

# Minimal Inhibitory Concentration of Ceftazidime and Co-trimoxazole for *Stenotrophomonas Maltophilia* using E-test

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## ABSTRACT

**Background:** *Stenotrophomonas maltophilia*, previously named as *Pseudomonas* or *Xanthomonas maltophilia*, is an important nosocomial pathogen. **Aim:** The purpose of the present study was to investigate the prevalence of *S. maltophilia* in Iranian hospitals and its susceptibility to available antimicrobial agents. **Setting and design:** A cross-sectional study in Imam Khomeini Hospital affiliated to Tehran University of Medical Sciences. **Materials and Methods:** All blood specimens were sent to the laboratory for blood culture and biochemical analysis. One hundred samples were positive for *S. maltophilia*. We used disk diffusion and E-test in order to determine minimal inhibitory concentration (MIC) of ceftazidime and co-trimoxazole as the first line antibiotics for *S. maltophilia*. The tests were performed and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI). **Statistical analysis:** Chi-square test and Kappa measurement of agreement were applied as appropriate. **Results:** *S. maltophilia* was the most frequent pathogen (895 specimens; 38.9%) isolated from the samples which were mostly from emergency ward (780 specimens; 33.9%). Ceftazidime MIC<sub>50</sub> and MIC<sub>90</sub> were 2 and 32 µg/ml, respectively (sensitive ≤8 µg/ml and resistant ≥32 µg/ml according to CLSI guideline). MIC<sub>50</sub> and MIC<sub>90</sub> for co-trimoxazole were 0.5 and 2 µg/ml, respectively (sensitive ≤2 µg/ml and resistant ≥4 µg/ml according to CLSI guideline). **Conclusion:** *S. maltophilia* is the most frequent pathogen in our hospital with a high susceptibility to both ceftazidime and co-trimoxazole.

**Key words:** Ceftazidime, Co-trimoxazole, Minimal inhibitory concentration, *S. maltophilia*, Susceptibility testing

## INTRODUCTION

*Stenotrophomonas maltophilia*, also named as *Pseudomonas* or *Xanthomonas maltophilia*, is an important nosocomial pathogen.<sup>[1,2]</sup> It is capable of infecting various systems such as urinary tract, respiratory tract, skin, soft tissues, and specially bloodstream.<sup>[3,4]</sup> This opportunistic pathogen is multidrug resistant which is due to its inherent enzymes including β-lactamases<sup>[5]</sup> and cephalosporinase.<sup>[6]</sup>

Bacteremia is a life-threatening condition requiring urgent attention;<sup>[7]</sup> so it is highly recommended to perform antibiogram and blood culture before antibiotic therapy which leads to lower mortality and bacterial resistance.<sup>[8]</sup> Empiric therapies which are not in accordance to blood

culture or antibiogram reports lead to poor therapeutic results. There are many reports of emergence of bacteremia caused by *S. maltophilia*.<sup>[5]</sup> As a result, identifying the most effective antibiotic is of great clinical importance.

Studies have demonstrated that co-trimoxazole is the treatment of choice for *S. maltophilia*,<sup>[9,10]</sup> however, emergence of resistance to this antibiotic is widely reported.<sup>[9-12]</sup> Various antibiotics are being tested to find an appropriate alternative. Ceftazidime, a third-generation cephalosporin antibiotic, is one of these recommended alternatives.<sup>[10]</sup>

Microtiter broth dilution testing is known as the standard susceptibility method for all organisms;<sup>[8]</sup> however, E-test is more practical in routine laboratory testing and is a reliable alternative.<sup>[12]</sup> Disk diffusion is in concordance with the dilution method while having the advantages of simplicity and low cost.<sup>[8]</sup>

The changing spectrum of microbial pathogens along with wide spread emergence of these pathogens within

Access this article online	
Quick Response Code: 	Website: <a href="http://www.jgid.org">www.jgid.org</a>
	DOI: 10.4103/0974-777X.83531

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the hospital environment which are resistant to antibiotics results in potentially life-threatening infections. This led us to conduct this study in order to provide crucial information on different pathogens and antimicrobial resistance in the hospitals of Iran, in order to improve our ability in controlling nosocomial blood pathogens. The Infectious Disease Control Committee of the hospital recommended *S. maltophilia* as the most emerging resistant pathogen. Ceftazidime and co-trimoxazole (according to CLSI-2007 guideline) as inexpensive and available antibiotics which are routinely used in our hospital were selected. Also, the prevalence of main pathogens isolated from blood cultures in patients admitted to Imam Khomeini Hospital affiliated to Tehran University of Medical Sciences was determined. Besides, the sensitivity to the disk diffusion method was compared to that of the E-test.

