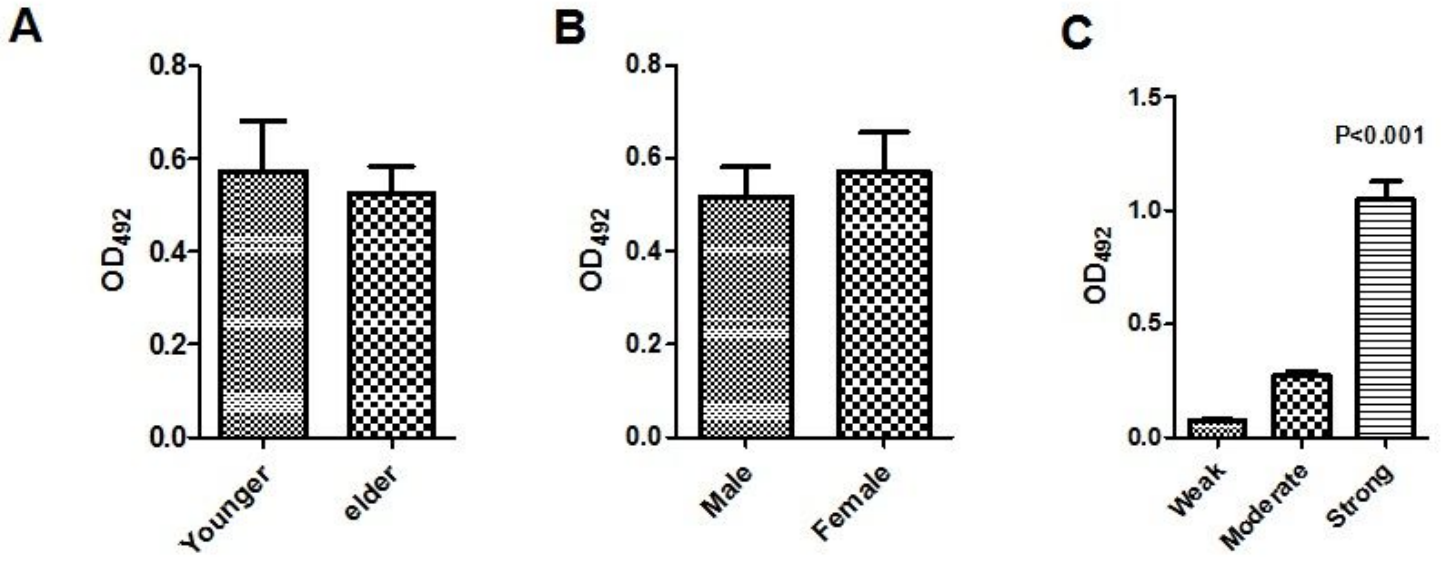

Figure 3

Biofilm formation abilities of S. maltophilia in the different genders and ages Histogram illustrating the ability of biofilm formation. There are no difference in different genders and ages. But the number of strains forming strong biofilms is significantly more than the weak and moderate ones.

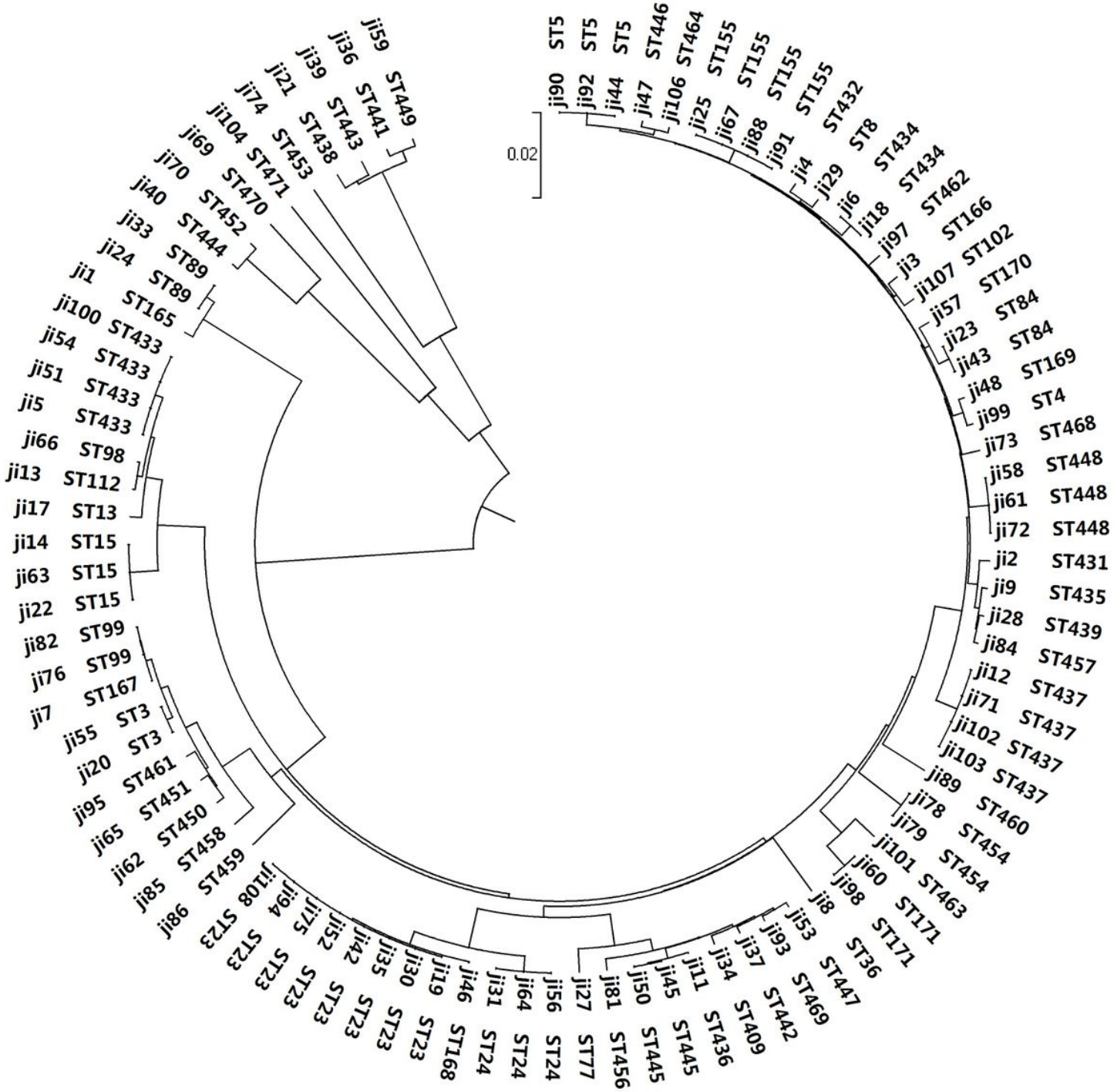

Figure 4

The point mutation of spgM in bacterial strains with different biofilm forming abilities The spgM gene mutations in strains with strong biofilm formation abilities, are significantly different from that with the

weak ones. The mutated parts of the DNA bases are shown in the red box.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryfig1.tif
- supplementarytable1.doc