

Multilocus Sequence Typing for Molecular Epidemiology of *Stenotrophomonas maltophilia* Clinical and Environmental Isolates from a Tertiary Hospital in West of Iran

Sinasadat Emami^{1*}, Jamileh Nowroozi¹, Ramin Abiri² and Parviz Mohajeri²

¹Microbiology Department of Islamic Azad University, North Tehran Branch, Iran;

²Department of Microbiology, Kermanshah University of Medical Science, Kermanshah, Iran

OPEN ACCESS

Received: 22 February 2021

Accepted: 9 April 2021

Published online: 26 January 2022

ABSTRACT

Background: *Stenotrophomonas maltophilia* is an opportunistic bacterium, contributing to different hospital-acquired infections and can be acquired from different hospital setting sources. Epidemiological study of *S. maltophilia* in the hospital also demonstrates the intrahospital distribution of certain strains of bacteria in healthcare facilities. The aim of the current study was to identify the molecular epidemiology of *S. maltophilia* isolates from clinical and environmental sources within a hospital.

Methods: A total of 400 samples (clinical and environmental) were collected from the different settings of hospital. Following the standard biochemical testing and 23S rRNA genotyping, the molecular typing of *S. maltophilia* isolates was determined using the MLST technique. Also, the frequencies of *zot* and *entF* virulence genes among *S. maltophilia* isolates were examined by PCR technique.

Results: Based on the biochemical testes and PCR targeting 23S rRNA gene, 22 *S. maltophilia* isolates were identified. The MLST analysis demonstrated that these isolates were assigned to 14 ST, and 6 out of 14 STs were common among clinical and environmental samples. All 22 isolates were identified in the PubMLST database. The PCR screening demonstrated that none of 22 *S. maltophilia* isolates had *zot* virulence gene, while the *entF* gene with the 59% frequency was observed in 13 out of 22 isolates. Among these 13 isolates, 6 STs were common in clinical and environmental isolates.

Conclusion: Our study showed the clonal relatedness between clinical and environmental sources of the *S. maltophilia* isolates in a hospital. Further studies are required to understand the epidemic situation of this pathogen in the clinic and the environment. DOI: 10.52547/ibj.26.2.142

Citation:

Emami S, Nowroozi J, Abiri R, Mohajeri P. Multilocus Sequence Typing for Molecular Epidemiology of *Stenotrophomonas maltophilia* Clinical and Environmental Isolates from a Tertiary Hospital in West of Iran. *Iranian biomedical journal* 2022; 26(2): 142-159.

Keywords: Environment, Multilocus sequence typing, *Stenotrophomonas maltophilia*

Corresponding Author: Jamileh Nowroozi

Vafadar Microbiology Department of Islamic Azad University, North Tehran Branch, Tehran, Iran; Tel.: (+98-21) 77009836;

Fax.: (+98-21) 77317998; E-mail: nowroozij@yahoo.com

INTRODUCTION

Stenotrophomonas maltophilia, as an anaerobic, non-fermentative and Gram-negative bacteria, is a ubiquitous species of the gamma subdivision of Proteobacteria^[1,2]. This environmental bacterium is

found in foods, animals, plant rhizospheres, and aqueous environments, as well as in contaminated medical care fluids and water sources^[1,3]. *S. maltophilia*, as an opportunistic pathogen, has been isolated from various water-based sources either inside or outside of the hospital environment or clinical

List of Abbreviations:

MLST, multilocus sequence typing; **PFGE**, pulsed-field gel electrophoresis; **Rep-PCR**, repetitive extragenic palindromic-PCR; ***S. maltophilia***, *Stenotrophomonas maltophilia*; **ST**, sequence types; **UPGMA**, unweighted pair group method with arithmetic mean

settings, with a broad geographical distribution^[4,5]. Despite the limited pathogenicity of this emerging bacterium^[4,6], it is responsible for different community-acquired and hospital-related diseases, especially in immunocompromised individuals, with a mortality rate of 37.5%^[4,7]. The prevalence of the bacterium among tracheal samples in Iran is estimated to be about 4.5%.

S. maltophilia can infect different organs and tissues^[8] and is associated with numerous clinical manifestations such as bacteremia, pneumonia, arthritis, sepsis, meningitis, endocarditis, endophthalmitis, urinary and respiratory tract infections^[9,10]. Patients hospitalized at intensive care units are more vulnerable to the *S. maltophilia* infection than healthy people^[11-13]. Based on a report from Iran, the high frequency of bacteria was found in bloodstream infections (88.6%). However, a lower rate (11.4%) of the bacteria was detected in general medicine wards^[14]. Hence, *S. maltophilia* is not a prevalent pathogen, and its imputed virulence factors, including biofilm formation, motility, adhesion capacity, hydrophobicity, and synthesis of extracellular enzymes, are responsible for the inflammatory response^[6,15]. Recently, increment of *S. maltophilia* isolation from different hospitals has contributed to uncontrolled and even exploited administration of antibiotics^[4,15]. Since this bacterium has a role in the high level of antibiotic resistance^[10,16,17], there is an increasing demand for new treatment options^[10,18].

High genetic diversity strains have been identified among *S. maltophilia* using a variety of biomolecular techniques. To discover the relationship of clinical isolates with environmental sources, several genotypic profiling methods have been employed. A number of these methods include whole genome sequencing analyses, amplified fragment length polymorphism fingerprinting, PCR-restriction fragment length polymorphism, the gyrase B gene analysis, and PCR-based fingerprinting methods, such as BOX-A1R-based repetitive extragenic palindromic-PCR, Rep-PCR, enterobacterial repetitive intergenic consensus PCR, PFGE analysis of XbaI genomic digests, and MLST^[4,19]. The MLST is administered to find the source of infections along with the distribution patterns of pathogens isolated from hospitalized patients. Therefore, it can provide reputable data from epidemiological distribution of the bacteria. Moreover, due to the availability of MLST data in public databases, the obtained results can be compared with other laboratories^[20]. The aim of the current study was to identify *S. maltophilia* isolated from clinical and environmental sources within a hospital using MLST technique in order to analyze the molecular and

epidemiological characteristics of this bacterium and examine the clonal relatedness between clinical and environmental specimens of the *S. maltophilia* isolates collected from hospital.

