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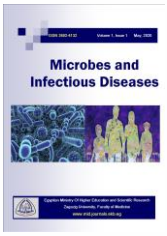

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Original article

Occurrence of biofilm forming and antibiotic resistance of clinical *Stenotrophomonas maltophilia* in Ninawa hospitals, Iraq

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ABSTRACT

Background: *Stenotrophomonas maltophilia* is an emerging multiresistant nosocomial pathogen. **Aim:** This study aimed to isolate *S. maltophilia* and estimate the frequency of biofilm forming as a virulence factor contributing with resistance to some antibiotics. **Methods:** A total of 350 samples were collected from (100) patients in different hospitals of Mosul city during the period from April 2022 to June 2023. Using conventional methods and Vitek 2 automated system. **Results:** The highest isolation rate of *S. maltophilia* was from blood samples at rate of 51.5%, urine and wound 28.5% , 20% respectively while there was no isolates in pharynx samples, the disc diffusion method used to confirm the resistant of bacterial strains against 10 antimicrobial agents, most of them showed high resistant rates to Amikacin (57.1%), Ceftazidime (62.8%), Ciprofloxacin (85.7%), Piperacillin (77.1%), Cefepime and Gentamicin (71.4%), while it was low against Imipenem (42.8), Meropenem (45.7%) and Cefotaxime (45.71%) also for Piperacillin-tazobactam (48.5%), The resistance level was > 60 % for the most tested antimicrobial agents. The biofilm formation was implemented using microtiter plate method, about (31.43%) of the isolates induced strong level of biofilm formation, (42.85%) moderate while only (25.72%) showed weak intensity of biofilm formation, **Conclusion:** The high resistance of *Stenotrophomonas maltophilia* to most of the antibiotics used is an indicator that requires attention, in addition most of the resistant isolates had strong biofilm production properties which refer to the virulently of this bacteria relating with serious nosocomial infections.

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Introduction

Stenotrophomonas maltophilia is an accident opportunistic nosocomial pathogen causing several infections, such as bloodstream, wounds, catheter-associated infections, septicemia, cystic fibrosis (CF), and pneumonia [1]. *S. maltophilia* is a Gram-negative, multidrug-resistant environmental microorganism that is widely linked to respiratory diseases worldwide [2]. *S. maltophilia* are intrinsically resistant to many wide spectrum antibiotics, such as aminoglycosides, carbapenems, and beta lactams [3,4]. Due to *S. maltophilia*'s limited resistance to aminoglycosides, macrolides and β-lactams which has been revealed in prior investigations, immunocompromised patients' attempts to eradicate infections have failed,

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increasing mortality rate [5]. It is concerning that nosocomial strains of *S. maltophilia* carry efflux pumps, aminoglycoside-modifying enzymes and proteases such aph, aac, AhtpxA, and clpA as well as blaL2 (Ambler class A β-lactamase) and β-lactamases like blaL1 (metallo-β-lacta-mase) [6,7].

The virulence factors that contribute to the severity of infections include gelatinases, lecithinases, lipases, hyaluronidase, hemolysin, and DNase [8,9]. However, the strains also develop biofilm, which shields the bacteria from harsh environmental factors, the host immune system, and drugs [10,11].

This microbe is significant because of its multiresistance to different antibiotics, minimal pathogenicity and sustained isolation in individuals who are already at risk. In addition, few researches have assessed its clinical significance and epidemiology. The persistent illnesses are a result of biofilm formation that pose challenges to treat [12,13].

The biofilm forming on a variety of biotic and abiotic surfaces is a crucial virulence trait, even though *S. maltophilia* is a pathogen with low pathogenicity, most bacteria share this pathway for biofilm development [14]. Its wide ecological spread and presence in a variety of anthropogenic and natural environments of people, animals, and plants make it a ubiquitous organism [6,15]. There are little researchers consider studying the antibiotic profile of *S.maltophilia* and prevalence of biofilm forming. This study aimed to identify *S.maltophilia* isolates regarding antibiotics resistance profile and biofilm forming capacity of these bacteria in Mosul, Iraq.

