International Journal of Infection Control
www.ijic.info ISSN 1996-9783

ORIGINAL ARTICLE

Multidrug resistant *Stenotrophomonas maltophilia*: an emerging cause of hospital acquired infections in Assiut University Hospitals, Egypt

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doi: 10.3396/IJIC.v13i1.001.17

Abstract

Stenotrophomonas maltophilia is an opportunistic multidrug resistant pathogen causing hospital-acquired infections (HAIs) with limited treatment options. We aimed to determine the prevalence of *S. maltophila* causing HAIs and environmental contamination in the intensive care units (ICUs) and wards of Assiut University Hospitals. We determined the antibiotic resistance profiles of, production of metallo-β-lactamases (MBLs) by, and the presence of the *sul II* gene in these isolates. The study included 362 patients with HAIs and 4151 environmental samples from the ICUs and wards. Antibiotic sensitivities were tested by the disc diffusion method; imipenem minimum inhibitory concentration (MIC) was determined using the E-test. Metallo-β-lactamase enzymes (MBLs) were detected phenotypically by combined disc test (CDT) and double disc synergy test (DDST). The *sul II* gene was detected by polymerase chain reaction. The percentages of *S. maltophilia* causing infections and environmental contamination were found to be 9.7% and 0.67% respectively. Respiratory tract infection was the most common infection (17.97%). Isolates were highly resistant to aztreonam, penicillins, carbapenems, quinolones, cephalosporins, aminoglycosides, chloramphenicol and tetracyclines, and least resistant to trimethoprim- sulfamethoxazole (SXT). All imipenem resistant isolates (82.54%) showed MBL phenotypically by both tests. For imipenem sensitive isolates (17.46%), MBL was detected by DDST and CDT in 36.36% and 18.18% respectively. Isolates resistant to SXT had *sul II* genes. In conclusion, *S. maltophilia* is a significant hospital pathogen at Assiut University Hospitals with high percentages of resistance to many antimicrobials, making the possibility of dissemination worrisome. In our setting, SXT is the agent of choice for the treatment of *S. maltophilia* infections.

Keywords: *Stenotrophomonas maltophilia*; healthcare-acquired infections; drug resistance; health facility environment; Egypt

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Introduction

Stenotrophomonas maltophilia is an emerging multidrug-resistant (MDR) pathogen in healthcare facilities worldwide.[1] Although it is sometimes thought to be a colonizer, it can cause infections in susceptible hosts with multiple risk factors.[1,2] Due to the increase in the patient population at risk, the incidence of *S. maltophilia* infections may be increasing.[3]

Stenotrophomonas maltophilia is intrinsically resistant to antibiotics.[4] Antibiotics with *in vitro* activity against *S. maltophilia* include trimethoprim-sulfamethoxazole (SXT), fluoroquinolones (FQs), tetracyclines, ticarcillin-clavulanate, and ceftazidime; however, there are limited clinical data on the use of these agents.[5,6]

Even though SXT is the drug of choice for *S. maltophilia* infections, treatment may not be possible due to allergies, toxicities, resistance, or drug shortages.[6] Resistance may be due to class 1 integrons containing the *sul1* sulfonamide resistance gene and insertion element common region elements containing the *sul2* resistance gene that can transfer intra- and inter-generically.[4,7]

Fluoroquinolones are an attractive alternative for treating *S. maltophilia* infection, as they are well-tolerated, effective, and have low rates of microbial resistance.[7] Although carbapenems are considered the last resort for treatment of critically ill patients, many mechanisms of resistance have evolved.[8,9] Metallo-β-lactamases (MBLs) are one of the most worrisome resistance mechanisms as they limit treatment options and their genes are carried on highly mobile elements, allowing easy dissemination.[9] Metallo-β-lactamase producing strains are reported to be responsible for prolonged HAI outbreaks, with serious infections and higher morbidity and mortality.[8,10] Rapid detection of MBLs is essential to help modify therapy and to initiate effective infection control policy to prevent further dissemination.[11]

Environmental *S. maltophilia* isolates usually have lower levels of resistance to antibiotics than clinical strains. However, in some instances, MDR environmental isolates have been isolated, which constitute a health risk.[12]

This study aimed to determine the prevalence of *S. maltophila* causing healthcare associated infections (HAIs) and environmental contamination in the intensive care units (ICUs) and wards of Assiut University Hospitals. In addition, this study investigated the pattern of antimicrobial resistance, production of MBLs and detection of the *sul 2* gene among *S. maltophilia* isolates.

Patients, Materials and Methods

This cross sectional study included patients with clinical signs and symptoms of HAIs according to the Centers for Disease Control and Prevention (CDC) definitions.[13] The Ethical Committee of Faculty of Medicine, Assiut University approved this study.

Clinical samples

A total of 690 clinical samples were obtained from 362 patients who developed criteria of HAIs. These samples included endotracheal swabs (n=205), blood (n=199), urine (n=114), surgical wound swabs (n=86), sputum (n= 69), rectal swabs (n=12), and bed sore swabs (n=5).

Environmental samples

A total number of 4,151 environmental samples were collected from surfaces, walls, furniture, beds, trolleys and the surroundings of patients in ICUs and wards.