The purpose of the present study was to investigate the prevalence of *S. maltophilia* in Iranian hospitals and its susceptibility to available antimicrobial agents.

## MATERIALS AND METHODS

All blood specimens from the patients hospitalized at Imam Khomeini Hospital sent for blood culture testing from December 2008 to November 2009; if there were more than one positive blood culture for a patient, the first one was considered as positive.

After incubating the specimens for 24 hours in 35°C, blood cultures were passaged on blood agar and chocolate agar plates 24 and 48 hours after incubation, respectively. The plates were incubated for further 24 hours at the same temperature and if there were any colonies, they were isolated for final identification. *S. maltophilia* is characterized as a non-fermentative gram negative motile bacillus with negative cytochrome oxidase reaction, positive DNase reaction, Alk/Alk reaction on the surface of triple sugar iron agar, and also positive for esculin, gelatin hydrolysis, and lysine decarboxylase. A sample size of 100 isolates of *S. maltophilia* was selected randomly and recruited in the final study.

The disk diffusion and E-test were performed according to the procedure outlined by Clinical Laboratory Standards Institute (CLSI).<sup>[13]</sup> The *S. maltophilia* stored at -70°C in a tube enriched by trypticase-soy broth (Himedia, India) and glycerol 15% were defrosted in trypticase-soy broth solution in the environmental temperature for 24 hours according to the manufacturer instructions.

The isolates were incubated for another 24 hours at 35°C

after being transferred to a Mueller-Hinton agar plate (Himedia, India) which was 150 mm in diameter and 5 mm in thickness. To achieve 0.5 McFarland turbidity and yield a suspension of  $1.5 \times 10^8$  cfu/ml, a few single colonies from each plate were suspended in 3 ml of normal saline. A sterile swab was dipped into the inoculum suspension, excess fluid was pressed out, and the Mueller-Hinton agar was swabbed carefully in three directions to make an even growth. According to E-test instructions, turbidity adjusted inoculums were used within 15 mins and inoculated plates were set for 10-15 mins before strip application.

A co-trimoxazole disk (Himedia, 1.25/23.75 mcg, India), a co-trimoxazole E-test strip (AB Biodisk, Solna, Sweden), a ceftazidime disk (Himedia, 30 mcg, India), and a ceftazidime E-test strip (AB Biodisk, Solna, Sweden) were applied on the surface of the agar plate at an appropriate distance. The plates were inversely put in the incubator, not allowing moisture to accumulate and interfere with reading the results, for 20-24 hours at 35°C. When an even growth was achieved and the zone of inhibition could be clearly seen, the plates were put in a dark field with a direct light source over them.

The inhibitory zone was measured at the point where there was a sharp decline in the amount of colony growth. The results were interpreted according to the criteria of CLSI for *Pseudomonas spp.* as follows:

- Ceftazidime disk: sensitive  $\geq 18$  mm, intermediate = 15-17 mm, resistant  $\leq 14$  mm
- Co-trimoxazole disk: sensitive  $\geq 16$  mm, intermediate = 11-15 mm, resistant  $\leq 10$  mm

The minimal inhibitory concentration (MIC) for E-test was considered as the first point of significant inhibition ellipse intersected the scale on the strip, and was interpreted as follows:

- Ceftazidime E-test strip: sensitive  $\leq 8$   $\mu\text{g/ml}$ , resistant  $\geq 32$   $\mu\text{g/ml}$
- Co-trimoxazole E-test strip: sensitive  $\leq 2$   $\mu\text{g/ml}$ , resistant  $\geq 4$   $\mu\text{g/ml}$

MIC<sub>50</sub> and MIC<sub>90</sub> were defined as the minimal concentration of the antibiotic capable of inhibiting the growth of 50% and 90% of the isolates, respectively.

## Statistical analysis

The correlation between antimicrobial susceptibility of E-test and disk diffusion was statistically analyzed by SPSS version 15.0 for Windows using chi-square test and Kappa coefficient separately for each of the two antibiotics.

