Treatment Challenges and Prevalence of Antibiotic Resistance

*Stenotrophomonas maltophilia* an Emerging Pathogen Isolated from Leafy Greens and Clinical Samples

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**ABSTRACT:** *Stenotrophomonas maltophilia* is a commensal and an emerging pathogen earlier noted in broad-spectrum life-threatening infections among the vulnerable, but more recently as a pathogen in immunocompetent individuals. In the current study, *S. maltophilia* was identified from 78% of vegetable samples. Bacterial population count ranged from \(5.50 \times 10^4\) to \(4.3 \times 10^4\) cfu/g (mean \(8.9 \times 10^3\) cfu/g, median \(7.75 \times 10^3\) cfu/g). Based on a cross-sectional study performed during 2015-2016 \(n=35\) and 2016-2017 \(n=55\) *S. maltophilia* was isolated from clinical samples. The occurrence of *S. maltophilia* from clinical samples increased during 2016-2017 with a significant value of \(P=0.0008\). Out of the total of \(n=90\) *S. maltophilia*, clinical isolates (35.28%) were from tracheal aspirates, followed by cerebrospinal fluid (CSF) (17.70%), sputum (15.20%), blood (16.82%), and urine (15.0%). Sample sources were taken from different age groups of patients between 0-75 years. Almost all the isolates > 97% confirmed multiple drug resistance (MDR). Above 95% of the isolates are biofilm formers and the resistance rate increases among the biofilm formers.

**KEYWORDS:** Antibiotic resistance, biofilm formation, emerging pathogen, frequency of occurrence, *S. maltophilia*

1. **INTRODUCTION**

*Stenotrophomonas maltophilia* is a gram-negative bacillus, initially called *Bacterium bookeri*, when it was first identified in 1943 from a specimen of human pleural fluid (Miles Denton and Kevin G. Kerr, 1998). It was later classified as *Pseudomonas maltophilia* in 1961, then reassigned to the gamma-proteobacteria class as *Xanthomonas maltophilia* in 1983, and eventually classified as *Stenotrophomonas* in 1999 (Chang YT, et al., 2015). The name has Greek roots meaning “The narrow feeder - that loves malt.” The whole-genome sequence of representative isolate K279a genome sequence was published in 2008 (Said MS, et al., 2022). *S. maltophilia* is a commensal organism of supposedly low virulence, yet vibrant as an emerging pathogen (Anusree Thenissery, et al., 2022). It is frequently isolated from water and soil (Adjidé et al., 2010; Hutinel, M., et al., 2012) and from animals and plant materials (Jayol A, et al., 2018; Li, D., et al., 2019; Bin Cai, et al., 2020). The bacteria frequently colonize patients’ irrigation fluid (e.g., irrigation solutions, intravenous fluids, etc.) and patient body fluid (respiratory aerosols or mucus, urine, and wound exudates) (Waters V., et al., 2012). The bacteria's frequent colonization of fluids used in hospital settings, irrigation solutions, and/or invasive medical devices might become a vehicle to bypass normal host defenses to cause human infection (Brooke JS., 2012). This has similar pathophysiology or pathogenesis with other non-fermentative aerobic organisms, in the face of immune systems as impendence factors. *S. maltophilia* can cause a wide spectrum of serious infections. Its ubiquity is ascertained in the environment as a commensal and in the hospital as an opportunistic pathogen associated with high morbidity and mortality rates among immunocompromised patients or true pathogen in immunocompetent (Zając OM, et al., 2022; Yeshurun et al., 2010; Hentrich et al., 2014). *S. maltophilia* can also be detected as environmental commensals and as etiological agents respectively. A previous study indicated that two clinical strains, one from Spain and the other from Australia clustered with an environmental strain from Brazil. The clinical strains, which were, identified as D457 and AU12-09 respectively as well as strain JV3 from the rhizosphere showed that both the environmental strain and the clinical strains are closely linked (Lambert B, et al., 1909; Youenou et al.,2015). This ubiquity of the potential pathogen may have an effect on the epidemiology (Adegoke AA., et al.,2017).

*S. maltophilia* is also recognized as one of the underestimated important multi-drug resistant organisms in hospitals by the World Health Organization (WHO) (Brooke JS., 2014; Willssey GG, et al., 2019). It exhibits resistance to a broad array of antibiotics, including TMP-SMX, β-lactam antibiotics, macrolides, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems,
chloramphenicol, tetracyclines, and polymyxins (Polianciuc SI, et al., 2020). The low membrane permeability that contributes to resistance to β-lactams including cefepime, ticarcillin-clavulanate, ceftazidime, and piperacillin-tazobactam and the presence of chromosomally encoded multidrug resistance efflux pumps, β-lactamases, and antibiotic-modifying enzymes all contribute to the intrinsic antibiotic resistance of S. maltophilia (Chang YT, et al., 2015). The intrinsic resistance of S. maltophilia was suggested to have been acquired in natural nonhuman environments and is not due solely to the use of antibiotics in medical/clinical settings (Gil-Gil, T., et al.,2020). Environmental intrinsically resistant bacteria such as S. maltophilia were suggested to use their metabolic machinery to detoxify and break down harmful compounds (including antibiotics). The biochemical pathways used by these bacteria may enable the use of antibiotics as food sources (Tamma PD., et al., 2022). The contamination of the environment with antibiotics can enrich antibiotic-resistant bacteria and provide an opportunity for the acquisition of drug resistance by other bacterial pathogens (Hutinel, M., et al., 2022, Polianciuc SI, et al., 2020). The drug resistance mechanisms are acquired by the horizontal transfer of antibiotic resistance through plasmids, transposons, integrons, integron-like elements, insertion element common region (ISCR) elements, and biofilms (Hu L-F, et al., 2011).

