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Antibiotic Resistance Profile Among *Stenotrophomonas maltophilia* Clinical and Environmental Isolates

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Abstract

Background: This study was conducted to compare the resistance profile of *Stenotrophomonas maltophilia* isolates -collected from clinical and environmental sources in a hospital- for different antibiotics to clarify their clonal relatedness.
Methods: In this study, a total of 22 *S. maltophilia* isolates collected from 400 different clinical and environmental samples from Imam Reza Hospital were subjected to the analysis. Antibiotic susceptibility testing for each isolate was carried out by the disc diffusion method and according to the CLSI guidelines.
Results: Among 22 *S. maltophilia* isolates, ten isolates were obtained from clinical specimens, and 12 were obtained from the environment. The isolates showed the lowest and highest antibiotic resistance to chloramphenicol and trimethoprim-sulfamethoxazole and chloramphenicol (18.2%) and meropenem (100%), respectively, and resistance to the other antibiotics were as follows: Gentamicin 22.7%, tobramycin 50.0%, aztreonam 63.6%, amikacin 63.6%, ceftriaxone 68.2%, and ceftazidime 68.2%. The antibiotic profile of *S. maltophilia* strains differed from tobramycin, aztreonam, amikacin, ceftriaxone, trimethoprim-sulfamethoxazole, ceftazidime, chloramphenicol, and gentamicin between clinical and environmental samples.
Conclusions: Based on the high antibiotic resistance of *S. maltophilia* isolates and various responses to the selected antibiotic, chloramphenicol is the best therapeutic option, with 81.8% susceptibility. The early diagnosis and determination of antibiotic resistance patterns have the utmost importance.

Keywords: *Stenotrophomonas maltophilia*, Antibioticresistance, Environmental Source, Hospital Infection

1. Background

Stenotrophomonas maltophilia is a non-fermented gram-negative rod-shaped bacteria mainly isolated from the environment and clinical specimen, accounting for a third cause of nosocomial infections (1, 2). *Stenotrophomonas maltophilia* become a significant opportunistic pathogen in hospitalized patients worldwide (3). The clinical manifestation of bacteria in immunocompromised patients included endocarditis, cellulitis, bacteremia, pneumonia, sepsis, meningitis, and bone, joint, eye, wound, and urinary tract infections (4, 5). The bacteria are primarily colonized in patients with malignancy, immunodeficiency diseases, use of a catheter, broad-spectrum antibiotics, and hospitalized for a long term (6, 7). *Stenotrophomonas maltophilia* is mainly colonized in aqueous and humid environments, including soil, animals, plants, and water sources (like lakes, wells, and rivers) (8). The survival rate of bacteria in humid environments is drastically higher than in dried surfaces (7). This pathogen does not appear to be a prevalent pathogen, but it is a significant cause of hospital-acquired infections and community infections (2) because of its colonization in medical equipment (6) and even the hands of healthcare personnel (7).

In recent years, treatment strategies for *S. maltophilia* infections have failed due to increasing antibiotic resistance. This bacterium is considered one of the principal multidrug-resistant organisms in hospital settings because of displaying high levels of intrinsic and acquired resistance to various antibiotics (9-11). Therefore, *S. maltophilia* infection has been a significant challenge for patients and clinicians (1, 12).

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The multi-locus sequencing typing (MLST) technique has been broadly applied for typing genomic DNA containing housekeeping genes, mainly used for the distribution pattern of infection and finding the source of infection. Previously, molecular epidemiology and clonal relatedness between *S. maltophilia* isolates from clinical and environmental sources were studied within a hospital in Iran using the MLST technique. A total of 22 *S. maltophilia* strain isolates were assigned to 14 sequence types (ST), 6 of which were common among clinical and environmental samples, suggesting clonal relatedness between these two sources (13).

2. Objectives

This study aimed to determine the resistance profile of these 22 *S. maltophilia* isolates to different antibiotics (specified by CLSI). The antibiotic resistance profile of *S. maltophilia* isolates was compared with common STs among clinical and environmental samples to clarify their clonal relatedness more specifically.