MATERIALS AND METHODS

Sample collection

S. maltophilia isolates were collected from different clinical and environmental settings in Imam Reza hospital (a 515-bed university-affiliated tertiary hospital in Kermanshah, West of Iran) over a 12-month duration from May 2019 to May 2020. The clinical specimens, including sputum, blood, and urine, were obtained from patients hospitalized in the hospital. Environmental samples were collected from equipment, surfaces, and solutions by rubbing sterile swabs on the 10-cm² surface of each selected area^[21].

Biochemical identification of *S. maltophilia* isolates

Clinical isolates were subcultured on Blood and MacConkey agars (Merck, Germany). Environmental isolates were cultured on blood agar plates and then were incubated at 37 °C for 24 hours. The standard biochemical testing, such as oxidase and catalase tests, was employed for the laboratory identification of the isolates, along with deoxyribonuclease test, triple sugar iron agar, and Sulfide Indole Motility (Merck). *S. maltophilia* ATCC 13637 was used as the control strain. All the isolates were stored in a Luria Bertani broth containing 20% glycerol at -70 °C^[22].

Molecular testing of *S. maltophilia* isolates

DNA extraction

The *S. maltophilia* isolates were cultured with aeration in Luria Bertani at 37 °C overnight. Next, total DNA extraction was performed using the high pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's protocol. The concentrations of all DNA samples were determined by NanoDrop ND-100 (NanoDrop Technologies, USA).

PCR analysis

The molecular identification of the *S. maltophilia* strain was confirmed by the detection of 23sr RNA using a common PCR method. Primers, including 23srRNA forward: 5'CTGGATTGGTTCTAGAAAA CGC3' and 23srRNA reverse: 5'ACGCAGTCACTCCT TGCG3', were applied in PCR reaction using a PCR kit (QIAGEN, Hilden, Germany) with the following thermal cycles: 94 °C (5 min) followed by 36 cycles of 94 °C (45 s), 58 °C (45 s), 72 °C (45 s), and final extension step at 72 °C (5 min). The PCR products were separated and visualized on 1% agarose gel.

Thereafter, samples showing the related fragments were verified by sequencing (Bioneer, Korea).

Molecular typing of *S. maltophilia* isolates by MLST technique

The MLST technique was performed for the molecular typing of *S. maltophilia* isolates as described earlier^[23].

Selection of housekeeping gene

Seven pair primers targeting the conserved regions of seven housekeeping genes of *S. maltophilia* were selected from MLST website (<http://pubmlst.org/smaltophilia/>). The genes included H (+)-transporting two-sector *atpD*, *gapA*, *guaA*, *mutM*, *nuoD*, *ppsA*, and *recA*. The PCR amplification of target fragments was performed as follows: initial denaturation at 94 °C (5 min) followed by 36 cycles of denaturation at 94 °C (45 s), annealing at 58.5 °C (45 s) for *atpD*, 57 °C for *gapA*, 57.5 °C for *mutM* gene, 56 °C for *mutM* and *nuoD* genes, 60 °C for *ppsA* gene, and 59.5 °C for *recA* gene, and extension step at 72 °C (45 s), with a final extension step at 72 °C (5 min). The PCR products were analyzed on agarose gel and were then sequenced by Bioneer Company (South Korea).

Data analysis

The obtained sequences were submitted to PubMLST database (<https://pubmlst.org/>) to determine the allele number and specific ST^[24]. All unique sequences were assigned with an allele number, and each unique combination of seven alleles in each isolate was assigned a ST^[25].

Phylogenetic analysis

The statistical analysis of allele profiles and sequence data was conducted using START 2.0 software to calculate guanine-cytosine content, frequencies of alleles, number of variable sites, and the dN/dS ratio (nonsynonymous per synonymous substitutions)^[24]. Phylogenetic tree was constructed by the neighbor-joining method in MEGA v.7 software (www.megasoftware.net). The dendrogram was constructed based on the UPGMA.

Frequency of *zot* and *entF* virulence genes among *S. maltophilia* isolates

The frequency of *zot* and *entF* genes among *S. maltophilia* isolates was examined by PCR with the following primer sets: *zot* forward: 5' GAATCGTGCTT TGATCTGC 3' and *zot* reverse: 5'AGAATACCGAGGT GTACGA 3' primers and were used for the amplification of *zot* gene with following program:

94 °C (5 min) and 35 cycles of 94 °C (45 s), 60 °C (45 s), 72 °C (45 s), and final extension step at 72 °C (5 min). The primers *entF* forward: 5' GATTACGCAAC GCTGGAAG 3' and *entF* reverse: 5' CACGATGTTC GATGACCACG 3' were used for the PCR amplification of *entF* gene similar to *zot* except for the annealing temperature, which changed to 56 °C.

RESULTS

Bacterial isolates

During one year of the study, 400 samples were collected from different sites of the hospital. Twenty-two *S. maltophilia* isolates were identified based on the biochemical tests and confirmed by PCR targeting 23S rRNA gene (Fig. 1). These isolates were obtained from clinical specimens and environmental sites; 10 out of 22 isolates were identified from clinical specimens (sputum, blood, and urine). The rest was obtained from dry (catheter, dialysis machine, manometer, ventilator, thermometer, stethoscope, suction tubes, and patient beds) and moist (bathtub, ice maker, tap, water reservoir, refrigerators, showerheads, sink drains, iodine, and detergents) sites. Among the clinical specimens, *S. maltophilia* isolates obtaining from sputum samples had the highest frequency, while the frequency of *S. maltophilia* isolated from different environmental samples was almost equal.

MLST analysis

Amplification of seven housekeeping genes was conducted by PCR, and the results were observed on 1% agarose gel (supplementary Fig. 1). The fragments of these selected genes, ranged from 514 bp (*nuoD*) to 800 bp (*gapA*), were successfully sequenced and analyzed by MLST for all the obtained isolates. These sequences were submitted to PubMLST database for the determination of the allele number and ST. According to different alleles, 22 isolates were assigned to 14 STs, including ST 14, ST 15, ST 34/194, ST 84/482, ST 85/99, ST 92, ST 143, ST 186, ST 186/252, ST 178, ST 196, ST 300, ST 451/461, and ST 477. All the 22 isolates consisted of existing types in the PubMLST database. The details of ST and allele numbers are shown in Table 1. The MLST results of 22 *S. maltophilia* isolates were demonstrated in phylogenetic tree using a neighbor-joining tree analysis for the concatenated data in all seven housekeeping genes of the 22 isolates (Fig. 2). UPGMA dendrogram demonstrated the similarity between these 22 isolates and other *S. maltophilia* strains obtaining from NCBI database (Fig. 3).