Material and methods

Sample collection

(350) clinical samples (Blood, urine, wounds and pharynx swaps) were gathered from patients with various infections in different hospitals of Mosul city, Iraq during April 2022–June 2023, and brought to Microbiology laboratory in Research Center of Northern Technical University for bacterial isolation.

Bacterial identification

Samples cultured onto EMB agar and MacConky agar, incubated at 37C° for 24-48 hrs. Gram-staining and biochemical tests (catalase positive, oxidase negative, oxidation of glucose and maltose, negative reaction of urease and indole) to differentiate from other Gram negative bacteria, and verified by using VITEK2 automated system [11].

Antibiotic susceptibility test

Ten antimicrobial agents (meropenem, cefotaxime, piperacillin, imipenem, cefepime, ciprofloxacin, ceftazidime, gentamicin, amikacin and piperacillin-tazobactam screening) were tested in this study according to CLSI 2021, and determined by disc diffusion method, after incubation for 42 hrs [16]. Using Muller-Hilton agar plates at 37C°, the diameter of the inhibition zone was calculated. *Pseudomonas aeruginosa* ATCC 15442, *S.aureus* ATCC 25923 and *E.coli* ATCC25922 standard strains were employed as the test's quality control [12].

DNA extraction

Utilizing commercial complete DNA extraction kits (Favorgen, Taiwan), genomic DNA was isolated. DNA was found using gel electrophoresis and a UV transilluminator. Using the procedure outlined by [16], the PCR assay was used to identify the (16S rRNA and 23S rRNA) genes for *Stenotrophomonas maltophilia* [17,18].

Molecular identification

Using polymerase chain reaction (PCR), the 16S rRNA gene of *S. maltophilia* was amplified by forward (F): AGTTTGATCCTGGCTC, and reverse (R): CCTACGTATTACCGCGGC. and (primers F and R of 23S rRNA gene) include F: GCTGGATTGGTTCTAGGAAAACGC, R:ACGCAGTCACTCCTTGCG] (5′GCTGGATTGGTTCTAGGAAAACGC-3′) and reverse (5′-ACG- CAGTCACTCCTTGCG 3′) and primers of 23S rRNA gene [1,6]. The thermal cycle programmer for 23S rRNA and for 16S rRNA gene perford by Azimi et al. procedure [17].

Biofilm formation

The test for biofilm formation was carried out according to Sun et al. description for Gram-negative organisms [14]. A bacterial cultivation overnight (1 × 107 CFU/mL) was adjusted to 1.0 McFarland Standard intended in trypticase soy broth containing 1% glucose then diluted 1:100 with broth medium, 20 µL was inserted to 96 well plates after being taken before incubating at 37 C° for 24 h. Three rounds of sterile phosphate-buffered saline (PBS, pH 7.3) washings were performed on microplates, after drying, the isolates were fixed using 200 µL of 2% formaldehyde, the wells were filled with crystal violet for five minutes at room temperature. After that, they were washed with

water and left to dry. After staining with 200 ml of 33% glacial acetic acid for about (15) min., optical density (OD) was measured by using a microtiter plate reader (BioTek, Germany), the cut -off value (ODc) has been categorized as three deviations (SD) above the mean OD of the negative control. The isolates were divided into four groups: non- biofilm producer, weak- biofilm producer, moderate-biofilm producer and strong- biofilm producer isolates [19,20].

Results

Isolation of *S. maltophilia* from clinical samples

Of 350 samples from (100) patients with various infection, 35 (14%) of *S. maltophilia* isolates were gathered from (Blood, urine, wounds and pharynx swaps), the high rates of isolation were from blood 18 (51.5%), the rest were from urine 10 (28.5%), wound 7 (20%) while no isolates were recovered from pharynx swaps as it shown in **Table1**.

