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Threatening Problem of *Stenotrophomonas maltophilia* Producing Extended-Spectrum Beta-Lactamases: Prevalence and Automated Antibiotic Susceptibility Pattern

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Abstract

Background: *Stenotrophomonas maltophilia (S. maltophilia)* is one of the most common emerging multi-drug resistant organisms. It is associated with difficult detection, treatment, and increased mortality particularly among immune compromised and debilitated individuals.

Objectives: To determine the prevalence and antibiotic susceptibility pattern of *S. maltophilia* as emerging problem from different clinical isolates.

Methods: A cross sectional study was conducted on 14000 cases. The isolates of Gram negative bacilli were identified based on conventional microbiological procedures. Extended spectrum beta lactamases (ESBLs) producing *S. maltophilia* identification and antibiotic susceptibility was further confirmed using automated BD Phoenix system where the panel of Gram negative antibiotics was tested.

Results: Out of 14000 reviewed cases, 2100 were proved to be culture positive. Among Gram negative isolates, 1.5% was proved to be *S. maltophilia.* One hundred fourteen of Gram negative isolates were proved to be ESBL producers. More than half of ESBL isolates were *E-coli*.

S. maltophilia represents (8.77%) of total Gram negative ESBL producers, while ESBL producing *S. maltophilia* represent (83.33%) among the total number of *S. maltophilia* isolates.

Half number of *S. maltophilia* isolates was obtained from blood. About 83 % of *S. maltophilia* isolates showed resistance to all Gram negative Panels of antibiotics, while 100% of isolates were sensitive to Trimethoprim-sulfamethoxazole (TMP – SXT). 16.67% of isolates showed sensitivity to ciprofloxacin (CIP), ceftazidime (CAZ).

Conclusion: ESBL producing *S. maltophilia* is frequently resistant to a wide range of commonly used antimicrobials. Routine sensitivity reporting is required to detect these threatening, multidrug resistant organisms. Trimethoprim-sulfamethoxazole (TMP – SXT) is recommended as the agent of choice for the treatment of *S. maltophilia* infections. Alternatively, fluoroquinolones and ceftazidime (CAZ) may be used.

Keywords: Prevalence; Extended spectrum beta lactamase (ESBL); Phoenix; Antibiotic; Susceptibility pattern

Introduction

Gram negative beta lactamase producing organisms exhibited resistance to beta lactam antibiotics (e.g. penicillin, cephalosporins, monobactams) were developed during the last 2 decades [1]. *Stenotrophomonas maltophilia* (*S. maltophilia*) has recently emerged as a threatening nosocomial pathogen and difficult to be treated as it is intrinsically resistant to a wide range of commonly used drugs. Infection by this organism is predominant mainly in immune compromised individuals with different types of organs affection [2].

As a result of continuous point mutations in TEM-1, TEM-2 and SHV-1 genes found among gram negative bacilli, ESBLs emerged which are enzymes first identified in1983 and mediated resistant to third generation cephalosporins (e.g. ceftazidime, ceftriaxone and cefotaxime) and monobactams (e.g. azetreonam) antibiotics and have been found in a wide range of Gram-negative bacilli [3,4]. ESBLs are associated with many problems including multidrug resistance, difficult detection and treatment, and increased mortality among patients. So, early detection of these enzymes is necessary to avoid high morbidity and mortality rates [5].

S. maltophilia is naturally resistant to many broad-spectrum antibiotics (including all carbapenems) and is thus often difficult to eradicate. Many strains of *S. maltophilia* are sensitive to co-trimoxazole and ticarcillin, though resistance has been increasing. It is not usually sensitive to piperacillin, and sensitivity to ceftazidime is variable [6]. Resistance to beta-lactams is conferred by two inducible beta-lactamases, a zinc-containing penicillinase (L1) and a cephalosporinase (L2) [7].

Susceptibility testing of *S. maltophilia* poses certain problems.