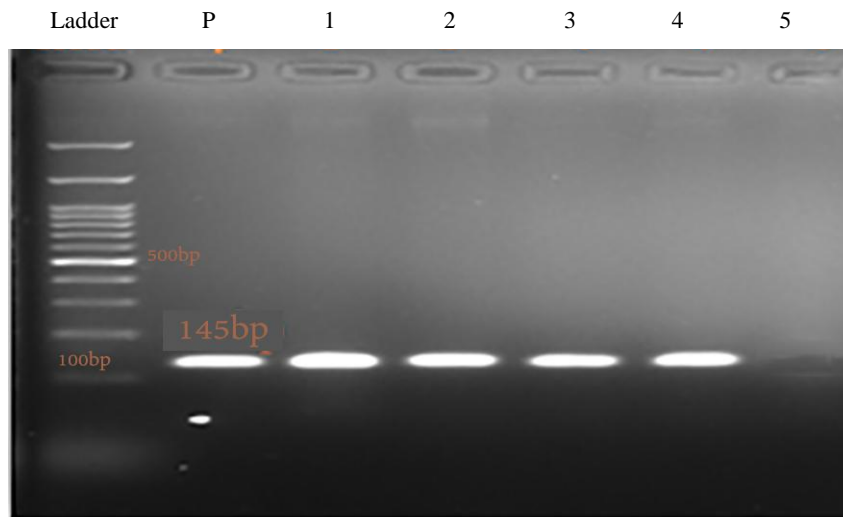


Fig. 1. The PCR products of 23S rRNA gene electrophoresed on 1% agarose gel. The related fragment (145 bp) was observed and verified by sequencing. P, positive control; lanes 1-4, experimental samples; lane 5, negative control; ladder (100 bp)

Determination of *zot* and *entF* gene frequency in *S. maltophilia* isolates

The PCR method was employed to determine the frequency of *zot* and *entF* virulence genes among 22 isolates of *S. maltophilia*. None of 22 isolates were positive for the presence of *zot* gene, indicating the absence of *zot* virulence factor. However, 13 out of 22 isolates were positive for the *entF* gene, representing the frequency of 59% for this virulence factor among

22 *S. maltophilia* isolates (Fig. 4). These isolates were allocated to ST 15, ST 92, ST 178, ST 186, ST 196, ST 85/99, ST 186/252, ST 84/482, ST 300, ST 451/461, and ST 477. It should be noted that the *entF* gene was observed only in sputum specimen. The detailed information on the determination of *zot* and *entF* genes frequency in *S. maltophilia* isolates is available in Table 2.

Table 1. Allele numbers and STs of *S. maltophilia* isolates obtained by MLST

| Sampling site | Allelic profile | | | | | | | ST number |
|-------------------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------|
| | <i>recA</i> | <i>guaA</i> | <i>gapA</i> | <i>nuoD</i> | <i>ppsA</i> | <i>MutM</i> | <i>atpD</i> | |
| Clinical specimen | 66 | 138 | 96 | 7 | 38 | 46 | 5 | 196 |
| | 62 | 82 | 94 | 28 | 4 | 3 | 5 | 84/482 |
| | 141 | 223 | 66 | 62 | 68 | 45 | 2 | 300 |
| | 16 | 284 | 70 | 18 | 15 | 33 | 28 | 85/99 |
| | 80 | 265 | 124 | 72 | 111 | 112 | 13 | 477 |
| | 80 | 49 | 124 | 92 | 144 | 83 | 13 | 186/252 |
| | 6 | 18 | 1 | 1 | 4 | 6 | 1 | 178 |
| | 6 | 23 | 23 | 26 | 5 | 16 | 17 | 14 |
| | 16 | 166 | 8 | 4 | 167 | 33 | 116 | 451/461 |
| | 26 | 10 | 10 | 23 | 1 | 14 | 10 | 34/194 |
| Dry environment | 5 | 223 | 66 | 62 | 68 | 45 | 2 | 300 |
| | 80 | 265 | 124 | 72 | 111 | 112 | 91 | 477 |
| | 1 | 82 | 1 | 1 | 1 | 3 | 1 | 92 |
| | 16 | 166 | 8 | 4 | 167 | 33 | 116 | 451/461 |
| | 10 | 21 | 29 | 32 | 32 | 21 | 10 | 15 |
| | 6 | 138 | 104 | 7 | 80 | 46 | 3 | 196 |
| Moist environment | 1 | 82 | 1 | 1 | 1 | 3 | 1 | 92 |
| | 106 | 18 | 1 | 1 | 4 | 6 | 1 | 178 |
| | 80 | 97 | 124 | 140 | 110 | 74 | 13 | 143 |
| | 5 | 223 | 66 | 62 | 68 | 45 | 2 | 300 |
| | 80 | 290 | 99 | 92 | 111 | 83 | 13 | 186 |
| 1 | 82 | 1 | 1 | 23 | 3 | 10 | 92 | |