Bacterial identification and susceptibility testing

Bacterial identification was done by conventional bacteriological methods and confirmed by API 20 NE (Biomerieux, France) system.[14] The Kirby-Bauer disc diffusion method of susceptibility testing was used with the following antimicrobial discs (HiMedia, India): ampicillin (10 µg), amoxacillin-clavulanic acid (20-10 µg), piperacillin (100 µg), aztreonam (30 µg), cefazolin (30 µg), cefaclor (30 µg), cefoperazone (75 µg), ceftriaxone (30 µg), amikacin (30 µg), tobramycin (10 µg), netilmicin (30 µg), tetracycline (30 µg), tigecycline (30 µg), ciprofloxacin (5 µg), lomefloxacin (10 µg), levofloxacin (5 µg), nalidixic acid (30 µg), imipenem (10 µg), meropenem (10 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (25 µg). Interpretation was accordance with Clinical and Laboratory Standards Institute (CLSI) 2011 guidelines.[15] Imipenem susceptibilities were performed using the E-test (BioMerieux, France), with a cutoff point of ≥16

µg/ml used to define imipenem resistance and ≤ 4 µg/ml to define imipenem susceptibility.[16]

Phenotypic detection of Metallo-β-lactamase enzymes (MBL)

The combined disc test (CDT) was performed as previously described by Jesudason *et al.*,[17] and the double disc synergy test (DDST) as described by Franklin *et al*.[18]

Polymerase chain reaction (PCR)

DNA cell extracts were prepared by the boiling method according to Caylan *et al*.[19] Amplification of the *16S rRNA-23S rRNA gene* was carried out as follows. The PCR mixture contained 1 µM concentration of each primer (Invitrogen, Lifetechnologies, USA) (Table I), 3 µl of genomic DNA, a 200 µM concentration of each of the nucleotides dATP, dTTP, dCTP and dGTP, and 1.25 µl of Taq DNA polymerase in a total volume of 50 µl. Amplification was carried out using the thermocycler (Techne-Progene, Cambridge, UK) according to the following conditions: initial denaturation at 95°C for 5 minutes with subsequent 30 cycles of amplification consisting of annealing at 58° C for 10 seconds, extension at 72°C for 60 seconds, and deanaturation at 95°C for 10 seconds. For the last cycle, the extension step was at 72°C for 2 minutes.

Amplification of the sul2 gene was conducted as follows. The PCR mixture contained: 2.5 µl of template DNA, 2.5 µl of 10× PCR buffer (Perkin Elmer); 2.5 µl of each nucleotide; 2 µl of $MgCl_2$ (25 mM); 0.25 µl of Ampli Taq DNA polymerase (50 µM; Perkin Elmer); 1.2 µl of each primer *Sul II*-F, and *Sul II*-R (2 µM) (Invitrogen, Lifetechnologies, USA) (Table I); and distilled water to reach 30 µl volume. Amplification was carried out by heating for 2 minutes at 94°C, followed by 35 cycles of 94°C for 1 minute, 60°C for 1 minute and 72°C for 1 minute followed by one cycle at 72°C for 10 minutes.

Gene products were detected by agarose gel electrophoresis (1.5%) stained with ethidium bromide. The amplicon sizes of 16S RNA –23S rRNA gene and for *sul 2* gene are shown in Table I.

Statistical analysis

Statistical analysis was performed using SPSS version 16 (IBM Corp., Somers, NY). Data were presented as numbers and percentages. Chi-square test was used to compare quantitative variables between groups.

Results

A total of 35 non-duplicate isolates of *S. maltophilia* were recovered from 362 patients who developed HAIs in different ICUs (9.7%; Table II). The organism was most commonly isolated from respiratory tract specimens. The highest proportion of patients infected with *S. maltophilia* were from the chest ICU (14.75%) (Figure 1).

Environmental contamination by gram-negative bacilli was confirmed in 12.29% (510/4151) of samples. A total of 28 *S. maltophilia* isolates (0.67% of total) were recovered from environmental samples from different ICUs, and wards in Assiut University Hospitals. The general ICU showed the highest percentage of *S. maltophilia* isolation (4/96, 4.17%). The details of distribution are presented in Tables III and IV, and Figure 2.

Table I. Primers used and amplicon size

Primer	Sequence	Target gene	Amplicon size
SM1-F	5- CAGCCTGCAAAAGTA-3	16S rRNA –	531 bp
SM2-R	5-TTAAGCTTGCCACGAACAG-3	23S rRNA gene	
***sul II*- F**	5- TGTGCGGATGAAGTCAGCTCC -3	*sul II* gene	
***sul II*- R**	5- AGGGGGCAGATGTGATCGAC -3		626 bp

Table II. Distribution of *S. maltophila* and other Gram negative bacilli in different clinical samples

Samples collected	No. of samples collected	Gm-ve bacilli					
		Lactose fermenters		Non Lactose fermenters			
				S. maltophilia		Others*	
		No.	% #	No.	% #	No.	% #
Endotracheal swabs	205	177	86.34%	19	9.27%	115	56.10%
Blood culture	199	30	15.08%	3	1.51%	10	5.03%
Urine	114	39	34.21%	1	0.88%	16	14.04%
Surgical wound swab	86	67	77.91%	6	6.98%	43	50%
Sputum	69	45	65.22%	6	8.70%	17	24.64%
Rectal swabs	12	12	100%	-	0%	8	66.67%
Bed sores	5	5	100%	-	0%	4	80%
Total	690	375	54.35%	35	5.07%	213	30.87%

* Others include *Pseudomonas, Proteus*, and *Acinetobacter spp*.

The percentage was calculated against the total number of clinical samples collected from the infection sites.