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These are related to the methods used and the differing results that they produce [8]. The rapid identification and susceptibility testing procedure have a positive impact on patient care and may reduce the levels of consumption of antibiotics, resulting in a decrease in overall health care costs [9]. Automated methods for bacterial identification (ID) and susceptibility testing in parallel have further improved, and machines such as the VITEK system (bioMérieux) and the PHOENIX Automated Microbiology System (PHX system; BD) are widely accepted and distributed in clinical microbiology laboratories [10].

The Phoenix 100 ID/AST system (Becton Dickinson Co., Sparks, Md.) is an automated system for the identification and antimicrobial susceptibility testing of bacterial isolates and falls short of being an acceptable new method for the identification of the *Enterobacteriaceae* including *S. maltophili* [11]. In the 8th edition of the *Manual of Clinical Microbiology*, it is recommended that the accuracy of a system exceed 90% in its overall ability to identify common and uncommon bacteria normally seen in the hospital laboratory and that the system be able to identify commonly isolated organisms with at least 95% accuracy compared with the accuracies of conventional methods [12].

Many studies worldwide reported the prevalence and antibiotic susceptibilities of S. *maltophilia*, but, up to our knowledge very few studies were done in Saudi Arabia regarding the prevalence and susceptibility pattern of S. *maltophilia*, using automated methods particularly in AL- Madinah region. This study aimed to focus on the threatening condition, prevalence and antibiotic susceptibility pattern of S. *maltophilia* from different clinical samples in governmental referral hospital in AL-Madina region in Saudi Arabia.

Material and Methods

This study was conducted at the Maternity and Children Hospital, a 500-bed referral hospital in Medina region, Saudi Arabia. A cross sectional study was conducted over the period from June 2011 to March 2012; where 14000 cases (either inpatients or attending outpatient clinics) were reviewed. A specially designed check list was used.

Ethics Review Committee reviewed and approved the proposal. Samples received from patients (including urine, pus, sputum, blood, tracheal aspirates) were initially Gram stained, inoculated on blood agar and Mac Conkey`s agar. Urine samples were cultured on cysteine lactose electrolyte deficient agar (CLED), microbial growth from blood specimens were detected by using the automated BACTEC™ system and sputum samples were inoculated on chocolate. The isolates of Gram negative bacilli were further identified by the conventional microbiological methods including morphology on Gram's staining, their pattern of growth on media, different biochemical tests (oxidase test, methyl red test, Vogues-Proskauer test, , catalase, indole and urease production, nitrate reduction and sugar fermentation.

The sensitivity of the isolates to the following drugs was done using the Kirby-Bauer disc diffusion method: ceftazidime (30 µg), amikacin (30 mg), gentamycin (10 µg), imipenem (10 µg), amikacin (30 µg), aztereonam (30 µg) and ciprofloxacin (5 µg).

The isolates that showed an inhibition zone size of ≤ 22 mm with ceftazidime (30 µg), and aztreonam< 27 were identified as potential ESBL producers and were further tested for confirmation of ESBL production. A bacterial suspension of 0.5 McFarland turbidity standards of the potentially ESBL producing isolate was swabbed on Muller Hinton agar. Disks containing the standard 30 µg of ceftazidime and cefotaxime were placed 20 mm apart (center to center) from each other and from a disc containing amoxicillin-clavulanate (20/10 µg) as the inhibitor of beta lactamase and incubated overnight at 37°C [13]. Clear extension of the edge of the inhibition zone of cephalosporin toward amoxicillin-clavulanate disc caused by the synergy with clavulanate was interpreted as positive for ESBL. *Escherichia* **coli** ATCC 25922 and *Klebsiella* pneumoniae ATCC 700603 were used as negative and positive controls respectively as per the clinical and laboratory standards institute (CLSI) recommendations [14]. ESBL producing S. *maltophilia* identification and antibiotic susceptibility was further confirmed using automated BD Phoenix (Becton Dickinson Diagnostic Systems [BD], Pont de Claix, France).