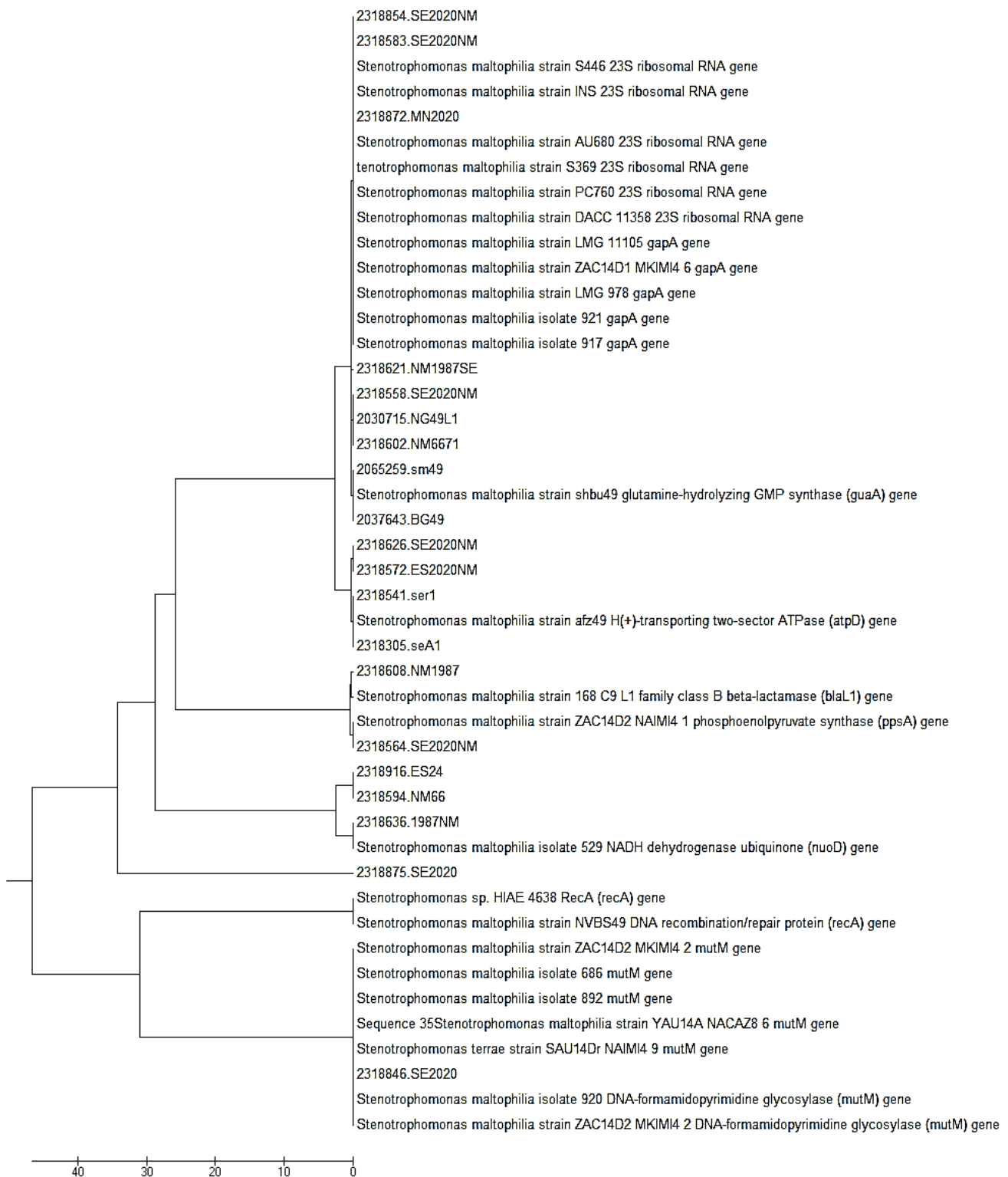


Fig. 2. Phylogenetic trees based on the concatenated data for all seven housekeeping genes of the 22 *S. maltophilia* isolates.

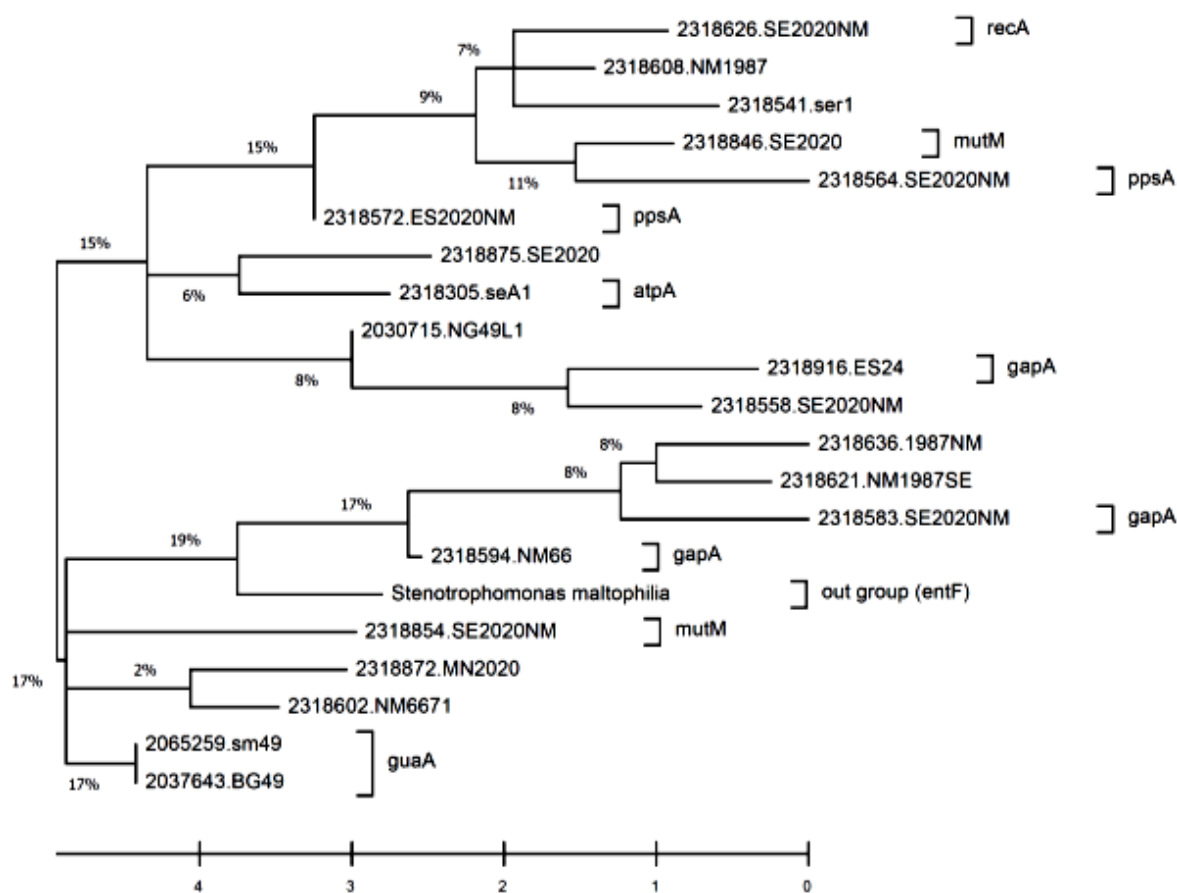


Fig. 3. Dendrogram using UPGMA method applied by the MEGA v.7 program to show similarity between 22 isolates and other *S. maltophilia* strains obtaining from NCBI database.

DISCUSSION

In the past decades, the prevalence of *S. maltophilia* has increased worldwide due to the misuse of antibiotics, especially in hospitals^[8,15,18]. This nosocomial bacterium form a biofilm to be resistant against antibiotics, resulting in a high prevalence of antibiotic resistant strains^[23]. The clinical importance of *S. maltophilia*, as a mere colonizer or infectious agent, often remains unresolved^[24]. Hence, the origin and the transmission way of this pathogen between patients are necessary to be elucidated.