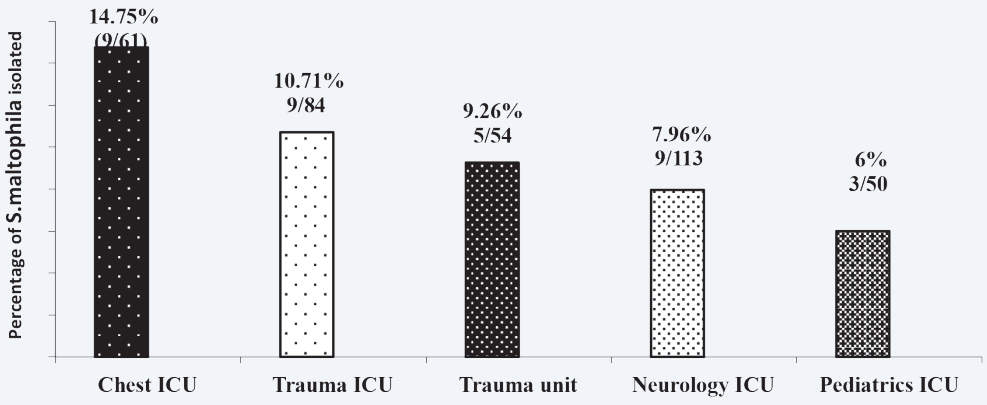

Figure 1. Percentage of *S. maltophilia* as a cause of hospital acquired infection

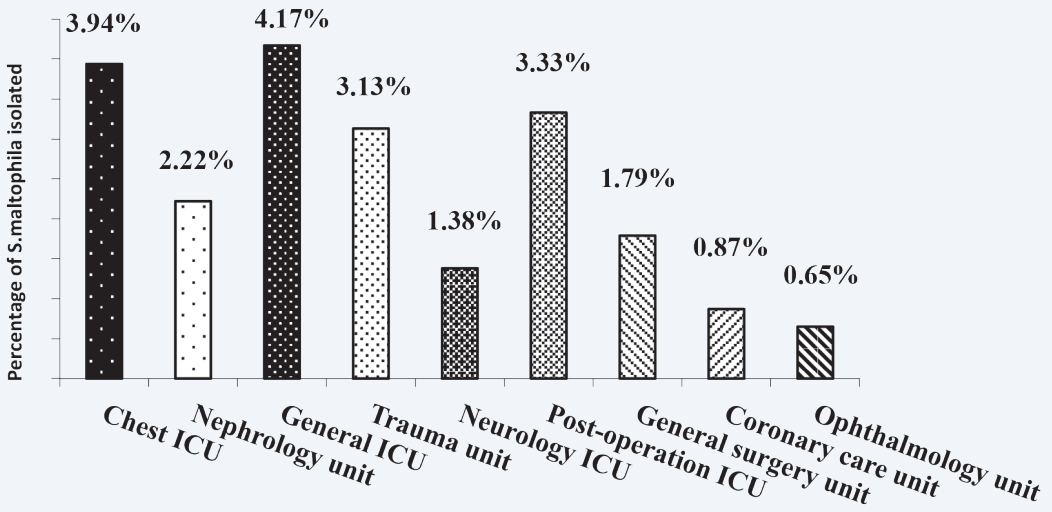

Figure 2. Frequency of *S. maltophilia* among different environmental samples collected from ICUs and wards

Table III. Distribution of *S. maltophilia* and other Gram negative bacilli among different environmental samples collected from ICUs

Samples collected	No. of samples collected	Gm-ve bacilli					
		Lactose fermenters		Non Lactose fermenters			
				S. maltophilia		Others*	
		No.	% #	No.	% #	No.	% #
Internal Medicine ICU	292	31	10.62%	-	0%	6	2.05%
Neurology ICU	217	43	19.82%	3	1.38%	24	11.06%
Paediatrics ICU	202	13	6.44%	-	0%	6	2.97%
Trauma ICU	200	46	23%	-	0%	12	6%
Gynaecology ICU	148	8	5.41%	-	0%	2	1.35%
Coronary care ICU	130	6	4.61%	-	0%	6	4.62%
Chest ICU	127	12	9.45%	5	3.94%	11	8.66%
Neurosurgery ICU	111	17	15.32%	-	0%	7	6.31%
General ICU	96	18	18.75%	4	4.17%	9	9.38%
Post-operation ICU	90	15	16.67%	3	3.33%	9	10%
Tropical ICU	85	20	23.53%	-	0%	3	3.53%
Ear, nose & throat ICU	8	-	0%	-	0%	-	0%
Plastic surgery ICU	8	1	12.50%	-	0%	-	0%
Nephrology ICU	2	-	0%	-	0%	-	0%
Total	1716	230	13.4%	15	0.87%	95	5.54%

*Others included *Pseudomonas, Proteus*, and *Acinetobacter spp*.

The percentage was calculated against the total number of environmental samples collected from the different ICUs and wards

Table IV. Distribution of *S. maltophila* and other Gram negative bacilli among different environmental samples collected from wards

Samples collected	No. of samples collected	Gm-ve bacilli					
		Lactose fermenters		Non Lactose fermenters			
				S. maltophilia		Others*	
		No.	% #	No.	% #	No.	% #
Gynaecology unit	721	39	5.41%	-	0%	10	1.39%
Paediatrics unit	336	9	2.68%	-	0%	7	2.08%
Orthopaedic unit	279	11	3.94%	-	0%	4	1.43%
Nephrology unit	225	7	3.11%	5	2.22%	6	2.67%
Ear, nose & throat unit	161	5	3.11%	-	0%	4	2.48%
Ophthalmology unit	153	1	0.65%	1	0.65%	3	1.96%
Trauma unit	128	2	1.56%	4	3.13%	5	3.90%
Coronary care unit	115	4	3.48%	1	0.87%	3	2.61%
General surgery unit	112	15	13.39%	2	1.79%	6	5.35%
Neurosurgery unit	80	1	1.25%	-	0%	2	2.50%
Emergency room	77	3	3.90%	-	0%	-	0%
Plastic surgery unit	40	-	0%	-	0%	2	5%
Internal Medicine unit	8	8	100%	-	0%	-	0%
Total	2435	105	8.31%	13	0.53%	52	2.14%

The Analytical Profile Index (API) showed that many clinical isolates had the same pattern number as the environmental isolates (5 isolates in the chest unit and 4 in the trauma unit). All isolates of *S. maltophilia* demonstrated the 16S rRNA-23S rRNA gene at 531 bp (Figure 3).

