

ORIGINAL ARTICLE

# Molecular Characterization of Trimethoprim Sulpha-methoxazole Resistant *Stenotrophomonas maltophilia* at Sohag University Hospital

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## ABSTRACT

### Key words:

**Molecular, Trimethoprim Sulpha-methoxazole, *Stenotrophomonas maltophilia***

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**Background:** *Stenotrophomonas maltophilia* (*S. maltophilia*) is an opportunistic human pathogen that is intrinsically multidrug resistant causing serious infections in humans and its emerged resistance to trimethoprim-sulfamethoxazole (SXT) is worldwide reported. **Objectives:** This work aimed to determine the occurrence of SXT resistance among *S. maltophilia* isolated from Sohag University Hospitals and to assess the association of sul genes with SXT-resistant isolates. **Methodology:** This study carried during the period from December 2015 to November 2016 in the microbiology laboratory of Sohag University Hospital on 65 *Stenotrophomonas maltophilia* isolates collected from 380 inpatients samples admitted to the Intensive care units (ICUs) of Sohag university hospital through this period. Identification and antibiotic susceptibility of *S. maltophilia* was done by the **Vitek- 2 colorimetric compact system (bioMérieux, France)** then multiplex PCR was done to detect the presence of *SUL1*, *SUL2*, *SUL3* genes in the isolates. **Results:** Among the 65 *S. maltophilia* isolates, 17(26.2%) were resistant to SXT. All SXT-resistant isolates were found to harbor *sul1* gene (17/17, 100%), one of these isolates had *sul2* gene (1/17,5.9%). Only 2 of the 48 SXT-susceptible isolates were found to yield positive PCR results for *sul* genes, one of them gave positive result for *SUL1*(1/48,2%) the other for *SUL2* (1/48, 2%) genes. Meanwhile, *sul3* gene was not detected in any of the isolates. **Conclusions:** Our study reported that *S. maltophilia* is among the common causes of infections occurring in the ICUs of Sohag university hospital specially those of the respiratory tract. Presence of SXT resistance among clinical *S. maltophilia* isolates from Sohag University Hospital, in which *sul1* gene was found to have a major role. Tigecycline and levofloxacin are the antimicrobials of choice for treatment of infections caused by *S. maltophilia*.

## INTRODUCTION

*Stenotrophomonas maltophilia* is a glucose non fermentative Gram-negative aerobic motile bacillus, generally found in aquatic environments, which causes human disease in immunocompromised patients<sup>1</sup>, behind *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, *S. maltophilia* is the third most common non-fermenting Gram-negative bacillus responsible for healthcare-associated infections<sup>2</sup>.

The organism was first isolated in 1943 and named *Bacterium bookeri*. In 1961, it was reclassified as a member of the genus *Pseudomonas*, then *Xanthomonas* in 1983 and finally *Stenotrophomonas* in 1993<sup>3</sup>. It is an environmental multidrug resistant organism (MDRO) can survive on almost any aqueous surface forming biofilm and can colonize areas of the body without causing infection<sup>4</sup>. But, in immunocompromised, hospitalized patients, *S. maltophilia* can cause a wide range of serious infections, including nosocomial pneumonia, bacteremia, urinary tract infections, wound

infections, skin and soft tissue infections, meningitis, and endocarditis<sup>3</sup>.

The incidence of hospital-acquired *S. maltophilia* infections is increasing, and cases of community-acquired *S. maltophilia* have also been reported<sup>6</sup>. The risk for *S. maltophilia* infection is increased in ICU patients, patients with long hospital stay, HIV infection, cancer, cystic fibrosis, recent surgery, trauma, mechanical ventilation, and previous therapy with broad-spectrum antibiotics<sup>7</sup>.

*S. maltophilia* has high level intrinsic resistance to many antibiotics because of its multidrug-efflux pumps and low outer membrane permeability<sup>5</sup>. In addition, it can acquire antibiotic resistance by horizontal transfer of resistance genes which carried on plasmids, transposons and Integrons making infections difficult to treat<sup>8</sup>. The World Health Organization classify *S. maltophilia* as one of the causative multidrug resistant organisms (MDROs) of infection in hospital settings<sup>9</sup>

Trimethoprim/sulfamethoxazole (SXT, cotrimoxazole) is considered the first-line agent recommended for the treatment of *S. maltophilia*<sup>7</sup>.