Healthcare-associated infections with *S. maltophilia* have been originated from different hospital setting sources, such as hemodialysis water and dialysis machine^[26], endoscopes^[27], contact lens solutions^[28], and contaminated disinfectants^[29], as well as handwashing soap^[30], sinkholes^[31], sink drains, and showers^[32]. The transmission of pathogen could be performed by direct contact with these infected clinical settings and/or by the healthcare personnel's hands^[21]. The study on the epidemiology of *S. maltophilia* in

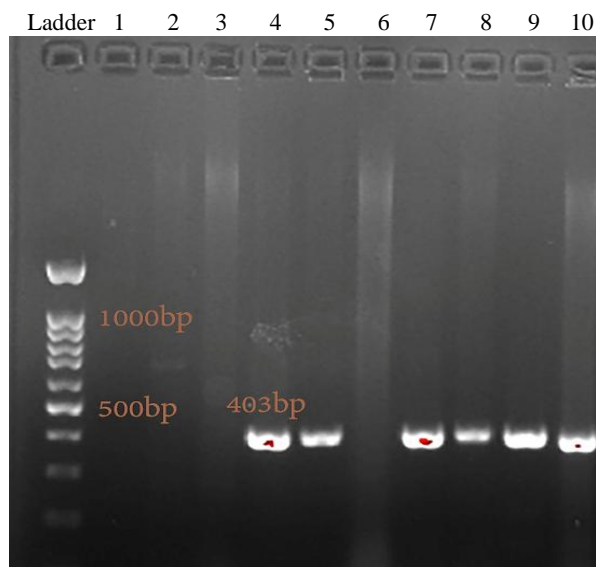


Fig. 4. The PCR products of *entF* gene. The related fragment (403 bp) observed on 1% electrophoresed agarose gel. Lane 1, negative control; lane 10, positive control; lanes 2-9, experimental samples; lanes 4, 5, 7, 8, 9, experimental samples containing *entF* gene; ladder (100 bp)

Table 2. Distribution of *zot* and *entF* virulence genes among STs of *S. maltophilia*

| Source | Virulence genes | | ST number |
|-------------------|-----------------|-------------|---|
| | <i>zot</i> | <i>entF</i> | |
| Clinical specimen | - | + | ST 196, ST 84/482, ST 300, ST 85/99, ST 186/252, ST 178, ST 451/461 |
| Dry environment | - | + | ST 477, ST 451/461, ST 15, ST 196 |
| Moist environment | - | + | ST 92, ST 186 |

hospital can demonstrate the intrahospital dissemination of distinct isolates of this pathogen in the clinic and environment. This study is helpful to control the increasing frequency of pathogen among hospitalized patients.

Genotyping methods have been used successfully in the molecular epidemiology of *S. maltophilia* and have revealed the high genodiversity of this species^[25]. Since MLST is considered as one of the best methods to study molecular epidemiology and population structure of bacteria^[25,33], it can be used to investigate the epidemiology of *S. maltophilia* in healthcare units such as hospitals. In a study conducted by Bostanghadiri *et al.*^[4], the genotypic characterization of 164 *S. maltophilia* isolates, collected from hospitalized patients in various regions of Iran, were determined by MLST and Rep-PCR methods. For the evaluation of genetic diversity, all 164 *S. maltophilia* isolates were divided into 16 common types and 114 single types, using Rep-PCR fingerprinting. For the first time in Iran, they showed that five TMP-SMX-resistant *S. maltophilia* isolates belonged to two different STs, including ST139 and ST259, using MLST analysis. ST259 with allelic profile (26,14,140,103,3,8,11) has not previously been reported. Their study also demonstrated the diversity among the isolates, suggesting the increment of antibiotic resistance and alternation of biofilm genes in clinical *S. maltophilia* isolates in Iran. However, TMP-SMX is still an effective antibiotic against *S. maltophilia*^[4].

In the current study, we used MLST with seven housekeeping genes on 22 *S. maltophilia* isolates obtained from patients and clinical settings of a tertiary hospital in the west of Iran (Kermanshah), to analyze the molecular epidemiology as well as clonal relatedness between clinic and environment sources of the *S. maltophilia* isolates. Among 22 isolates, 10 were identified from sputum, blood, and urine as clinical specimens, and the rest was obtained from dry and moist sites of the hospital. Among clinical specimens, 80% of *S. maltophilia* isolates were obtained from sputum samples. Moreover, the frequency of *S. maltophilia* isolates collected from dry and moist sites was almost equal. Using MLST analysis, we showed

that these 22 isolates were assigned to 14 STs, which all types existed in the PubMLST database. As shown in Table 2, the ST300 was observed in clinical specimens and also in samples obtaining from dry and moist sites of the hospital. The ST196, ST477, and ST451/461 were obtained from both clinical specimens and dry sites, while ST178 was obtained from clinical specimens and moist sites. In addition, ST92 observed in both dry and moist sites. We also found that 6 out of 14 STs were common among clinical specimens and samples obtained from dry sites and moist sites of the hospital. It can be concluded that there is a clonal relatedness between the clinic and environment sources of the *S. maltophilia* isolates obtained from the hospital.

Recently, a study conducted in Iran^[34] demonstrated the clonal relatedness between environmental and clinical *S. maltophilia* isolates using PFGE method, indicating a wide range of genetic diversity of *S. maltophilia* strains among the clinical and environmental sources. In that study, a total of 150 *S. maltophilia* isolates from patients and 1108 environmental samples were collected from three hospitals in Tehran (capital of Iran). At first, 150 clinical and 18 environmental isolates were confirmed using phenotypic tests, then the species were confirmed by PCR of the 23S *rRNA* gene. PFGE analysis displayed 24 common pulsotypes and 32 single pulsotypes. Only a small cluster was common among the clinic and environment within a hospital, indicating the existence of a common source for *S. maltophilia* to disseminate between different wards. Therefore, that study demonstrated the intra-hospital dissemination of certain isolates of *S. maltophilia* among the clinic and environment^[34].