Antibiotic susceptibility testing

Among clinical specimens, the highest resistance was to imipenem and piperacillin (80%) and the lowest resistance was to SXT (8.6%) (Table V). Twenty- two of the 35 isolates (62.9%) were MDR (resistant to ≥3 classes of antibiotics). The highest resistance among environmental samples was to aztreonam and cefoperazone (89.3%) and imipenem (85.7%), and the lowest resistance was to SXT (10.7%) (Table VI). Eighteen isolates (64.3%) were MDR. The percentages of resistance of clinical and environmental samples

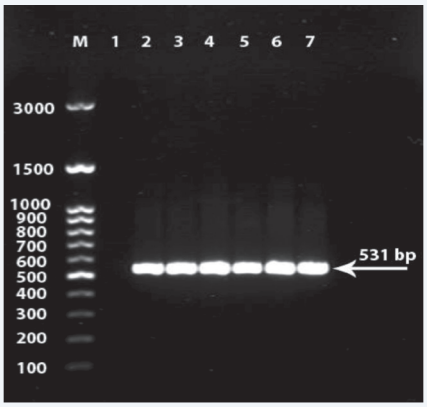

Figure 3. PCR for detection of 16S rRNA-23S rRNA gene of *S. maltophilia*
M: DNA marker
Lane 1: negative control
Lane 2: positive control
Lanes 3 to 7: positive results for the gene

Table V. Antibiotic susceptibility testing of *S. maltophilia* isolated from clinical samples

		***S. maltophilia* isolated from clinical samples**					
		S		**I**		**R**	
Group	**Members**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillin derivatives	Ampicillin	5	14.29%	3	8.57%	27	77.14%
	Amoxacillin-Clavulanic acid	7	20%	6	17.14%	22	62.86%
	Piperacillin	3	8.57%	4	11.43%	28	80%
Monobactams	Aztreonam	4	11.43%	5	14.29%	26	74.29%
Cephalosporines	Cefaclor	7	20%	3	8.57%	25	71.43%
	Cefoperazone	12	34.29%	0	0%	23	65.71%
	Ceftriaxone	12	34.29%	3	8.57%	20	57.14%
	Cefazolin	10	28.57%	0	0%	25	71.43%
Carbapenems	Imipenem	7	20%	0	0%	28	80%
	Meropenem	6	17.14%	2	5.71%	27	77.14%
Quinolones	Ciprofloxacin	8	22.86%	8	22.86%	19	54.29%
	Levofloxacin	10	28.57%	0	0%	25	71.43%
	Lomefloxacin	9	25.71%	6	17.14%	20	57.14%
	Nalidixic acid	11	31.43%	0	0%	24	68.57%
Aminoglycosides	Netilmicin	10	28.57%	1	2.86%	24	68.57%
	Amikacin	12	34.29%	5	14.29%	18	51.43%
	Tobramycin	9	25.71%	2	5.71%	24	68.57%
Tetracyclines	Oxytetracycline	9	25.71%	4	11.43%	22	62.86%

are shown in Figure 4. Regarding imipenem MICs, most isolates had MICs > 16 µg/ml (Table VII).

Phenotypic detection of metallo-β-lactamase enzyme

A total of 54 out of 63 *S. maltophilia* isolates were positive (i.e. harboured the MBL enzyme) by CDT; these included all imipenem resistant isolates and 18.2% of imipenem susceptible isolates (Table VII). With the double disk synergy test, 56/63 isolates were positive, including all imipenem resistant and 36.4% of imipenem susceptible isolates (Table IX).

Detection of *sul II* gene in *S. maltophilia* by PCR

All *S. maltophilia* isolates that were resistant to SXT by disc diffusion method had the *sul II* gene at 626 bp as shown in Figure 5. None of the sensitive isolates harboured the gene.