3. Methods

3.1. Isolation of Bacteria

The 22 *S. maltophilia* strains were isolated from 400 different clinical and environmental samples selected from Imam Reza Hospital (Kermanshah, Iran) between May 2019 - 2020. As reported in our previous study, all 22 *S. maltophilia* strains were identified by biochemical and molecular tests, and the ST of each *S. maltophilia* isolate was determined using the MLST technique. Then, the isolates were stored in a Luria Bertani (LB) broth containing 20% glycerol at - 70°C until further study (13).

3.2. Antibiotic Susceptibility Testing

The fresh LB culture media were prepared for the bacterial suspension for antibiotic susceptibility on MHA (Mueller-Hinton agar). The 0.5-McFarland standard was used to compare the bacterial suspension's turbidity to achieve appropriate density to evaluate the effect of antimicrobial agents. The disc diffusion method was used to determine the antibiotic susceptibility pattern of the isolates. The tests were examined after 24 h incubation at 37°C and were repeated if they were found to be discordant. The antibiotics for *S. maltophilia* strain susceptibility tests, recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines, were used in the study (14). These antibiotics include chloramphenicol, tobramycin, meropenem, gentamicin, ceftazidime, imipenem, aztreonam, amikacin, ceftriaxone, and trimethoprim-sulfamethoxazole. *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were control strains for the culture media. (All media and antibiogram discs were purchased from Merck, Germany).

4. Results

A total of 22 *S. maltophilia* isolates, assigned to fourteen STs, were recovered from clinical and environmental samples. Ten out of 22 isolates were obtained from clinical specimens, while the rest were obtained from dry and moist sites in the hospital. Antibiotic susceptibility was investigated in all isolates considering ten different antibiotics. Antibiotic resistance is high in *S. maltophilia* isolates in Imam Reza Hospital. With 81.8% sensitivity, chloramphenicol was the most effective antibiotic for eradicating environmental and clinical *S. maltophilia* infections (Table 1).

The isolates' minimum and maximum resistance rates (RR) were observed for trimethoprim-sulfamethoxazole (18.2%) and meropenem (100%), respectively. Resistance to the other antibiotics was as follows: chloramphenicol (18.2%), tobramycin (50.0%), gentamicin (22.7%), ceftazidime (68.2%), aztreonam (63.6%), amikacin (63.6%), ceftriaxone (68.2%), and trimethoprim-sulfamethoxazole (18.2%) (Table 2).

In addition, the intermediate resistance (4.54%) was only observed in isolate seven with ST number 451/461 (from the dry environment) in response to trimethoprim-sulfamethoxazole. In contrast, ST 451/461 in isolate 19 (from a blood sample) was susceptible to this antibiotic (Table 1). ST300, ST196, ST477, ST451/461, and ST178 were common among clinical and environmental samples, but different susceptibilities were observed in their antibiotic profile between clinical and environmental samples. The different exposures to antibiotics were as follows:

- ST300 for tobramycin, aztreonam, amikacin, ceftriaxone, and trimethoprim-sulfamethoxazole
- ST196 for tobramycin, ceftazidime, and amikacin
- ST477 for aztreonam
- ST451/461 for chloramphenicol, tobramycin, aztreonam, amikacin, ceftriaxone, and trimethoprim-sulfamethoxazole
- ST178 for chloramphenicol, gentamicin, ceftazidime, aztreonam, amikacin (Table 1).