Despite the growing significance of *S. maltophilia* infections, little is known about its pathogenicity and virulence factors^[35-37]. Studies have indicated that *S. maltophilia* strains possess traits that link them to virulence in other bacteria^[8,38,39]. The genomes of clinical and environmental *S. maltophilia* isolates encode three homologous proteins, EntA, EntC, and EntF^[38]. These proteins are involved in the biosynthesis of enterobactin as a catecholate

siderophore that is made by enteric bacteria (e.g. *E. coli*) and *Streptomyces* sp.^[38,40,41]. The pathogenic role of siderophores has been shown in many bacteria^[38,42-45]. Moreover, there is evidence that the *S. maltophilia* siderophore is likely important in pathogenesis^[22,38,46-50]. Toxin-related factor has been studied in *S. maltophilia* strains as a virulence factor, and a phage-encoded zonula occludens-like toxin has been considered in this pathogen^{[[35-37]}. In cholera toxin defective strains of *V. cholera*, a second enterotoxin, *zot* (zonula occludens toxin), causes diarrhea in affected patients^[51]. Interestingly, the occurrence of a *zot*-like gene has been reported in *S. maltophilia* strains of clinical origin by Hagemann *et al.*^[36] in 2006. In the current study, we performed PCR screenings to check the distribution of the *zot* and *entF* genes as virulence factors among 22 *S. maltophilia* isolates. The results demonstrated none of 22 *S. maltophilia* isolates have *zot* virulence gene, encoding zonula occludens-like enterotoxin. However, the *entF* gene with 59% frequency was observed in 13 out of 22 isolates. Significantly all six common STs, including ST300, ST196, ST477, ST451/461, ST178, and ST92, among clinical and environmental isolates from hospital represented the *entF* gene (Fig. 4 and Table 1).

In summary, this study investigated the molecular epidemiology of *S. maltophilia* isolates from environmental and clinical sources within a hospital using the MLST technique. PCR screenings was also employed to check the distribution of the *zot* and *entF* genes, as virulence factors, among *S. maltophilia* isolates. Our findings revealed a clonal relatedness between environmental and clinical specimens of the *S. maltophilia* isolates from hospital, which may be helpful in providing necessary groundwork for the prevention and treatment of *S. maltophilia* infections.

ETHICAL STATEMENT

The above-mentioned sampling was in accordance with Helsinki Declaration of 2013 and approved by the Ethics Committee of Imam Reza Hospital, Kermanshah, Iran. All participants signed informed consent forms.

DATA AVAILABILITY STATEMENT

Data supporting this article are included within the article and supplementary file.

AUTHOR CONTRIBUTION

SSE contributed to all experimental work, data and statistical analysis, and interpretation of data. JN contributed to conception and design. For overall supervision, RA drafted the manuscript, and PM conducted the MLST analysis and microbial

evaluation. All authors have read and approved the final manuscript

CONFLICT OF INTEREST

None declared.

FUNDING/SUPPORT

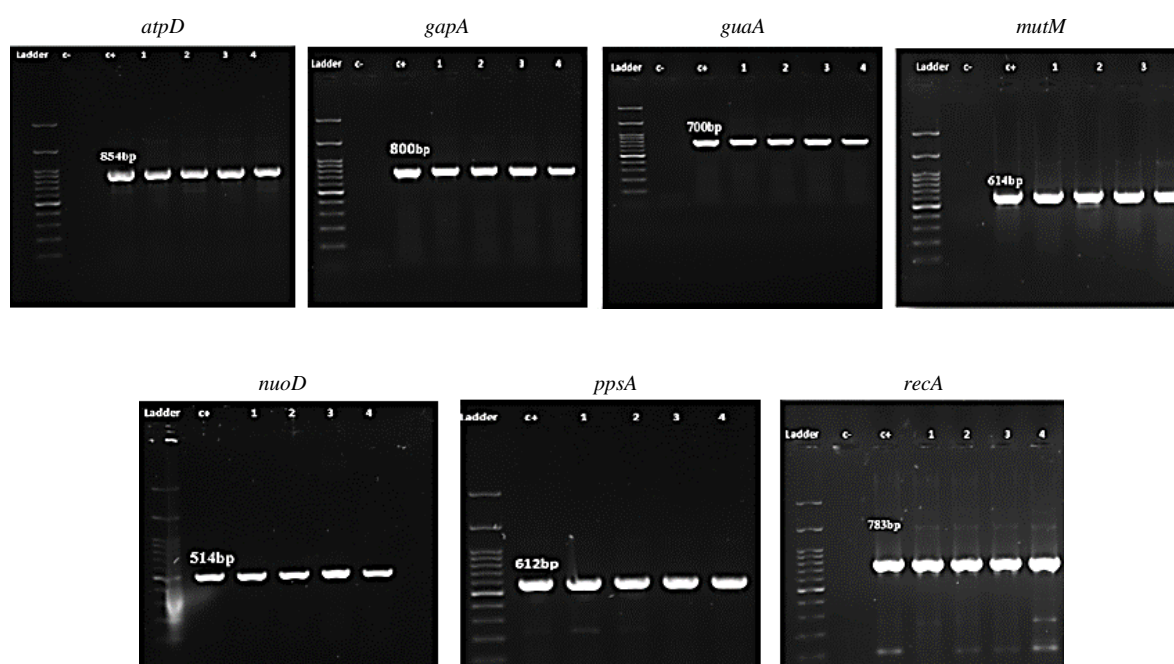
This study has received no funding or support.

REFERENCES

1. Igbinsosa EO, FE Oviasogie. Multiple antibiotics resistant among environmental isolates of *Stenotrophomonas maltophilia*. *Journal of applied sciences and environmental management* 2014; **18**(2): 255-261.
2. Hayward AC, Fegan N, Fegan M, Stirling G. *Stenotrophomonas* and *Lysobacter*: ubiquitous plant-associated gamma-proteobacteria of developing significance in applied microbiology. *Journal of applied microbiology* 2010; **108**(3): 756-770.
3. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, Van Der Lelie D, Dow JM. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature reviews microbiology* 2009; **7**(7): 514-525.
4. Bostanghadiri N, Ghalavand N, Fallah F, Yadegar A, Ardebili A, Tarashi S, Pournajaf A, Mardaneh J, Shams S, Hashemi A. Characterization of phenotypic and genotypic diversity of *Stenotrophomonas maltophilia* strains isolated from selected hospitals in Iran. *Frontiers in microbiology* 2019; **10**: 1191.
5. Pompilio A, Pomponio S, Crocetta V, Gherardi G, Verginelli F, Fiscarelli F, Dicuonzo G, Savini V, D'Antonio D, Di Bonaventura G. Phenotypic and genotypic characterization of *Stenotrophomonas maltophilia* isolates from patients with cystic fibrosis: genome diversity, biofilm formation, and virulence. *BMC microbiology* 2011; **11**(1): 1-17.
6. Di Bonaventura G, Pompilio A, Zappacosta R, Petrucci F, Fiscarelli E, Rossi C, Piccolomini R. Role of excessive inflammatory response to *Stenotrophomonas maltophilia* lung infection in DBA/2 mice and implications for cystic fibrosis. *Infection and immunity* 2010; **78**(6): 2466-2476.
7. Falagas ME, Kastoris AC, Vouloumanou EK, Rafailidis PI, Kapaskelis AM, Dimopoulos G. Attributable mortality of *Stenotrophomonas maltophilia* infections: a systematic review of the literature. *Future microbiology* 2009; **4**(9): 1103-1109.
8. Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clinical microbiology reviews* 2012; **25**(1): 2-41.
9. Sumida K, Chong Y, Miyake N, Akahoshi T, Yasuda M, Shimono N, Shimoda S, Maehara Y, Akashi K. Risk factors associated with *Stenotrophomonas maltophilia* bacteremia: a matched case-control study. *PLoS one* 2015; **10**(7): e0133731.