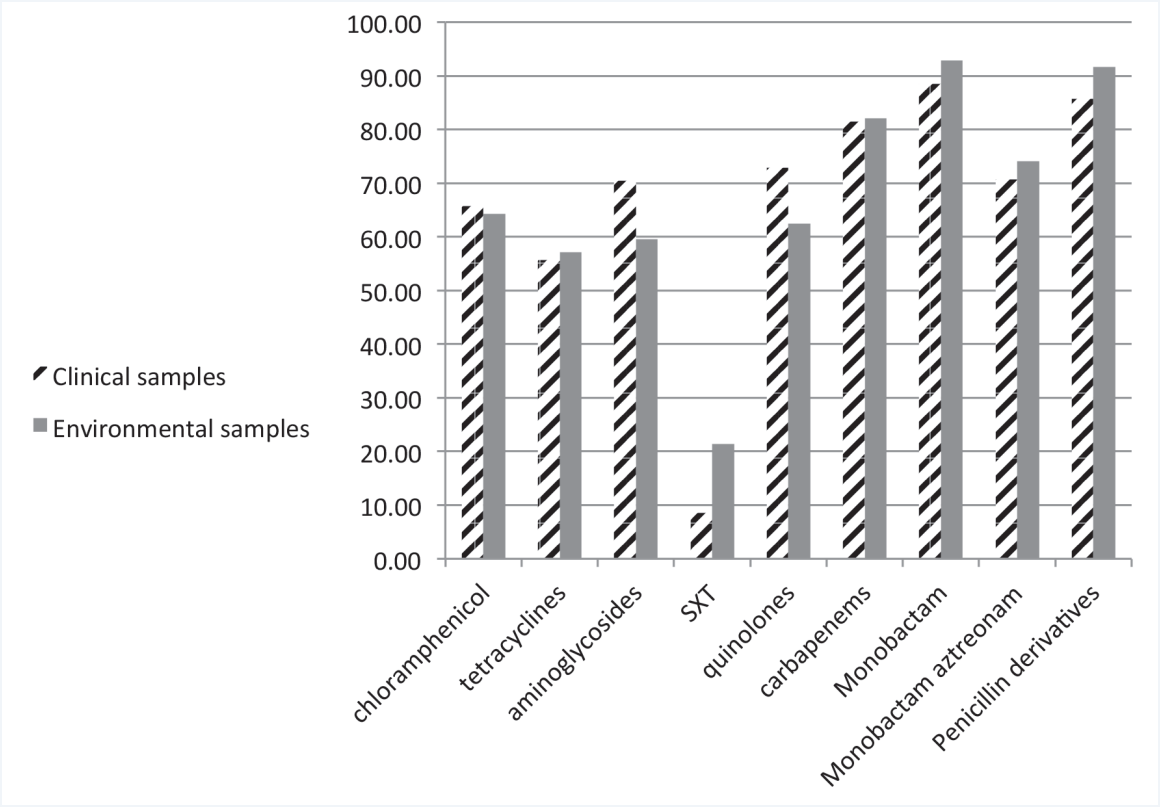

Figure 4. The mean percentage of antimicrobial non-susebtibilaty in clinical and environmental samples

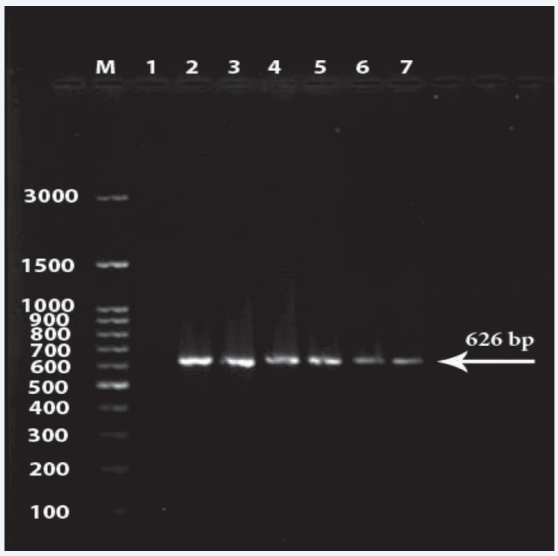

Figure 5. PCR for detection of *Sul II* gene of *S. maltophilia*

M: DNA marker
Lane 1: negative control
Lane 2: positive control
Lanes 3 to 7: positive results for the gene

Discussion

In the current study, *S. maltophilia* caused 9.7% of HAIs (35/362) and comprised 6% (35/588) of the gram-negative bacilli isolated from both clinical and environmental specimens. The most frequent type of infection was respiratory tract infection (9.1%), and endotracheal samples were the most common specimens (9.3%). Other studies have reported the same finding, but with higher percentages (64%, 65% and 67%).[20-22] Although we found that blood samples were not frequent isolation sites, other studies have detected *S. maltophilia* in blood at higher percentages (32%, 14% and 16%).[20-22]

Lower rates of *S. maltophilia* infections are reported worldwide compared with our results. In a previous Egyptian study, the prevalence of *S. maltophilia* HAIs was 1.3% among adult cancer patients.[23] In Saudi Arabia, *S. maltophilia* isolates represented 1.5%, 1.8% and 5.7% of total gram-negative isolates causing infections in three different studies.[24-26] In the USA, a multi-hospital study of patient infections in the

Table VI. Antibiotic susceptibility pattern of *S. maltophilia* isolated from environmental samples

		***S. maltophilia* isolated from clinical samples**					
		S		**I**		**R**	
Group	**Members**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Penicillin derivatives	Ampicillin	3	10.71%	2	7.14%	23	82.14%
	Amoxacillin-Clavulanic acid	3	10.71%	3	10.71%	22	78.57%
	Piperacillin	1	3.57%	4	14.29%	23	82.14%
Monobactams	Aztreonam	2	7.14%	1	3.57%	25	89.29%
Cephalosporines	Cefaclor	10	35.71%	3	10.71%	15	53.57%
	Cefoperazone	3	10.71%	0	0%	25	89.29%
	Ceftriaxone	6	21.43%	5	17.86%	17	60.71%
	Cefazolin	10	35.71%	0	0%	18	64.29%
Carbapenems	Imipenem	4	14.29%	0	0%	24	85.71%
	Meropenem	6	21.43%	1	3.57%	21	75%
Quinolones	Ciprofloxacin	12	42.86%	2	7.14%	14	50%
	Levofloxacin	9	32.14%	2	7.14%	17	60.71%
	Lomefloxacin	10	35.71%	4	14.29%	14	50%
	Nalidixic acid	11	39.29%	0	0%	17	60.71%
Aminoglycosides	Netilmicin	10	35.71%	2	7.14%	16	57.14%
	Amikacin	14	50%	2	7.14%	12	42.86%
	Tobramycin	10	35.71%	4	14.29%	14	50%
Tetracyclines	Oxytetracycline	6	21.43%	4	14.29%	18	64.29%
	Tigecycline	18	64.29%	0	0%	10	35.71%
Chloramphenicol	Chloramphenicol	10	35.71%	4	14.29%	14	50%
Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	22	78.57%	3	10.71%	3	10.71%

