



Research Article

Isolation of *Stenotrophomonas maltophilia* from Clinical Samples in Some Hospitals in Shiraz, Iran

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ABSTRACT

Stenotrophomonas maltophilia is an emerging pathogenic non-fermentative Gram-negative Bacillus species. It has caused many nosocomial infections and can be isolated from various hospital wards and healthcare facilities. Research has shown that most of its strains are inherently resistant to many antibiotics and have multidrug resistance. This research intended to determine its occurrence frequency at some Hospitals in Shiraz, Iran. The present study was conducted in six months (from early spring to late summer 2019). Clinical samples (Blood, Urine and cerebrospinal fluid (CSF)) collected from 120 patients afflicted with various infections. The samples were transferred to the Laboratory and subjected to microbiological analysis. Identification of the isolates was carried out by phenotypic methods and *Stenotrophomonas maltophilia* isolates verified using molecular methods. In total, various bacteria were isolated from 84 clinical samples. The isolates were *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Stenotrophomonas maltophilia* was isolated from 17 (20.2%) positive samples and most of them were isolated from blood samples. Our finding indicated that *Stenotrophomonas maltophilia* isolated more from blood samples follow by CSF sample. In addition, our finding illustrated that *Stenotrophomonas maltophilia* can be considered as the common nosocomial agent at hospitals in Shiraz, Iran.

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Introduction

Stenotrophomonas maltophilia is a non-fermentative Gram-negative Bacillus species that was formerly classified in the genera *Pseudomonas* and *Xanthomonas*. At present, it is placed in the genus *Stenotrophomonas* [1]. *S. maltophilia* is an emerging opportunistic pathogen and one of the most important agents causing hospital-acquired infections [2]. It has been isolated from hospital sources such as medical equipment, hospital water supply, sinks, disinfectant solutions, dialysis devices, ventilators, central venous catheters, thermometers, blood gas analysers, distilled water dispensers and intra-aortic balloon pumps are among the main hospital devices and equipment from which it can be isolated. Although *S. maltophilia* has relatively low pathogenic power, it can cause a broad spectrum of dangerous infections such as pneumonia, bacteremia, endocarditis, meningitis, wound and soft tissue

infections, osteomyelitis, keratitis and urinary tract infections, especially in patients with immunodeficiency [3].

On the other hand, recent research report opined that 11% of Cystic Fibrosis (CF) patients can be infected by *S. maltophilia*. Thus, high incidence of infections due to *S. maltophilia* can be expected in immunocompromised patients [4]. Several factors such as intrinsic resistance to antimicrobial agents, production of proteolytic enzymes and other pathogenic extracellular enzymes such as DNase, RNase, elastase, lipase, hyaluronidase, mucinase and hemolysin [3]. Resistance in *S. maltophilia* to various antibiotics can lead to numerous problems in controlling hospital-acquired infections and threaten public health. That is why identification of this bacterial species is very useful in therapy management [5]. Therefore, based on foregoing evidence the present study conducted to determine frequency of occurrence of *S. maltophilia* at different hospitals in Shiraz, Iran in order to achieve maximum information regarding to occurrence of *S. maltophilia* in our area.

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Materials and Methods

I Sample Collection and Isolation

This cross-sectional study was carried out from early spring to late summer of 2019 at Shiraz Hospitals. In the present 107 clinical samples (Blood, Urine and Cerebrospinal fluid (CSF)) were collected from patients with various infections. Furthermore, to perform demographic study simple data (age and sex) with respected to personal privacy were recorded from each patient. All the samples were transferred to the Microbiology Laboratory within two hour in containers under completely sterile conditions. The samples were cultivated on selective and differential media such as blood and MacConkey agars and incubated for 24-48h at 37°C [6].

II Phenotypic and Genotypic Identification

Following the initial culture, the pure colonies subjected to microscopic examination and biochemical test analysis using Gram stain and oxidase, TSI, SIM, MR, VP, urease, motility, citrate, sugar fermentation (glucose, lactose, maltose, mannitol), amino acid metabolism (lysine, arginine), pigment production, DNase and polymyxin susceptibility tests [7].

Assuming *S. maltophilia* isolates were authenticated using specific primers (Table 1) and genotypic methods. To perform genotypic identification, DNA extraction was carried out using the standard kit (Cinnagene, Iran). Then PCR mixture was prepared by purified extracted DNA, master mix (Cinnagene, Iran) and *S. maltophilia* specific primers. DNA thermal cycler (Bio-Rad, USA) according to the following reaction conditions: initial denaturation at 94°C for 5 min, 35 × denaturation at 94°C for 30s, annealing at 60°C for 30s, extension at 72°C for 30s, and final extension at 72°C for 5min. Finally, PCR products loaded in agarose gel electrophoresis and isolation of *S. maltophilia* verified.

Table 1: Specific *S. maltophilia* primers [8].

Primer name		Sequence 3'-5'	Length
S.maltophilia-F	(forward)	GCTGGATTGGTTCTAGGAAAACGC	278bp
S.maltophilia-R	(reverse)	ACGCAGTCACTCCTTGCG	

III Statistical Analysis

A statistically significant difference between frequency of occurrence of *Stenotrophomonas maltophilia* and patient age groups and clinical samples was performed using one sample t-test was performed using SPSS v. 20 at a significance level of p value < 0.05.