However, SXT resistance in *S. maltophilia* has been widely increasing over recent years<sup>10</sup>. This constitutes a great clinical problem, as the range of effective antibiotics is even more limited in infections caused by co-trimoxazole-resistant *S. maltophilia*<sup>11</sup>.

Resistance to co-trimoxazole can result from mutations in the chromosomal dihydropteroate synthetase (DHPS) gene or more frequently from the acquisition of an alternative DHPS gene (*sul*), whose product has a lower affinity for sulfonamides<sup>12</sup>. The *sul1* gene is mostly found linked to other resistance genes in class 1 integrons, while *sul2*, *Sul3* are usually located on small plasmids. The genetic localization of *sul* genes on efficient mobile genetic structures probably contributes to the widespread of sulfonamides resistance<sup>13</sup>.

As no much information is currently available regarding the frequency of SXT resistance among *S. maltophilia* isolates in our hospital, this study aimed to determine the occurrence of SXT resistance among *S. maltophilia* isolated from Sohag University Hospital and to assess the association of *sul* genes with SXT-resistant isolates.

## METHODOLOGY

### Study Design and Patient Selection

Across-sectional study was carried out in the period from December 2015 to November 2016 during which samples were collected according to the site of infection from patients admitted to Sohag University Hospital. The study was conducted in the microbiology laboratory of Sohag University. Urine, sputum, endotracheal aspirates (ETA), blood and pus were collected from patients located in different ICUs according to the standard microbiological methods.

### Cultivation and Presumptive Identification of *Stenotrophomonas* Isolates

Samples were grown on blood and MacConkey's agar except urine samples, which were grown on CLED (Oxoid, UK). Blood samples were collected in blood culture bottles (Oxoid, UK) containing brain-heart infusion broth and then subcultured onto agar plates (Blood and MacConkey's agar). Non-lactose fermenting colonies were identified initially by Gram stain, catalase test, oxidase test, then confirmed to be *S. maltophilia* by Vitek-2 colorimetric compact system (bioMerieux, France). An overnight growth on blood agar of pure colonies was suspended in sterile saline to 0.5 McFarland Standard turbidity. The suspension was applied onto an ID GN Card (bioMerieux, Marcy l'Etoile, France) and the result was obtained on VITEK-2 compact (bioMerieux, Marcy l'Etoile, France) within 24 hours.

### Antibiotic Susceptibility Test

Vitek-2 was employed to determine the antibiotic susceptibility pattern of the isolates with the use of AST-GN card. All isolates were tested against, Ampicillin, Ampicillin/sulbactam, Piperacillin/Tazobactam, Cefazolin, Ceftriaxone, Cefepime, Aztreonam, Cefoxitin, Imipenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, Trimethoprim/Sulfamethoxazole.

### Assessment of SXT Resistance Genes

#### DNA extraction

A single colony was inoculated into Mueller-Hinton broth and incubated for 20 hours at 37°C. After centrifugation at 10000 round per minute for 10 minutes, each pellet was washed three times in 750µl TE buffer and then resuspended in 500µl TE buffer. The solution was boiled for 20 min and centrifuged at 10000 round per minute for 10min, and the supernatant was then used as a crude

DNA extract in PCR. Extracted DNA was stored at -20°C until further processing.<sup>14</sup>

#### *sul1*, *sul2* and *sul3* detection:

Multiplex PCR amplification of *SUL1*, *SUL2* and *SUL3* was conducted as described by Kern et al.<sup>15</sup> Amplification of *SUL1* was performed using the forward primer *SUL1f* (5'-CGG CGT GGG CTA CCT GAA CG-3') and reverse primer *SUL1r* (5'-GCCGATCGCGTGAAGTTCCG-3') (433bp). *SUL2* was identified using the forward primer *SUL2-F* (5'-GCG CTC AAG GCA GAT GGC ATT-3') and the reverse primer *SUL2-B* (5'-GCG TTT GAT ACC GGC ACC CGT-3') (293bp). *SUL3F* primer (5'-GAG CAA GAT TTT TGG AAT CG-3') and *SUL3R* primer (5'-CAT CTG CAG CTA ACC TAG GGC TTT GGA-3') (569bp)

The PCR mixture contained 5µl of template DNA, 5 µl of 10×PCR buffer, 10 µl of dNTP mix, 4 µl of MgCl<sub>2</sub>, 0.5 µl of Taq DNA polymerase, 1.25 µl of each primer *sul1-F*, *sul1-R*, *sul2-F*, *sul2-R*, *SUL3-F*, *SUL3R* and 18 µl of PCR water (*Invitrogen, UK*).