5. Discussion

Stenotrophomonas maltophilia strains confer resistance to a broad spectrum of antibiotics,

Table 1. The Antibiotic Resistance Profile of *Stenotrophomonas maltophilia* Isolates [a]

Isolate Number	Sample Type	ST Number	Antibiotics									
			A	B	C	D	E	F	G	H	I	J
1	C.S (sputum)	196	S	S	R	S	S	R	R	S	R	S
2	C.S (sputum)	84/482	S	S	R	S	R	R	R	S	S	S
3	C.S (sputum)	300	S	R	R	R	R	R	S	R	R	S
4	E.S (dry)	300	S	S	R	R	R	R	R	S	S	S
5	E.S (dry)	477	S	R	R	S	S	R	S	R	R	S
6	E.S (Moist)	143	S	S	R	R	R	R	R	R	R	S
7	E.S (dry)	451/461	R	S	R	S	S	R	R	S	S	I
8	E.S (dry)	15	S	R	R	S	R	R	R	R	R	S
9	E.S (Moist)	300	S	R	R	S	R	S	R	R	R	R
10	E.S (Moist)	92	R	R	R	S	R	S	S	S	S	S
11	C.S (sputum)	85/99	S	S	R	R	S	R	S	R	R	S
12	C.S (sputum)	477	S	R	R	S	S	R	R	R	R	S
13	C.S (sputum)	186/252	S	R	R	S	R	R	S	R	S	R
14	C.S (sputum)	178	S	R	R	R	R	R	S	S	R	S
15	E.S (Moist)	178	R	R	R	S	S	R	R	R	R	S
16	E.S (dry)	92	R	S	R	S	R	R	S	S	S	S
17	E.S (dry)	196	S	R	R	S	R	R	R	R	R	S
18	C.S (blood)	14	S	S	R	S	R	R	S	S	R	R
19	C.S (blood)	451/461	S	R	R	S	R	R	R	R	R	S
20	E.S (Moist)	186	S	S	R	S	R	R	R	R	R	R
21	E.S (Moist)	92	S	S	R	S	S	R	R	R	S	S
22	C.S (blood)	34/194	S	S	R	S	R	R	R	R	R	S

Abbreviations: S, susceptible; I, intermediate; R, resistant; C.S, clinical sample; E.S, environmental sample.
[a] A, Chloramphenicol; B Tobramycin, C, Meropenem; D, Gentamicin; E, Ceftazidime; F, Imipenem; G, Aztreonam; H, Amikacin; I, Ceftriaxone; J, Trimethoprim-sulfamethoxazole.

Table 2. The Resistance Rates of *Stenotrophomonas maltophilia* Isolates Based on the Selected Antibiotics

Antibiotic	Response (%)		
	Susceptible	Intermediate	Resistant
Chloramphenicol	81.8	-	18.2
Tobramycin	50.0	-	50.0
Meropenem	0.0	-	100.0
Gentamicin	77.3	-	22.7
Ceftazidime	31.8	-	68.2
Imipenem	9.1	-	90.9
Aztreonam	36.4	-	63.6
Amikacin	36.4	-	63.6
Ceftriaxone	31.8	-	68.2
Trimethoprim-sulfamethoxazole	77.3	4.54	18.2

such as aminoglycosides, β-lactams, carbapenems, chloramphenicol, fluoroquinolones, macrolides, tetracyclines, trimethoprim-sulfamethoxazole, and polymyxins (8). The intrinsic resistance of *S. maltophilia* strains to antibiotics is associated with efflux pumps, low membrane permeability, and the inherent β-lactamases L1 and L2, among other drug resistance determinants, such as aminoglycoside acetyl-transferase and enzymes that inactivate erythromycin (7), which shield these bacteria (1, 8, 12).

This study investigated the antibiotics resistance rates of 22 *S. maltophilia* isolates subjected in our previous study (13), considering antibiotics specified by CLSI, including chloramphenicol, tobramycin, meropenem, gentamicin, ceftazidime, imipenem, aztreonam, amikacin, ceftriaxone, and trimethoprim-sulfamethoxazole. All isolates were utterly susceptible to chloramphenicol (SR = 81.8%). However, the highest resistance rate (RR) = 100% was observed for meropenem (Table 2). Inappropriate use of broad-spectrum antibiotics like imipenem compromises a high-risk factor for *S. maltophilia* infections. At the same time, *S. maltophilia* can hydrolyze imipenem based on the reason for this high resistance rate (7).