Table VII. Detection of Imipenem MICs by IPM E-test

Sample type	**MIC (below 4 µg/ml)**		**MIC (above 16 µg/ml)**	
	No.	**%**	**No.**	**%**
Clinical Samples (n=35)	7	20%	28	80%
Environmental Samples (n=28)	4	14.29%	24	85.71%
Total (n=63)	11	17.46%	52	82.54%

Table VIII. Detection of metallo- β-lactamase enzymes in imipenem susceptible and resistant isolates by combined disc method

Samples type	Imipenem susceptible by E-test					Imipenem resistant by E-test				
	CDT				Total	CDT				Total
	Positive		Negative			Positive		Negative		
	No.	%	No.	%		No.	%	No.	%	
Clinical Samples (n=35)	1	14.29%	6	85.71%	7	28	100%	0	0%	28
Environmental Samples (n=28)	1	25%	3	75%	4	24	100%	0	0%	24
Total (n=63)	2	18.18%	9	81.82%	11	52	100%	0	0%	52

Table IX. Detection of metallo- β-lactamase enzymes in imipenem susceptible and resistant isolates by Double Disc Synergy test (DDST)

Samples type	Imipenem susceptible by E-test					Imipenem resistant by E-test				
	DDST				Total	DDST				Total
	Positive		Negative			Positive		Negative		
	No.	%	No.	%		No.	%	No.	%	
Clinical samples (n=35)	2	28.57%	5	71.43%	7	28	100%	0	0%	28
Environmental Samples (n=28)	2	50%	2	0%	4	24	100%	0	0%	24
Total (n=63)	4	36.36%	7	63.64%	11	52	100%	0	0%	52

ICU reported *S. maltophilia* as being 4.3% of the total gram-negative bacilli.[27] Data from the SENTRY Antimicrobial Surveillance Program revealed that the rate of recovery of *S. maltophilia* from hospitalized patients with pneumonia was 3.1%, with regional recovery rates of 3.3% for the United States, 3.2% for Europe, and 2.3% for Latin America.[28]

Antimicrobial therapy for *S. maltophilia* infections is problematic worldwide. Isolates are usually resistant to many agents including carbapenems, which makes infections difficult to treat.[4,29] In the present study, a very high percentage of clinical and environmental isolates were resistant to imipenem (~82%) using the E-test.

It was also noted in this study that MBLs were detected in all imipenem resistant isolates and in 18.2% and 36.4% of imipenem sensitive isolates by CDT and DDST respectively. These results were higher than those of a previous study in Egypt, where 83% of imipenem resistant isolates and 14% of imipenem sensitive strains were positive for MBL by the CDT assay.[23] That study was performed on non-fermenting gram-negative bacilli including *S. maltophilia*. An alarming finding in this study was the detection of MBLs in environmental isolates.

Frequent and unfounded use of the broad-spectrum antibiotics has led to the appearance of multidrug and even pan-resistant strains in hospitals.[30] In the

present study, 62.9% of clinical isolates and 64.3% of environmental isolates were MDR,[31] similar to another recent Egyptian study in which 63% of isolates were MDR.[23] Our low resistance rates to SXT (8.6%) is in concordance with many other studies.[26,35] Higher SXT resistance rates were reported in Egypt (24.4%),[20] Turkey (10%, 20.3% in two studies),[32,21] and Germany (65.6%).[33]

All *S. maltophilia* isolates that were resistant to SXT by disc diffusion method in the current study had the *sul 2* gene. This contradicts results of another Egyptian study, which reported that all SXT resistant *S. maltophilia* isolates were positive for the *sul1* gene with the complete absence of *sul2*.[20] This finding was also reported by Chung *et al.*[34] Previous analysis of an international collection of 25 SXT resistant *S. maltophilia* strains from six countries for *sul1* and *sul2* genes detected *sul1* in 68% of isolates and *sul*2 in 36% of isolates.[6] The importance of *sul2* genes is clonal spread that is responsible for dissemination among *S. maltophilia* isolates, and which could further disseminate among bacteria through horizontal gene transfer.[35]

There is a great possibility that the low resistance to SXT found in this study may increase over time, as the environmental isolates in the present study showed a higher percent of SXT resistance (21.4%). Environmental isolates showed a general increase in the mean percentage of resistance to all beta lactams (except carbapenems) and SXT compared to clinical isolates. Environmental *S. maltophilia* represents a major threat, as it has been proposed that antibiotic resistance gene acquisition occurs in these strains, and then upon gaining access to the clinical setting, the strains retain such gene(s).[36] This organism has the ability to persist in nutrient-poor aqueous environments, which may act as reservoirs if not properly decontaminated.[37]

Many studies reported that FQs were found to have success rates similar to that of SXT and were even reported to be alternative options for use as monotherapy for the treatment of patients with *S. maltophilia* infections when SXT administration is not possible.[7,38] Hankiewicz-Ziołkowska *et al.,* found the resistance rate for levofloxacin to be 4%,[39] and it was 7.6% in the study by El-Mahallawy, et al.[23] Even a previous Egyptian study reported that levofloxacin was the most active agent (9% resistance) against SXT resistant *S. maltophilia*, whereas ciprofloxacin had poor activity (90% resistance).[20] In contrast, our results demonstrated a high mean percentage of resistance to FQs (72.9%), with high resistance rates for all FQ drugs studied (69-74%), compared to only 8.6% for SXT. This highlights the need to study the mechanisms of this widespread resistance in our hospitals.