Amplification was carried out by heating for 5 min at 94°C, followed by 30 cycles of 94°C for 15s, 69°C for 30s and 72°C for 60s, followed by one cycle at 72°C for 7 min using Biometra T gradient thermal cycler (*Electrophoresis power supply-Biometra, Germany*).

#### Amplicon detection by agarose gel electrophoresis:

Ten µl of each amplified DNA & 1000 molecular weight marker (*Invitrogen, UK*) were separated on 2% agarose gel containing 0.3mg/ml of ethidium bromide. The bands were visualized using gel documentation system (*Ingenius Syngene, USA*).

## RESULTS

A total of 65 clinical isolates of *S. maltophilia* were obtained from 380 patients (65/380, 17%) admitted to different ICUs at Sohag University Hospital in the period from December 2015 to November 2016. Isolates were obtained from 35 male (53.8%) and 30 female (46.2%). The most frequent site of isolation was the respiratory tract (90.7%); including ETA (73.8%) & sputum (16.9%) followed by blood (6.2%), pus (3.1%), no *S. maltophilia* isolated from urine samples (Table 1).

The antimicrobial activities of 17 antibiotics against 65 *S. maltophilia* isolates are presented in Table (2) as tested by Vitek-2 automated system. All *S. maltophilia* isolates were sensitive to Tigecycline, while 48 (73.8%) isolates were sensitive to SXT, 55 (84.6%) isolates were sensitive to levofloxacin and all the isolates were completely resistant to the remaining tested antibiotics.

The presence of SUL genes was tested in all the 65 *S. maltophilia* isolates. All of the 17 SXT-resistant isolates harbored *sul1* gene while only one of the SXT sensitive isolates carried it, *sul2* gene was detected in one SXT resistant and one SXT sensitive isolate. *sul3* gene, on the other hand, had not been detected in any of the *S. maltophilia* isolates (Table 3).

**Table 1: Distribution of *Stenotrophomonas maltophilia* isolates in different samples.**

Specimen	No. (%)
Endotracheal aspirate	48(73.8)
Sputum	11(16.9)
blood	4(6.2)
pus	2(3.1)
urine	0(00.0)
Total	65(100%)



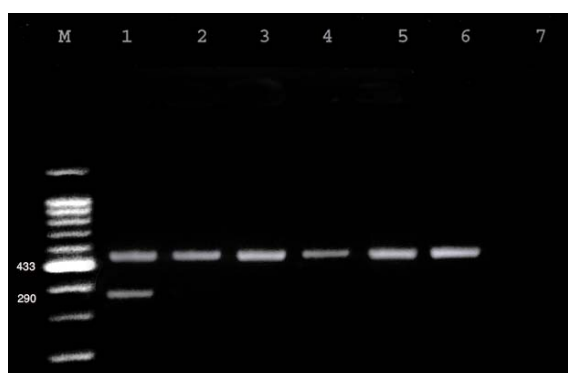
**Fig. 1: *Stenotrophomonas maltophilia* growth on blood agar**

**Table 2: Antimicrobials to which the sensitivity of *Stenotrophomonas maltophilia* was tested by Vitek-2 automated system.**

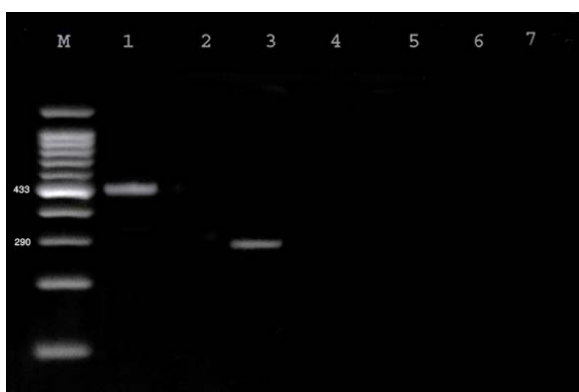
Antimicrobial	Sensitive		Resistant		MIC
	No.	%	No.	%	
Ampicillin	0	0	65	100	≥32
Ampicillin/sulbactam	0	0	65	100	≥32
Piperacillin/ Tazobactam	0	0	65	100	≥32
Cefazolin	0	0	65	100	≥64
Ceftriaxone	0	0	65	100	≥64
Cefepime	0	0	65	100	≥64
Aztreonam	0	0	65	100	≥64
Cefoxitin	0	0	65	100	≥64
Imipenem	0	0	65	100	≥64
Amikacin	0	0	65	100	≥16
Gentamycin	0	0	65	100	≥16
Tobramycin	0	0	65	100	≥16
Ciprofloxacin	0	0	65	100	≥8
levofloxacin	55	84.6	10	15.4	0.5
Tigecycline	95	100	0	0	2
Nitrofurantoin	0	0	65	100	≥512
Trimethoprim/Sulfamethoxazole	48	73.8	17	26.2	≤20