Results

I Isolation of Bacteria from Clinical Samples

The results on isolation of bacteria from clinical samples indicated that *Escherichia coli* 32(51.2%), *Stenotrophomonas maltophilia* 15 (20.2%), *Enterobacter aerogenes* 12 (14.2%), *Klebsiella pneumoniae* 12

(14.2%), *Staphylococcus aureus* 8 (9.5%) and *Pseudomonas aeruginosa* 5 (5.9%) were isolated. All presumptive *Stenotrophomonas maltophilia* were verified by PCR and gel electrophoresis (Figure 1).

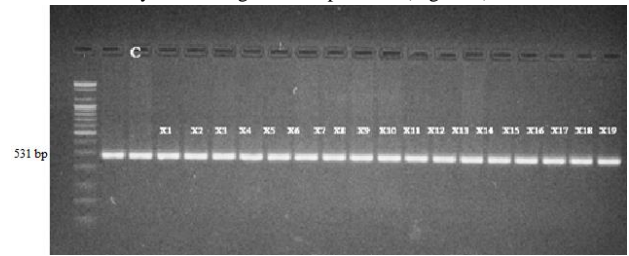


Figure 1: Gel electrophoresis of PCR product using specific *Stenotrophomonas maltophilia* primers.

II Demographic Study

As shown in (Figure 2) two age groups of > 1 and 74 < were relatively more infected. The results obtained indicated that age group of 74 < was relatively more infected. However, no significant difference was found between isolation of *S. maltophilia* and sex.

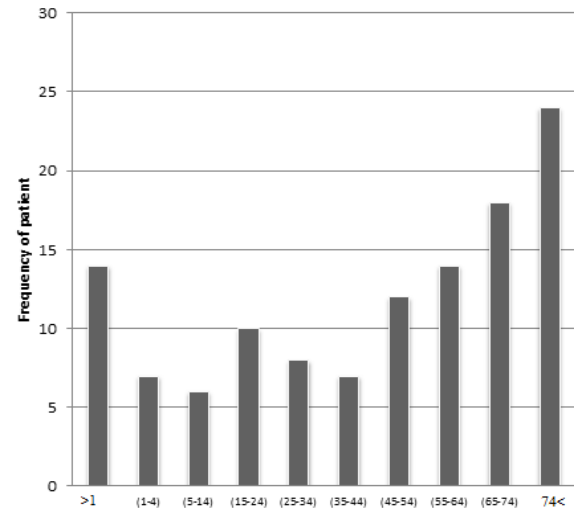


Figure 2: Frequency distribution of the samples based on age-group.

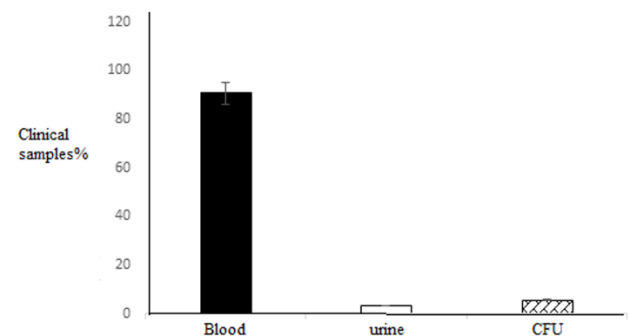


Figure 3: Frequency of occurrence of *S. maltophilia* based on the type of clinical samples.

The results obtained from determination of frequency of occurrence of *S. maltophilia* in clinical samples indicated that blood follow by CSF was more contaminated sample (Figure 3).

III Statistical Analysis

The results obtained from statistically analysis of the data indicated that significant relationship was found between frequency of occurrence of *Stenotrophomonas maltophilia* and age group patients and clinical samples. However, no significant difference was found between isolation of *S. maltophilia* and sex.

Discussion

Our finding illustrated high frequency of occurrence of *Stenotrophomonas* at hospitals in Shiraz, Iran. Although, frequency of *E.coli* isolation in different types of hospitals wards was relatively more, *Stenotrophomonas maltophilia* was isolated from 20.2% of positive samples. Therefore, this opportunistic pathogenic bacterium can be existed in the area of investigation with high frequency. Several reports indicated that *Stenotrophomonas maltophilia* can be existed in different environments because of its special characters such as intrinsic resistance character against antimicrobial agents and its ability for producing enzymes, which help to survive in different habitats. Even *Stenotrophomonas maltophilia* is abundance in burn patients [9].

Our results on determination of Frequency of isolation of *S. maltophilia* in different age groups indicated that two age groups of >1 and 74< were relatively more infected. This result clearly illustrated that infection by *S. maltophilia* is generally related to human immunity systems. It means the persons with immunocompromised system are more under risk of infection with opportunistic pathogen such as *S. maltophilia*. On the other hand, among all clinical samples, blood samples (Figure 3) Frequency of occurrence of *S. maltophilia* based on the type of clinical samples follow by CSF was relatively more contaminated. It is because blood and CSF compared to urine are more enriched and complex. Therefore, they are very suitable media for reproduction of *S. maltophilia*. In this regard, many factors are involved in transmission of *S. maltophilia* to susceptible individuals. These risk factors are host immune status, pathogen status, and the ability of the pathogen to cause infection [10].

Therefore, the patients with cancer, chronic respiratory disease, immunocompromised host and long-term hospitalization or ICU stay are under risk of *S. maltophilia* infection. Overall, *S. maltophilia* can be considered as the common nosocomial agent at hospitals in Shiraz, Iran.

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