Phoenix Identification and Antibiotic Sensitivity

The method used for Phoenix identification was described previously [15]. Briefly, the Phoenix system uses one ID and AST combination panel with the ID substrates on one side and the antimicrobial drugs on the other side of the panel. Bacterial isolates were subcultured on Trypticase soy agar supplemented with 5% sheep blood agar (bioMérieux) to ensure the exclusion of contaminants. The Phoenix ID broth was inoculated with several bacterial colonies from a pure culture adjusted to 0.5 to 0.6 McFarland standards by using a CrystalSpec nephelometer (BD). After the transfer of 25 µl of the ID broth suspension to the Phoenix AST broth, the suspension was poured into the ID side of the Combo panel. Once inoculated, the panel was logged and loaded into the Phoenix Automate, in which kinetic measurements of colorimetric and fluorimetric signals were collected every 20 min. The suspension was always subcultured on Trypticase soy agar plus 5% sheep blood to ensure inoculum's purity, followed by incubation for 18 h at 37°C.

The AST side of the combination panel contains dried antimicrobial panels of the following drugs(Amikacin, Gentamicin, Tobramycin, Meropenem, Cefepime, Ampicillin, Ticarcillin, Trimethoprim-sulfamethoxazole, Ciprofloxacin and tetracycline) and 1 growth control well. The assay is a broth-based microdilution test. The system uses a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. The previously described 25 µl of the standardized ID broth suspension was transferred to the AST broth, yielding a final concentration of approximately 5 × 105 CFU/ml. Quality control was performed according to the manufacturer's recommendations.

Statistical Analysis

Statistical Package for Social Sciences SPSS version 13 was used. Frequencies, percentages were calculated. Chi-square test was used. P value <0.05 was considered significant.

Results

Out of 14000 reviewed cases, 2100 (15.0%) was proved to be culture positive.1288 (61.3%) isolates proved to be Gram positive organisms, while the remaining 812 (38.7%) were Gram negative isolates. The medium age of the patients was six days; male to female ratio was 1:1.

Regarding the distribution of Gram negative bacilli isolates, *E.coli* showed the highest number of isolates among Gram negative bacilli {315 (38.8%)}, followed by *K. pneumonia* {205 (25.2%)}, while Salmonella species, *Serratia marcescens* and *Citrobacter* species showed the lowest distribution respectively {5 (0.6%), 8 (1.0%) and 9 (1.1%)}(Table1).

Among Gram negative isolates, 12 (1.5%) were proved to be *S. maltophilia.* One hundred fourteen 114(14.0%) of Gram negative isolates were proved to be ESBL producers. More than half of ESBL isolates (54.4%) were *E-coli*, 14.0% *Pseudomonas aeruginosa* (*P.*

%	Number	Organism
38.8%	315	*E-Coli*
25.2%	205	*Klebsiella pneumoniae*
2.2%	18	*Proteus species*
22.4%	182	*Pseudomonas*
1.1%	9	*Citrobacter species*
4.7%	38	*Enterobacter cloacae*
1.0%	8	*Serratia marcescens*
2.5%	20	*Acinetobacter baumannii*
0.6%	5	*Salmonella species*
1.5%	12	*Stenotrophomonas maltophila*

Table 1: Distribution of Gram negative bacilli isolates.

%	Number	Organism
54.4%	62	*E.coli*
14.0%	16	pseudomonous
13.2%	15	*K.pnumoniae*
5.3%	6	*Enterobacter. Cloacae*
2.6%	3	*Acineto.baumanni*
8.8%	10	*S.maltophilla*
1.8%	2	*Serratia.marscesens*
100.0%	114	Total ESBL producers

Table 2: Distribution of ESBL producing organisms.

%	Number	Sites of Isolation
34.30%	39.00	**Swabs**
42.50%	49.00	**Urine**
2.70%	3.00	**Sputum**
5.50%	6.00	**Blood**
15.10%	17.00	**Tracheal aspirate**

Table 3: Sites of isolation of ESBL producing organisms.

aeruginosa) and 13.2% were *Klebseilla pneumonia* (*K. pneumonia)* (Table 2).