## RESULTS

Among a total of 12922 blood specimens, 2300 specimens had a positive blood culture (17.7%); the specimens were collected early at hospitalization, as a result, blood samples were collected before initiation of any treatment. Not considering fungal growth, 21 microorganisms were recognized, with *S. maltophilia* being the most common (895 specimens; 38.9%). Other frequent pathogens included *S. epidermidis* (254 specimens; 11.1%), *Alcaligenes* (164 specimens; 7.1%), *S. aureus* (157 specimens; 6.8%), *Acinetobacter* (129 specimens; 5.6%), *Enterobacter* (129 specimens; 5.6%), *E. coli* (120 specimens; 5.2%) and, others (452 specimens; 19.7%). Most of the bacterial growth were reported for emergency ward 1 (780 specimens; 33.9%), intensive care unit (ICU) (350 specimens; 15.2%), emergency ward 2 (303 specimens; 13.2%), infectious disease ward (280 specimens; 12.2%), and others (587 specimens; 25.5%) with the highest rate of *S. maltophilia* isolated from emergency ward 1 (371 out of 895; 41.5%), emergency ward 2 (211 out of 895; 23.6%), infectious disease ward (102 out of 895; 11.4%), ICU (82 out of 895; 9.2%), and others (129 out of 895; 14.3%).

Among 100 specimens, randomly selected from 890 specimens with a positive *S. maltophilia* culture, there was 84 sensitive, 2 intermediate, and 14 resistant species in the disk diffusion method and 82 sensitive, 8 intermediate, and 10 resistant species in the E-test for ceftazidime. Also, there was 95 sensitive and 5 resistant species in both the disk diffusion method and E-test for co-trimoxazole [Table 1].

Reported MIC for ceftazidime ranged from 0.3 to 128 µg/ml and a mean MIC<sub>50</sub> and MIC<sub>90</sub> of 2 and 32 µg/ml were detected, respectively. The mean MIC<sub>50</sub> and MIC<sub>90</sub> for co-trimoxazole were 0.5 and 2 µg/ml, respectively, with an MIC range of 0.13 to 32 µg/ml [Table 2]. Considering ceftazidime, the MICs obtained by E-test correlated well with those determined by the disk diffusion method, with an overall agreement of 97.6% for sensitive bacteria and 87.2% for resistant ones (kappa=0.78) (including intermediate bacteria in the resistant group). For co-trimoxazole, the MICs obtained by E-test correlated well with those of the disk diffusion method just for sensitive bacteria with an overall agreement of 95.8%; however, the two tests showed a poor agreement of 20% for resistant bacteria (kappa=0.158).

Considering E-test as the gold standard test, very major error was determined as false-susceptible result, and major error as false-resistant result, by the disk diffusion test; any other discordance was considered as minor

**Table 1: Comparative susceptibility of ceftazidime and co-trimoxazole against *S. maltophilia* by E-test and disk diffusion**

	Susceptibility in disk diffusion			Susceptibility in E-test		
	Sensitive	Interme- diate	Resistant	Sensitive	Interme- diate	Resistant
Ceftazidime	84	2	14	82	8	10
Co-trimoxazole	95	2	3	95	-	5

**Table 2: E-test susceptibility result of *S. maltophilia* (n=100)**

Antibiotic	MIC <sub>50</sub> (µg/ml)	MIC <sub>90</sub> (µg/ml)	Range
Ceftazidime	2	32	0.3-128
Co-trimoxazole	0.5	2	0.13-32

error. Hence, two very major, one major and seven minor errors were detected for ceftazidime while two very major, one major and two minor errors were diagnosed for co-trimoxazole.

## DISCUSSION

The study showed that the most prevalent pathogens in the blood cultures were *S. maltophilia*, *S. epidermidis*, *Alcaligenes*, and *S. aureus*, respectively; generally gram-negative bacilli were the most prevalent bacterial types. This bacterial distribution pattern was different from the findings of other countries, as Fluit<sup>[14]</sup> showed that *E. coli* was the most frequent organism in Europe followed by *S. aureus*, coagulase negative staphylococci, and *Pseudomonas*. In a study by Pfaller<sup>[15]</sup> *E. coli*, *Kelebsiela* and *Pseudomonas* were the most prevalent microorganisms.

Emergency 1, ICU and emergency 2 wards were the most infected wards, respectively. This demonstrates the emergence of considering the epidemiology, transmission routes, and sources of the pathogens. As patients in emergency wards are mostly referred to different wards according to their main complaint, paying no attention to the high frequency of pathogens in the emergency ward may lead to inconceivable spread of the microorganisms. In the ICU, where patients are in critical conditions, this may cause a high mortality rate.