Identification by PCR

The *S. maltophilia* isolates which identified by microbiological methods (colony morphology, Gram stain and biochemical tests) and VITEK2 automated system were subsequently verified by molecular analysis after extraction of PCR products based on 16S rRNA gene that formed 569 bp fragments. The 23S rRNA target PCR produced 278 bp fragments as a result. All (35) positive *S.maltophilia* isolates were verified by PCR and gel electrophoresis.

Antibiotic susceptibly test of *S. maltophilia*

In this study, the ability of 35 *S.matolphilia* isolates to show resistance against the antimicrobial agents used indicated in **Table 2**. The resistant rate of *S. maltophilia* isolates against Ciprofloxacin as a member of fluoroquinolones was the highest 85.7%. The ratios were similar for other antibiotics, piperacillin and cefepime which belong to penicillin and beta-lactamase inhibitors also gentamicin in rates of 77,1% and 71.4% while the resistance rate for ceftazidime and amikacin (aminoglycoside) was 62.8% and 57.1% consecutively, and lower for Piperacillin-tazobactam (48.5%), for meropenem and cefotaxime show resistant at rate 45.75 for both. Imipenem which is one of the broad spectrum carbapenem showed the least resistance to tested *S. maltophilia* isolates.

Biofilm formation by *S. maltophilia*

The biofilm phenotypes accounted for 100% using the microtiter plate method, being distributed according to the severity of biofilm formation as follow: 9 isolates (25.72%) produced weak biofilm, 15 isolates (42.85%) produced moderate biofilm and 11(31.43%) produced strong biofilm formation.

Table 1. Distribution of *S. maltophilia* strains according to the samples from which they were isolated

Sample	No.	Rate %
Blood	18	51.5 %
Urine	10	28.5%
Wound	7	20%
Pharynx swaps	0	0%
Total	35	100 %

Table 2. Susceptibilty of *S. maltophilia* isolates to 10 antimicrobial agents

Antimicrobial agent	Antibiotics susceptibly test results			Resistant rate %
	R	I	S	
Cefepime	25	2	8	71.42
Cefotaxime	16	1	18	45.71
Ceftazidime	22	3	10	62.8
Amikacin	20	2	13	57.1
Gentamicin	25	1	9	71.4
Ciprofloxacin	30	2	3	85.7
Piperacillin	27	3	5	77.1
Piperacillin- tazobactam	17	0	18	48.5
Imipenem	15	1	19	42.8
Meropenem	16	0	19	45.7

R : Resistant to antimicrobial agents.
I :Intermediate sensitivity agents.
S : Sensitive to antimicrobial agents .

Table 3. Distribution of *S. maltophilia* strains according to the biofilm formation

Isolates No.	Intensity of biofilm formation	Rate %
9	Weak	25.72%
15	Moderate	42.85 %
11	Strong	31.43 0%
Total 35		100 %

Discussion

S.maltophilia cause some deleterious infections in many healthcare unites, and may be life-threatening in immunocompromised individuals [21]. Many researches at the years between 1997 and 2023 reported that the third most isolated non-fermentive bacterium was *S. maltophilia*, following *Pseudomonas aeruginosa* and *Acinetobacter*, with a rate of 8% from clinical specimens [22]. In this study, from about 350 samples of blood, urine, wounds and pharynx, only 35 (14%) isolates were identified as *S.maltophilia*, this result is approximate with the total isolation rate from critical care unit patients and mechanical ventilation as 29% [23], but was lower than the rate 38.2% reported by Alsuhaibani et al. isolated from hospitalized pediatric patients [22]

Isolation *of S.maltophilia* from clinical samples

The highest rate of isolation 51.5% was from blood samples, %28.5 and 20% respectively were from urine and wound samples while Pharynx swaps were free from this bacterium, these results were parallel with Arslan et al. who recorded the highest percentage (20.2%) from blood and CSF samples [18]. Another study indicated the most isolation rate from respiratory tract as (40%) followed by blood (21.5%) [2]. Bostanghadiri et al. isolated most of *S.maltopilia* (90.03%) from the blood approaching this study while the rest (9.97%) was from pharynx swabs which contradicts with what we have found [18]. The results of our research correspond with other research regarding the isolation of the bacteria from blood in percentage of 10.7%, urine 9.7% but contradicted with pharynx samples 5.3% [25].