**Table 3: Association between SUL genes and Trimethoprim/Sulpha-methoxazole(SXT) resistance.**

SXT susceptibility	No. Of Isolates	SUL1 NO.(%)	SUL2 NO.(%)	SUL3 NO.(%)
susceptible	48	1(2)	1(2)	0(0)
Resistant	17	17(100)	1(5.9)	0(0)



**Fig. 2:** Gel electrophoresis showing results of multiplex PCR of SXT resistant *S. maltophilia* isolates for detection of SUL1,2,3 genes lane 1(M): 100 bp marker, lane 2(Sample1): amplicons of both SUL1, SUL2 genes (433, 290 bp respectively), lane 3-7(samples 2,3,4,5,6) amplicon of SUL1 gene (433 bp), SUL3 gene was not detected in any of the samples, lane 8: negative control.



**Fig. 3:** Gel electrophoresis showing results of multiplex PCR of SXT sensitive *S. maltophilia* isolates for detection of SUL1,2,3 genes, lane 1(M): 100 bp marker, lane 2(Sample1): amplicon of SUL1 gene (433bp), lane 4 (sample 3): amplicon of SUL2 gene (290bp), other samples were negative for all SUL genes, lane 8: negative control.

## DISCUSSION

*S. maltophilia* is an emerging multidrug resistant opportunistic pathogen. Its intrinsic or acquired resistance to most antibiotics and its ability to colonize the surfaces of medical devices have made it a potentially dangerous pathogen<sup>16</sup>.

In our study, 65 *S. maltophilia* isolates were obtained from 380 inpatients admitted to different ICUs at Sohag University Hospital in the period between December 2015-November 2016 with a percentage of 17%. Lower percentages were reported in other Egyptian studies as it was 9.6% in a study carried in Assiut university hospitals by Amany et al<sup>17</sup>, 10.6% in a study carried in Zagazig University by Morsi et al.<sup>14</sup> while in a study carried in Mansoura University

Hospitals by Dalia et al.<sup>18</sup> the percentage was 14%. A much lower percentages detected by other researchers as a study carried in Saudia Arabia by Nada et al.<sup>19</sup>, the percentage of *S. maltophilia* among other bacterial isolates was 1.5%, and in an egyptian study carried by Hadir et al.<sup>20</sup> in National Cancer Institute of Cairo the percentage was 2%, a percentage of 1.3% found in another Egyptian study carried also in National Cancer Institute of Cairo by Hadir et al.<sup>21</sup>, in a Brazilian study carried by Gallo et al.<sup>22</sup> *S. maltophilia* represented 3% of the bacterial isolates. This variability in the percentage of *S. maltophilia* isolates in the different localities may be attributed to different patient population and different underlying risk factors and diseases.

In the present study, *S. maltophilia* isolation was most frequent from respiratory specimens (endotracheal aspirate and sputum) which come in agreement with other egyptian studies Morsi et al<sup>14</sup>, Dalia et al.<sup>18</sup>, Thabit et al.<sup>23</sup> who reported that the respiratory tract is the most popular for *S. maltophilia* isolation, also other studies from other countries come in agreement with our study in this point as Naeem et al.<sup>24</sup>, Samonis & Karageorgopoulos.<sup>25</sup>, Hsiu et al.<sup>26</sup>

The results of all mentioned studies in addition to ours agreed that although *S. maltophilia* may cause many types of human infections, the respiratory tract represents the most common site affected.