Our study demonstrated a high rate of resistance for all beta-lactam antibiotics studied. This is in concordance with a previous study in Egypt, which reported a high percentage of resistance to piperacillin (73%).[20] Poor activity of aminoglycosides against *S. maltophilia* (70.5% resistance for the clinical isolates) was also reported. This may be due to intrinsic resistance, and therefore these agents play virtually no role in monotherapy.[3]

Unfortunately, there is not a single drug alternative to SXT, but it may be possible to try to use different combination therapies that show i*n vitro* synergy as reported in many studies, in order to overcome the problem of resistance.[5]

In the current study, a total of 28 *S. maltophilia* isolates (0.67%) were recovered from environmental samples from different ICUs and wards in Assiut University Hospitals. By API, many clinical sample isolates showed the same biotype as those isolated from environmental samples. What amplifies the problem is that a high percentage of the environmental isolates were found to harbour *sul2* genes and produce MBLs. This highlights the importance of identifying these environmental sources to take preventive measures to control the spread of *S. maltophilia,* as well as the great need for proper implementation of infection control policies.

Conclusion

The study revealed that *S. maltophilia* causes a considerable percentage of HAIs, especially respiratory tract infections, and is an environmental contaminant in ICUs and wards. A high percentage of the bacteria is MDR. Trimethoprim-sulfamethoxazole is the single recommended agent of choice for the

treatment of *S. maltophilia* infections. The isolation of SXT resistant isolates harbouring *sul2* genes at our hospitals was alarming. Carbapenem resistance was significant with the detection of MBLs among clinical and environmental isolates.

References

1. Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev* 2012; **25:** 2–41. http://dx.doi.org/10.1128/CMR.00019-11
2. Safdar A, Rolston KV. *Stenotrophomonas maltophilia*: changing spectrum of a serious bacterial pathogen in patients with cancer. *Clin Infect Dis* 2007; **45:** 1602–1609. https://doi.org/10.1086/522998
3. Looney W J, Narita M, Mühlemann K. *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *Lancet Infect Dis* 2009; **9:** 312-323. https://doi.org/10.1016/S1473-3099(09)70083-0
4. Sanchez MB, Hernandez A, Martinez JL. *Stenotrophomonas maltophilia* drug resistance. *Future Microbiol* 2009; **4:** 655-60. https://doi.org/10.2217/fmb.09.45
5. Samonis G, Karageorgopoulos DE, Maraki S, *et al*. *Stenotrophomonas maltophilia* infections in a general hospital: patient characteristics, antimicrobial susceptibility, and treatment outcome. *PLoS One* 2012; **7:** e37375. https://doi.org/10.1371/journal.pone.0037375
6. Toleman M A, Bennett P M, Bennett D M, Jones R N, Walsh T R. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of sul genes. *Emerg Infect Dis* 2007; **13:** 559-565. https://doi.org/10.3201/eid1304.061378
7. Abbott IJ, Slavin MA, Turnidge JD, Thursky KA, Worth LJ. *Stenotrophomonas maltophilia*: emerging disease patterns and challenges for treatment. *Expert Rev Anti Infect Ther* 2011; **9:** 471-88. https://doi.org/10.1586/eri.11.24
8. Hodiwala A, Dhoke R, Urhekar AD. Incidence of metallo-beta-lactamase producing *Pseudomonas, Acinetobacter* and Enterobacterial isolates in hospitalised patients. *Int J Pharm Bio Sci* 2013; **3:** 79-83.
9. Omair M, Usman J, Kaleem F, Hassan A, Khalid A, Fahim Q. Evaluation of combined disc method for the detection of metallo-β-lactamase producing gram negative bacilli. *Malays J Microbiol* 2012; **8:** 21-25.
10. Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi PJ. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in gram negative bacilli. *Int J Biol Med Res* 2011; **2:** 775-777.
11. Mohanty S, Maurya V, Gaind R, Deb M. Phenotypic characterization and colistin susceptibilities of carbapenem-resistant of *Pseudomonas aeruginosa* and *Acinetobacter* spp. *J Infect Dev Ctries* 2013; **7:** 880-887. https://doi.org/10.3855/jidc.2924
12. Alouache S, Kada M, Messai Y, Estepa V, Torres C, Bakour R. Antibiotic resistance and extended-spectrum beta-lactamases in isolated bacteria from seawater of Algiers beaches (Algeria). *Microbes Environ* 2012; **27:** 80–86. https://doi.org/10.1264/jsme2.ME11266
13. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008; **36:** 309–332. https://doi.org/10.1016/j.ajic.2008.03.002
14. Winn WC, Allen S, Janda W, *et al*. (Eds.) *Koneman's Color Atlas and Textbook of Diagnostic Microbiology* Philadelphia: Lippincott Williams and Wilkins, 2006
15. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, informational supplement M100-S21, 21st ed., CLSI, Wayne, PA, 2011. https://doi.org/10.1128/JCM.02204-10
16. Livermore DM. The impact of carbapenemases on antimicrobial development and therapy. *Curr Opin Invest Drugs*. 2002; **3:** 218–224.
17. Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemase and metallo-β-lactamase production in clinical isolates. *Indian J Med Res* 2005; **121(6):** 780-783.
18. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-β-lactamase-producing gram-negative bacilli in the clinical laboratory. *J Clin Microbiol* 2006; **44:** 3139–3144.
19. Caylan R, Kaklikkaya N, Aydin K, *et al*. An epidemiology analysis of *Stenotrophomonas maltophila* strains in a university hospital. *Jpn J Infect Dis* 2004; **57:** 37-40. https://doi.org/10.1128/JCM.00879-06
20. Moemen DM, Mahfouz RA, El-Kinawy MF, Mesbah MR, El-Bayoumi MA. Nosocomial infection by trimethoprim/Sulfamethoxazole resistant *Stenotrophomonas maltophilia* in intensive care units of Mansoura University hospitals. *Egyptian Journal of Medical Microbiology* 2011; **20:** 91-98.
21. Çıkman A, Parlak M, Bayram Y, Güdücüoğlu H, Berktaş M. Antibiotics resistance of *Stenotrophomonas maltophilia* strains isolated from various clinical specimens. *Afri Health Sci* 2016; **16:** 149-152. https://doi.org/10.4314/ahs.v16i1.20
22. Çelebi S, Kavurt S, Hacımustafaoğlu M. Çocuklarda hastaneden edinilmiş *Stenotrophomonas maltophilia* infeksiyonları: Beş yıllık çalışma sonuçları. *Çocuk Enf Derg* 2008; **3:** 100-104. (Abstract in English)
23. El-Mahallawy HA, Hamid RMA, Hassan SS, Radwan S, Saber M. The increased frequency of carbapenem resistant non fermenting gram negative pathogens as causes of health care associated infections in adult cancer patients. *Journal of Cancer Therapy* 2015; **6:** 881-888. https://doi.org/10.4236/jct.2015.610096
24. Abdel-Aziz N, Morsy MMF, Amin SS, *et al*. Threatening problem of *Stenotrophomonas maltophilia* producing extended-spectrum beta-lactamases: prevalence and automated antibiotic susceptibility pattern. *Clin Microbiol* 2013; **2:** 108. https://doi.org/10.4172/2327-5073.1000108
25. Memish ZA, Shibl AM, Kambal AM, Ohaly YA, Ishaq A, Livermore DM. Antimicrobial resistance among non-fermenting Gram-negative bacteria in Saudi Arabia. *J Antimicrob Chemother* 2012; **67:** 1701-1705. https://doi.org/10.1093/jac/dks091
26. Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M, Memish Z. Prevalence of antimicrobial resistance among gram-negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. *Ann Saudi Med* 2010; **30:** 364-369. https://doi.org/10.4103/0256-4947.67073
27. Lockhart SR, Abramson MA, Beekmann SE, *et al*. Antimicrobial resistance among Gram negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. *J Clin Microbiol* 2007; **45:** 3352–3359. https://doi.org/10.1128/JCM.01284-07
28. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin Infect Dis* 2010; **51:** S81–S87. https://doi.org/10.1086/653053