Among ten antibiotics, intermediate resistance (I = 4.54%) was found only for trimethoprim-sulfamethoxazole (Table 2). Mutations or resistance-encoding genes acquired through horizontal gene transfer are other antibiotic resistance mechanisms in *S. maltophilia* (8, 15), and dihydropteroate synthase and dihydrofolate reductase genes are the main mechanisms of trimethoprim-sulfamethoxazole resistance in this bacterium (8). *Stenotrophomonas maltophilia* had a lower resistance to trimethoprim-sulfamethoxazole leading to the administration of these antibiotics to eradicate infection (4). Managing *S. maltophilia* infections has been increasingly demanding, with increased acquired resistance to this antibiotic (6). However, few strains of *S. maltophilia* in the study were resistant to trimethoprim-sulfamethoxazole (RR = 18.2%, SR = 77.3%), in accordance with Baseri et al. on 117 *S. maltophilia* isolates from hospitalized patients in Iran, indicating the lowest frequency of resistance (RR = 10.25%) to this antibiotic (6). Bostanghadiri et al. studied 85 clinical *S. maltophilia* isolates collected from several hospitals in Iran and observed about 2.35% resistance to trimethoprim-sulfamethoxazole (1). Another study over approximately five years in Turkey showed that 20.3% of 118 *S. maltophilia* clinical isolates were resistant to these antibiotics (7). Nikpour et al. observed the same frequency of trimethoprim-sulfamethoxazole resistance in *S. maltophilia* isolates from Jahrom Hospital with about 5.5% resistance to trimethoprim-sulfamethoxazole (16).

The reports have suggested that trimethoprim-sulfamethoxazole is still the best antibiotic with a favorable antimicrobial effect in treating nosocomial infections caused by *S. maltophilia* strains.

As reported previously, 22 *S. maltophilia* isolates were assigned to 14 ST in which ST300, ST196, ST477, ST451/461, and ST178 were common among clinical and environmental (moist and wet) samples, suggesting clonal relatedness between these two sources (13). Hence, the antibiotic profile of these common STs and different responses to tobramycin, aztreonam, amikacin, ceftriaxone, trimethoprim-sulfamethoxazole, ceftazidime, chloramphenicol, and gentamicin was found. In contrast, similar responses to meropenem and imipenem in clinical and environmental isolates were observed (Table 2). The minimum (SR = 0.0%) and maximum (SR = 81.8%) susceptibility were observed respectively to meropenem and chloramphenicol in all 22 isolates.

5.1. Conclusions

In clinical and environmental isolates, high antibiotic resistance was observed in *S. maltophilia* isolates from Imam Reza Hospital, Kermanshah, Iran. 100% resistance to meropenem and an 18.2% resistance to chloramphenicol and trimethoprim-sulfamethoxazole were found. The most effective antibiotic was chloramphenicol, with a sensitivity of 81.8%, suggesting the administration of the antibiotic mentioned above for *S. maltophilia* eradication. However, colonization of this organism in medical equipment and hospital settings is one of the leading causes of acquired resistance to various antibiotics, which facilitates the dissemination of *S. maltophilia*. Since this study was conducted on a few isolates and carried out in one center only, the significance of our data should be confirmed by further research in a multicenter setting with more *S. maltophilia* isolates.

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Footnotes

Authors' Contribution: Study concept and design: Jamileh Nowroozi. Acquisition of data: Sinasadat Emami. Analysis and interpretation of data: Sinasadat Emami, Parviz Mohajeri. Drafting of the manuscript: Jamileh Nowroozi, Sinasadat Emami. Critical revision of the manuscript for important intellectual content:

Jamileh Nowroozi. Statistical analysis: Ramin Abiri. Administrative, technical, and material support: Parviz Mohajeri, Ramin Abiri, Jamileh Nowroozi. Study supervision: Jamileh Nowroozi.

Conflict of Interests: Parviz Mohajeri might be the editorial board member of this journal.

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