The management of *S. maltophilia* infections represents a great challenge to Clinicians due to problems with in vitro susceptibility testing, lack of clinical trials to determine optimal therapy, and its intrinsic resistance to majority of antimicrobial agents, which greatly limits the effectiveness of commonly used empiric antimicrobial therapies.<sup>16</sup>

In our study, sensitivity of *S. maltophilia* isolates were tested against 17 antibiotic using the Vitec-2 automated machine only three antibiotics were effective in vitro namely Tigecycline, Levofloxacin, and SXT, Similar results were obtained by Morsi et al.<sup>14</sup>, Samonis & Karageorgopoulos<sup>25</sup> who reported that Tigecycline could be considered as new therapeutic option against *S. maltophilia* infections. This is also in line with Zhanel et al.<sup>27</sup> who stated that Tigecycline displayed good in vitro activity against MDR isolates of *S. maltophilia*. Chung et al.<sup>28</sup> reported that Tigecycline have shown good in vitro activity against clinical isolates of *S. maltophilia*.

It was found that 84.6% of *S. maltophilia* isolates were sensitive to Levofloxacin, one of the new fluoroquinolones which come in agreement with other Egyptian studies as a study made by Dalia et al.<sup>18</sup> in Mansoura University Hospitals where 86% of their *S. maltophilia* isolates were sensitive to levofloxacin and also another study carried out in Zagazig University by Morsi et al.<sup>14</sup> found that 81.3% their *S. maltophilia* isolates were sensitive to levofloxacin.

Lower Levofloxacin susceptibility rates among *S. maltophilia* isolates ranging from 64–69.6% have been reported in Canada<sup>27</sup>, Korea<sup>29</sup>, and China<sup>30,31</sup>.

Trimethoprim/sulfamethoxazole has been considered as the mainstay of therapy against *S. maltophilia* infections. This is primarily based on in vitro susceptibility data rather than clinical studies.

However, increasing resistance to trimethoprim/sulfamethoxazole has been reported by several studies and has been mostly related to the horizontal spread of mobile genetic elements carrying resistance genes<sup>25</sup>.

In our study, 26.2% of *S. maltophilia* isolates showed SXT resistance. This comes much higher than a previous Egyptian study done in Mansoura city, which revealed SXT-resistance only in 4.55% of their isolates<sup>32</sup>. This also comes in contrast to Chung et al.<sup>10</sup> who stated that resistance rates vary geographically but are generally less than 10%. Two Egyptian studies come in agreement with us as regards the increased SXT resistance rate among *S. maltophilia* isolates which was 37.5% in a study done by Morsi et al.<sup>14</sup>, done in Zagazig university and 24.4% in a study done by Dalia et al.<sup>18</sup> in Mansoura university. However, various rates of resistance to SXT have been reported in several countries, including Taiwan, Japan, Korea, Thailand, Spain, Mexico, Saudi Arabia, Turkey, and Canada (16–78.8%)<sup>33</sup>.

In the current study the sul genes were tested in all the 65 *S. maltophilia* isolates. All of the 17 SXT-resistant isolates harbored sul1 gene. One isolate among them was additionally positive for sul2 gene. On the other hand, sul3 gene has not been detected in any of our isolates. We come in agreement with the study carried by Morsi et al.<sup>14</sup> in Zagazig university hospitals where SUL1 genes detected in all SXT *S. maltophilia* isolates and in two of SXT susceptible group and SUL2 gene detected in one of the SXT resistant group and not detected in the susceptible group, and SUL3 not detected in any of the isolates.

Several other studies have reported that sul1 gene is the major mechanism of SXT resistance in *S. maltophilia*<sup>28,34,35</sup>.

Only 2 of the 48 SXT-susceptible isolates were found to yield positive PCR results for sul gene, one of them had SUL1 and the other carried SUL2. This is in line with other researchers who reported the presence of sul genes in SXT susceptible *S. maltophilia* isolates<sup>36,37</sup>. However, others reported absence of sul genes in SXT-susceptible isolates<sup>34,38</sup>.

It worth mentioning that the presence of SUL genes in *S. maltophilia* can further lead to the development of multi-drug resistance and may act as a potential source for the dissemination of resistance. This indeed confirms the importance of strict application of infection control measures in order to decrease the incidence of infections caused by this serious worldwide intrinsically drug-resistant pathogen.

## CONCLUSIONS

In conclusion, this study highlighted the presence of Trimethoprim- sulphamethoxazole resistance among *S. maltophilia* isolated from Sohag University Hospital, which was much associated with sul1 gene. This necessitates continuous surveillance of antimicrobial drug resistance and careful epidemiological monitoring of SXT resistance, which has the potential to spread by means of mobile genetic elements as well as strict adherence to infection control.

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