Although in other studies ICU has been mentioned as the most infected ward by *S. maltophilia*,<sup>[16,17]</sup> here we report the highest rate of infection by this microorganism from the emergency wards (47.5% in emergency 1, 69.6% in emergency 2) which necessitates identifying the characteristics of the bacteria. This pathogen is isolated from water sources;<sup>[18]</sup> as a result, it seems necessary to perform frequent sampling from dialysis systems,

disinfectant solution, ventilator systems, blood sampling devices, and various water sources in hospital, the recommendation suggested by the previous studies.<sup>[19]</sup> The microorganism ability to attach and colonize on plastic surfaces makes intravenous catheters potential sites for the pathogen colonization.<sup>[20]</sup>

In this study we selected co-trimoxazole and ceftazidime from the various antibiotics recommended by CLSI, after consulting infectious disease specialist, in order to compare their antibacterial susceptibility. The results indicated that the susceptibility of *S. maltophilia* against ceftazidime using E-test was 82% and the MIC<sub>50%</sub> and MIC<sub>90%</sub> of this antibiotic was respectively 2 and 32 µg/ml. In a study by Pfaller,<sup>[15]</sup> the susceptibility in Canada, United States and Latin America was respectively 27%, 64.7%, and 93.3% and Tatman<sup>[21]</sup> in Turkey showed the susceptibility of 67% for this drug. This variety in results indicates that the susceptibility of *S. maltophilia* is not the same in different countries and even different hospitals, obligating health care centers to evaluate the antibacterial susceptibility of various antibiotics by their own. The high susceptibility for ceftazidime in the present study confirms that this antibiotic is an effective drug in the treatment of *S. maltophilia* infections in our center.

Ninety-five percent of *S. maltophilia* were susceptible against co-trimoxazole and the MIC<sub>50%</sub> and MIC<sub>90%</sub> for this antibiotic was measured 0.5 and 2 µg/ml, respectively. Although in different studies by Tatman<sup>[21]</sup> and Nicomedeo<sup>[5]</sup> the susceptibility was reported to 98% and 98.6%, respectively, Wang *et al*<sup>[22]</sup> found a susceptibility of about 60% for this drug. We believe that 5% antibacterial resistance for co-trimoxazole as the treatment of choice for *S. maltophilia* infection is acceptable; however, future consecutive reevaluation of the pathogen resistance is necessary. Besides, this drug is a bacteriostatic antibiotic with increasing toxicity in higher dosage, as a result, it is recommended to be used in combination with a bactericidal antibiotic like ceftazidime.<sup>[9]</sup>

Several studies have compared disk diffusion and E-test susceptibility methods, obtaining paradoxical findings<sup>[5,8,21]</sup>; some have reported correlation between the two tests,<sup>[8]</sup> while others mentioned disk diffusion as an unreliable test<sup>[21]</sup> and introduced E-test as an appropriate practical susceptibility test for *S. maltophilia*.<sup>[5]</sup> In our study the correlation between these two methods for susceptibility against ceftazidime in sensitive and resistant isolated *S. maltophilia* were respectively 95.2% and 95.8%, while against co-trimoxazole they were measured to be 87.2% and 20%, respectively for the sensitive and resistant isolated

bacteria. These results show that when ceftazidime and co-trimoxazole are used against sensitive *S. maltophilia*, reports by disk diffusion are reliable; however, for resistant *S. maltophilia*, E-test should be applied according to patient's clinical condition and manifestation.

Based on various reports about *S. maltophilia* resistance against multiple antibiotics<sup>[9-12]</sup>, it is vital to run consecutive studies in this field. However, application of these findings depends on the active interaction between the physicians in clinic and laboratory. While there are several studies evaluating *in vitro* susceptibility of antibiotics, it seems indispensable to design studies about the effect of antibiotics in patient clinic.

## CONCLUSION

According to the reported MIC and susceptibility for ceftazidime and co-trimoxazole, *S. maltophilia* is the most frequent reported pathogen in our hospital with a high susceptibility to both antibiotics. However, it is of great importance to investigate the reason for such a high prevalence of this pathogen in the hospital, especially in the emergency ward and find the epidemiologic sources of the microorganism.

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**How to cite this article:** Jamali F, Boroumand MA, Yazdani F, Anvari MS, Pourgholi L, Mahfouzi S, Khak M. Minimal inhibitory concentration of ceftazidime and Co-trimoxazole for *Stenotrophomonas maltophilia* using E-test. J Global Infect Dis 2011;3:254-8.

**Source of Support:** Nil. **Conflict of Interest:** None declared.

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