S. maltophilia represents (8.77%) of total Gram negative ESBL producers, while ESBL producing *S. maltophilia* represent (83.33%) among the total number of *S. maltophilia* isolates .More than two fifths (42.50%) of ESBL producing organisms were isolated from urine, 34.30% from swabs and 15.10% from tracheal aspirate (Table 3).

All *S. maltophilia* isolates were obtained from neonates admitted at neonatal care unit was suspected clinical septicaemia. Blood was the major source of isolates {6 (50.00%)}, 4 isolates from tracheal aspirates and 2 were from urine.

Half number {6 (50.00%)} of *S. maltophilia* isolates were obtained from blood, {4 (33.33%)} from tracheal aspirate and {2 (16.67%)} from urine.Regarding *S. maltophilia* antibiotic susceptibility to various antibiotics, 12 (100%) of isolates showed sensitivity to Trimethoprim-sulfamethoxazole (TMP – SXT). Ten (83.33%) isolates out of twelve isolates showed resistance to all Gram negative Panels of antibiotics (except of Trimethoprim-sulfamethoxazole), while two of them (16.67%) showed sensitivity to ciprofloxacin (CIP), ceftazidime (CAZ).

Discussion

Stenotrophomonas maltophilia, previously named as Pseudomonas or *Xanthomonas maltophilia*, has recently emerged as an important nosocomial pathogen that causes infections mainly in immune compromised patients and in many studies showed high resistance to multiple antimicrobial drugs [16]. *Stenotrophomonas maltophilia* (*S. maltophilia*) has recently emerged as an important nosocomial pathogen. Treatment of invasive infections caused by this organism is difficult as the bacterium is frequently resistant to a wide range of commonly used antimicrobials. Hu et al. reported that most of the S. *maltophilia* isolated were multidrug-resistant [17].

In the present study, S. *maltophilia* isolates represented (1.5%) of total Gram negative isolates. Our results are in agreement with other results from Saudi Arabia which reported that, Stenotrophomonas maltophilia represented (1.8%) of total Gram negative isolates [18]. While, others from Saudi Arabia reported that, *S. maltophilia* isolates represent 5.7% from the total gram negative isolates [19].

In the present study, 5.4% of total positive culture isolates proved to be ESBL producers. In another study from Saudi Arabia, a total of 6,750 Gram-negative organisms, ESBL was detected in 6% of isolates [20]. In Saudi Arabia in 2007, out of 400 *K. pneumoniae* isolates investigated, 55% were positive for ESBLs [21]. In our study, we demonstrated that, more than two fifths (42.5%) of ESBL producing organisms were isolated from urine, 34.3% from swabs and 15.1% from tracheal aspirate.In another study from Saudi Arabia, the ESBL-producing strains were mostly from aspirates (25%) followed by sputum (20%) and blood (18.2%) [22].

Regarding antibiotic susceptibility testing for *S. maltophilia,* we found agreement between both double disc diffusion and phoenix automated methods in detection of ESBL production by *S. maltophilia* isolates as our results showed that the most effective antimicrobials against *S. maltophilia* were SXT, as (100%) of isolates showed sensitivity to it. Our results strongly agree with results published by several authors [23,24]. Others reported that gentamicin effectiveness in *S. maltophilia* decreased dramatically, while trimethoprim-sulfamethoxazole remained the most effective antimicrobial agent [19].

However, Al-Jasser reported first isolation of *S. maltophilia* resistant to TMP – SXT from two patients in Saudi Arabia [6]. Resistance rates among *S. maltophilia* reported by others were: 20.5%; and 38.9% for trimethoprim/sulfamethoxazole and ciprofloxacin respectively. In conclusion, ESBL producing *S. maltophilia* are rapidly increasing and representing a problem as nosocomial infections.

Treatment of invasive infections caused by this organism is difficult as the bacterium is frequently resistant to a wide range of commonly used antimicrobials. Early diagnosis and treatment is essential for their control which is a therapeutic challenge and the choice of antibiotics should be made according to the susceptibility results. Trimethoprim-sulfamethoxazole (TMP – SXT) is recommended as the agent of choice for the treatment of *S. maltophilia* infections.

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