10. Hu LF, Chen GS, Kong QX, Gao LP, Chen X, Ye Y, Li JB. Increase in the prevalence of resistance determinants to trimethoprim/sulfamethoxazole in clinical *Stenotrophomonas maltophilia* isolates in China. *Plos one* 2016; **11**(6): e0157693.
11. Borsi SH, Shoushtari MH, Raji H, Ghalavand F. Prevalence of Gram-negative bacteria isolated from patients with ventilator-associated pneumonia in intensive care units of Imam Khomeini Hospital, Ahwaz, Iran. *Novelty in biomedicine* 2018; **6**(4): 167-173.
12. Thomas R, Hamat RA, Neela V. Extracellular enzyme profiling of *Stenotrophomonas maltophilia* clinical isolates. *Virulence* 2014; **5**(2): 326-330.
13. Araoka H, Baba M, Yoneyama A. Risk factors for mortality among patients with *Stenotrophomonas maltophilia* bacteremia in Tokyo, Japan, 1996–2009. *European journal of clinical microbiology and infectious diseases* 2010; **29**(5): 605-608.
14. Ebrahim-Saraie HS, Heidari H, Soltani B, Mardaneh J, Motamedifar M. Prevalence of antibiotic resistance and integrons, sul and Smqr genes in clinical isolates of *Stenotrophomonas maltophilia* from a tertiary care hospital in Southwest Iran. *Iranian journal of basic medical sciences* 2019; **22**(8): 872.
15. Madi H, Lukić J, Vasiljević Z, Biočanin M, Kojić M, Jovčić B, Lozo J. Genotypic and phenotypic characterization of *Stenotrophomonas maltophilia* strains from a pediatric tertiary care hospital in Serbia. *PloS one* 2016; **11**(10): e0165660.
16. Rutter WC, Burgess DR, Burgess DS. Increasing incidence of multidrug resistance among cystic fibrosis respiratory bacterial isolates. *Microbial drug resistance* 2017; **23**(1): 51-55.
17. Sánchez MB. Antibiotic resistance in the opportunistic pathogen *Stenotrophomonas maltophilia*. *Frontiers in microbiology* 2015; **6**: 658.
18. Brooke JS. New strategies against *Stenotrophomonas maltophilia*: a serious worldwide intrinsically drug-resistant opportunistic pathogen. *Expert review of anti-infective therapy* 2014; **12**(1): 1-4.
19. Ferjani S, Saidani M, Hamzaoui Z, Alonso CA, Torres C, Maamar E, Slim AF, Boutiba BBI. Community fecal carriage of broad-spectrum cephalosporin-resistant *Escherichia coli* in Tunisian children. *Diagnostic microbiology and infectious disease* 2017; **87**(2): 188-192.
20. Cho HH, Sung JY, Kwon KC, Koo SH. Expression of Sme efflux pumps and multilocus sequence typing in clinical isolates of *Stenotrophomonas maltophilia*. *Annals of laboratory medicine* 2012; **32**(1): 38-43.
21. Denton M, Todd NJ, Kerr KG, Hawkey PM, Littlewood JM. Molecular epidemiology of *Stenotrophomonas maltophilia* isolated from clinical specimens from patients with cystic fibrosis and associated environmental samples. *Journal of clinical microbiology* 1998; **36**(7): 1953-1958.
22. Dalbøge C, Hansen C, Pressler T, Høiby N, Johansen H. Chronic pulmonary infection with *Stenotrophomonas maltophilia* and lung function in patients with cystic fibrosis. *Journal of cystic fibrosis* 2011; **10**(5): 318-325.
23. Waite T, Georgiou A, Abrishami M, Beck C. Pseudo-outbreaks of *Stenotrophomonas maltophilia* on an intensive care unit in England. *Journal of hospital infection* 2016; **92**(4): 392-396.
24. Kaiser S, Biehler K, Jonas D. A *Stenotrophomonas maltophilia* multilocus sequence typing scheme for inferring population structure. *Journal of bacteriology* 2009; **191**(9): 2934-2943.
25. Dan T, Liu W, Sun Z, Lv Q, Xu H, Song Y, Zhang H. A novel multi-locus sequence typing (MLST) protocol for *Leuconostoc lactis* isolates from traditional dairy products in China and Mongolia. *BMC microbiology* 2014; **14**(1): 150.
26. Arvanitidou M, Vayona A, Spanakis N, Tsakris A. Occurrence and antimicrobial resistance of Gram-negative bacteria isolated in haemodialysis water and dialysate of renal units: results of a Greek multicentre study. *Journal of applied microbiology* 2003; **95**(1): 180-185.
27. Kovaleva J, Degener J, Van der Mei H. Mimicking disinfection and drying of biofilms in contaminated endoscopes. *Journal of hospital infection* 2010; **76**(4): 345-350.
28. Furuhashi K, Ishizaki N, Kawakami Y, Fukuyama M. Bacterial contamination of stock solutions in storage cases for contact lens, and the disinfectant-resistance of isolates. *Biocontrol science* 2010; **15**(3): 81-85.
29. Wishart MM, Riley TV. Infection with *Pseudomonas maltophilia* hospital outbreak due to contaminated disinfectant. *Medical journal of australia* 1976; **2**(19): 710-712.
30. Klausner JD, Zukerman C, Limaye AP, Corey L. Outbreak of *Stenotrophomonas maltophilia* bacteremia among patients undergoing bone marrow transplantation: association with faulty replacement of handwashing soap. *Infection control and hospital epidemiology* 1999; **20**(11): 756-758.
31. De la Rosa García S, Muñoz García A, Barahona Pérez L, Gamboa Angulo M. Antimicrobial properties of moderately halotolerant bacteria from cenotes of the Yucatan peninsula. *Letters in applied microbiology* 2007; **45**(3): 289-294.
32. Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, Pace NR. Opportunistic pathogens enriched in showerhead biofilms. *Proceedings of the national academy of sciences* 2009; **106**(38): 16393-16399.
33. Liang J, Ducatelle R, Pasmans F, Smet A, Haesebrouck F, Flahou B. Multilocus sequence typing of the porcine and human gastric pathogen *Helicobacter suis*. *Journal of clinical microbiology* 2013; **51**(3): 920-926.
34. Kardan Yamchi J, Hajjhasani A, Talebi M, Khodaparast S, Azimi A, Rahbar M, Fallah F, Douraghi M. Intra-hospital dissemination of clinical and environmental isolates of *Stenotrophomonas maltophilia* from Tehran. *Letters in applied microbiology* 2020; **72**(3): 325-331.
35. De Oliveira Garcia D, Dall'Agnol D, Rosales M, Azzuz AC, Alcántara N, Martinez NB, Girón JA. Fimbriae and adherence of *Stenotrophomonas maltophilia* to