29. Zer Y, Karaoğlan İ, Çevik S, Erdem M. Evaluation of antibiotic susceptibility of *Stenotrophomonas maltophilia*. *Klimik Derg* 2009; **22:** 21 -4. (Abstract in English)
30. Öztürk R. Antimicrobial treatment for infectious diseases with multi-drug resistant *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*. *ANKEM Derg* 2008; **22:** 36-43. (Abstract in English).
31. Vaze ND, Emery CL, Hamilton RJ, Brooks AD, Joshi SG. Patient demographics and characteristics of infection with carbapenem-resistant *Acinetobacter baumannii* in a teaching hospital from the United States. *Adv Infect Dis* 2013; **3:** 10-16. https://doi.org/10.4236/aid.2013.31002
32. Türk Dağı H, Arslan U, Tuncer İ. Kan kültürlerinden izole edilen *Stenotrophomonas maltophilia* suşlarının antibiyotik direnci. *ANKEM Derg* 2011; **25:** 27-30.
33. Meyer E, Schwab F, Gastmeier P, Rueden H, Daschner FD. Surveillance of antimicrobial use and antimicrobial resistance in German intensive care units (SARI): a summary of the data from 2001 through 2004. *Infection* 2006; **34:** 303-309. https://doi.org/10.1007/s15010-006-6619-x
34. Chung HS, Kim K, Hong SS, Hong SG, Lee K, Chong Y. The *sul1* gene in *Stenotrophomonas maltophilia* with high-level resistance to trimethoprim/sulfamethoxazole. *Ann Lab Med* 2015**;** **35:** 246-249. https://doi.org/10.3343/alm.2015.35.2.246
35. Hu LF, Xu XH, Yang HF, Ye Y, Li JB. Role of *sul2* gene linked to transposase in resistance to trimethoprim/sulfamethoxazole among *Stenotrophomonas maltophilia* Isolates. *Ann Lab Med* 2016; **36:** 73-75. https://doi.org/10.3343/alm.2016.36.1.73
36. Berg G, Eberl L, Hartmann A. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol* 2005; **7:** 1673–1685.
37. Carmody LA, Spilker T, LiPuma JJ. Reassessment of *Stenotrophomonas maltophilia* phenotype. *J Clin Microbiol* 2011; **49:** 1101–1103. https://doi.org/10.1128/JCM.02204-10
38. Wang Y L, Scipione MR, Dubrovskaya Y, Papadopoulos J. Monotherapy with fluoroquinolone or trimethoprim-sulfamethoxazole for treatment of *Stenotrophomonas maltophilia* infections. *Antimicrob Agents Chemother* 2014; **58:** 176–182. https://doi.org/10.1128/AAC.01324-13
39. Hankiewicz-Ziołkowska K, Mikucka A, Gospodarek E. Clinical strains isolation and antibiotic susceptibility of *Stenotrophomonas maltophilia*. *Med Dosw Mikrobiol* 2010; **62(2):** 127 -34.