Antibiotic susceptibly test of *S. maltophilia*

The resistance rate of isolated *S.maltophilia* strains in our study were high for four wide spectrum antimicrobial agents which include (Ciprofloxacin, Cefapime, Gentamicin and Piperacilllin). The strain's resistance profile did not depended on its source [26]. These results are compatible with Emami et al. who isolated the bacteria from different sources in some Turkish hospitals and recorded a high resistance percentage against Imipenem, Gentamicin, Cefepime and Piperacillin [27]. Another research support our results regarding resistant to carbapenems, such as fluoroquinolones, meropenem (92.4%-94%), while it varied from (45% to 100%) against imipenem, due to the fact that ciprofloxacin resistance in *S. maltophilia* isolates is largely caused by active efflux pumps [2].

While a small percentage of the bacterial isolates were susceptible for piperacillin, cefotaxime and imipenem as an approximate percentage (45.7 %), as in some studies which refer to the sensitivity rates of about (32.1% - 93%) for the mentioned antibiotics [28]. Recent studies have reported contrary to our results raising in the resistance rates against many antimicrobial agents such as imipenem and cefotaxime as 85% and 61%. Moreover, our findings relating with the susceptibility of *S.maltophilia* against piperacillin 45.7% which are compatible with a study by El Baradei et al. showed the susceptibility rate as 32.51% in Europe [29]. In contrast, Insuwanno et al. reported the susceptibility rate of 96.1% against this drug [15]. It is understood that antibiotic resistance is caused by a multitude of genes, Moreover, it has been suggested that *S. maltophilia* may develop resistance by horizontal gene transfer-induced mutations in resistance genes [30].

Biofilm formation by *S.maltophilia*

One of the various mechanisms underlying antibiotic resistance in bacteria is the development of biofilms, this characteristic has established the standard for looking for alternative treatments, the ability to fight infections, as well as proving that *S. maltophilia* genes implicated in biofilm formation appear to be linked to antibiotic resistance [31,32], the results of this research referred that most of the isolates that showed resistance to ciprofloxacin, fluroquinolons, gentamicin and piperacillin are consistent with the development of strong biofilms, as Saleh et al. mentioned that almost 58% of *S. maltophilia* isolates that showed resistance to ciprofloxacin were strong biofilm forming isolates [33].

At physiological pH, *S. maltophilia* has a positive surface charge, it adheres more readily to materials that are negatively charged, such as glass and teflon [34]. Due to its capacity to stick to various polymeric materials, mainly in hospital environments, this makes it possible to link the process of colonization and infection with surgical material, as well as tracheotomy techniques and catheterization [35,36]. As in our results relating to biofilm forming capacity include weak (25.72%), moderate (42.85%) and strong biofilm forming isolates (31.43%) Shahid et al. also found similar results which include weak (28.23%), (37.655%) and (34.12%) respectively for weak, moderate and strong biofilm forming nosocomial originated isolates [2], in another study , most of *S.maltophilia* clinical isolates resistant to antimicrobial drugs like fluoroquinolones showed that these isolates were associated with strong biofilm forming capability in rate of about 51%, moderate 33% and weak level 16% strains. Previously, the virulent biofilm-producing *S.maltophilis* has been isolated from Mexican hospital Cruz- cordova et al. [35] Another important point is that in vitro methods for biofilm measurement may not accurately represent in vivo circumstances [13] **.** *S. maltophilia* possesses a huge number of virulence factors and antibiotics resistant, the elaboration of these characteristics have clinical significance to the health [29] . The specific properties of this bacterium and increasing of its infection rates noticeably between patients with immunocompromised conditions reflect the difficulties in therapeutic approaches. This affects health and requires further studies regarding the virulence factors and other characteristics of these bacterium.

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Conflicts of interest

The authors declare that they do not have any conflict of interest.

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