- epithelial cells and to abiotic surfaces. *Cellular microbiology* 2003; **5**(9): 625-636.
36. Hagemann M, Hasse D, Berg G. Detection of a phage genome carrying a zonula occludens like toxin gene (zot) in clinical isolates of *Stenotrophomonas maltophilia*. *Archives of microbiology* 2006; **185**(6): 449-458.
 37. Windhorst S, Frank E, Georgieva DN, Genov N, Buck F, Borowski P, Weber W. The major extracellular protease of the nosocomial pathogen *Stenotrophomonas maltophilia* characterization of the protein and molecular cloning of the gene. *Journal of biological chemistry* 2002; **277**(13): 11042-11049.
 38. Nas MY, Cianciotto NP. *Stenotrophomonas maltophilia* produces an EntC-dependent catechol siderophore that is distinct from enterobactin. *Microbiology* 2017; **163**(11): 1590.
 39. Pompilio A, Crocetta V, Ghosh D, Chakrabarti M, Gherardi G, Vitali LA, Fiscarelli E, Di Bonaventura G. *Stenotrophomonas maltophilia* phenotypic and genotypic diversity during a 10-year colonization in the lungs of a cystic fibrosis patient. *Frontiers in microbiology* 2016; **7**: 1551.
 40. Raymond KN, Dertz EA, Kim SS. Enterobactin: an archetype for microbial iron transport. *Proceedings of the national academy of sciences* 2003; **100**(7): 3584-3588.
 41. Fiedler HP, Krastel P, Müller J, Gebhardt K, Zeeck A. Enterobactin: the characteristic catechol siderophore of Enterobacteriaceae is produced by Streptomyces species. *FEMS microbiology letters* 2001; **196**(2): 147-151.
 42. Crouch MLV, Castor M, Karlinsey JE, Kalthorn T, Fang FC. Biosynthesis and IroC-dependent export of the siderophore salmochelin are essential for virulence of *Salmonella enterica* serovar Typhimurium. *Molecular microbiology* 2008; **67**(5): 971-983.
 43. Wyckoff EE, Allred BE, Raymond KN, Payne SM. Catechol siderophore transport by *Vibrio cholerae*. *Journal of bacteriology* 2015; **197**(17): 2840-2849.
 44. Lawlor MS, O'Connor C, Miller VL. Yersiniabactin is a virulence factor for *Klebsiella pneumoniae* during pulmonary infection. *Infection and immunity* 2007; **75**(3): 1463-1472.
 45. Allard KA, Dao J, Sanjeevaiah P, McCoy-Simandle K, Chatfield CH, Crumrine DS, Castignetti D, Cianciotto NP. Purification of legiobactin and importance of this siderophore in lung infection by *Legionella pneumophila*. *Infection and immunity* 2009; **77**(7): 2887-2895.
 46. Ghysels B, Ochsner U, Möllman U, Heinisch L, Vasil M, Cornelis P, Matthijs S. The *Pseudomonas aeruginosa* pirA gene encodes a second receptor for ferrienterobactin and synthetic catecholate analogues. *FEMS microbiology letters* 2005; **246**(2): 167-174.
 47. Singh V, San Yeoh B, Xiao X, Kumar M, Bachman M, Borregaard N, Joe B, Vijay-Kumar M. Interplay between enterobactin, myeloperoxidase and lipocalin 2 regulates *E. coli* survival in the inflamed gut. *Nature communications* 2015; **6**(1): 1-11.
 48. Coburn B, Wang PW, Caballero JD, Clark SD, Brahma V, Donaldson S, Zhang Y, Surendra A, Gong Y, Tullis DE. Lung microbiota across age and disease stage in cystic fibrosis. *Scientific reports* 2015; **5**: 10241.
 49. Pompilio A, Crocetta V, De Nicola S, Verginelli F, Fiscarelli E, Di Bonaventura G. Cooperative pathogenicity in cystic fibrosis: *Stenotrophomonas maltophilia* modulates *Pseudomonas aeruginosa* virulence in mixed biofilm. *Frontiers in microbiology* 2015; **6**: 951.
 50. Holden VI, Lenio S, Kuick R, Ramakrishnan SK, Shah YM, Bachman MA. Bacterial siderophores that evade or overwhelm lipocalin 2 induce hypoxia inducible factor 1 α and proinflammatory cytokine secretion in cultured respiratory epithelial cells. *Infection and immunity* 2014; **82**(9): 3826-3836.
 51. Fasano A, Baudry B, Pumphlin DW, Wasserman SS, Tall BD, Ketley JM, Kaper J. *Vibrio cholerae* produces a second enterotoxin, which affects intestinal tight junctions. *Proceedings of the national academy of sciences* 1991; **88**(12): 5242-5246.



Supplementary Fig. 1. The PCR products of seven housekeeping genes amplification electrophoresed on 1% agarose gel. The related fragments of *atpD* (854 bp), *gapA* (800), *guaA* (700 bp), *mutM* (614 bp), *nuoD* (514), *ppsA* (612 bp), and *recA* (738 bp) were verified by sequencing. C+, positive control; C-, negative control; lanes 1-4: experimental samples; ladder (100 bp)