Despite the abundance of global surveillance studies published, there are only a few reports assessing the microbiological and clinical significance of S. maltophilia in Ethiopia. The aim of this study was to assess the prevalence of distribution and frequency of occurrence of S. maltophilia isolates, characteristics, biofilm-forming ability, and the implication of the commensal S. melophilia in infections and their antibiotic regime.

2. METHODS AND MATERIALS

Isolation of S. maltophilia from leafy greens and clinical specimen Samples

The environmental samples were cauliflower, lettuce, spinach, and cabbage (produced by local irrigation farms, around Gondar) purchased from a local market (outside farm markets) and transported to the laboratory in a cooler bag. Twenty-five samples of cauliflower and lettuce and twenty-four samples of spinach and cabbage were collected during the summer of 2015 to 2016 and from 2016 to 2017. The leafy green samples were stored in a refrigerator at approximately 4°C prior to the start of the experiment within the day of sampling. Twenty-five grams of each type of leafy green vegetable was stomached with 99 mL of Buffered Peptone Water (BPW) for 2 min using a stomacher. Serial dilution of the stomached solutions was inoculated onto VIA (Stenotrophomonas Selective Agar with vancomycin, imipenem, and amphotericin B) plates and incubated at 30°C for 48 hrs. Presumptive S. maltophilia colonies (dark green, circular, non-mucoid) were picked out, streaked on new VIA agar plates, and incubated at 30°C for another 48 hrs. This was to make a secondary purification of the selected colonies of S. maltophilia on the VIA agar plates: a dark, translucent, green smear with small and circular single colonies. A total of 76 S. maltophilia isolates were identified from vegetable samples. All the clinical samples were collected from the Gondar referral hospital. A total of 90 clinical isolates from all samples including tracheal aspirates, blood, cerebrospinal fluid (CSF), urine, and sputum were collected from patients with various infections. Furthermore, to perform demographic study simple data (age and sex) with respect to personal privacy were recorded from each patient. All the samples were transferred to the Microbiology Laboratory within two hours in containers under completely sterile conditions. The samples were cultivated on selective and differential media such as blood and MacConkey agars and incubated for 24-48h at 37°C.

Confirmation of S. maltophilia by API 20 NE

For each identified S. maltophilia isolates a loopful of bacteria was streaked onto a Tryptone Soya Agar (TSA) plate and incubated at 30°C for 24 hrs. Colonies on TSA plates were picked and transferred to 5 mL of 0.85% physiological saline solution until the bacterial suspension reached turbidity that matched the 0.5 McFarland standard. The 0.5 McFarland standard was prepared by adding 0.05 mL of 1.175% barium chloride dihydrate solution to 9.95 mL of 1% sulfuric acid solution. The bacterial suspensions were inoculated into the API 20NE strips following the instructions from the manufacturer. The strips were incubated at 30°C for 24 hrs. prior to the reading of the results. A seven-digit profile number was generated by comparing the phenotyping results from the strip with the reading table supplied in the kit, and a significant taxa percentage was generated based on the seven-digit profile number by the apiweb™ database. ((https://apiweb.biomerieux.com)
Strain DNA isolation and characterization

The *S. maltophilia* strain was cultured onto brain heart infusion broth for genomic DNA extraction under anaerobic conditions at 37 °C for 24 h. The strain was extracted with the use of a Gene-Jet Genomic DNA Extraction Kit according to the manufacturer’s instructions. The 16S rDNA was amplified using Taq polymerase with 0.2 μM of 27F and 1492R primers in a 50 μl reaction (27F: AGAGTTTGTACMTGCTGCTCAG, 1492R: TACCTTGTTACGACTT). The optimal thermocycling conditions were as follows: 95°C hold for 15 min, 35 cycles of 94°C for 1 min, 52°C for 1.5 min, and 70°C for 1.5 min, followed by 5 min at 70°C. PCR products were visualized by electrophoresis using a 1% agarose gel and staining with Floro-Safe DNA stain.

### Antimicrobial Resistance Disk Diffusion Test

For each confirmed *S. maltophilia* isolate the antibiotic susceptibility test using the Kirby Bauer method was performed by standard disk diffusion on Mueller–Hinton agar and incubated at 37°C for 24 hrs, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M100 breakpoint values (obtained in November 2018) (Biswas S, Berwal A. et al., 2020). A total of 25 antibiotics (Oxoid, Basingstoke, UK) were classified into 11 different groups according to the WHO (Organization, 2007): amoxicillin (AMC, 30 μg), ampicillin (AMP, 10 μg), cefepime (FEP, 10 μg), Cefoxitin (FOX, 30 μg), Cefazolin (CFZ) (30 μg), ceftazidime (CAZ, 30 μg), amikacin (AK, 30 μg), gentamicin (CN, 10 μg), kanamycin (K, 30 μg), Cefaclor (CEC) (25 μg), Cefotaxime (CTX) (30 μg), clindamycin (DA, 2 μg), Doripenem (DOR) (30 μg), Imipenem (IPM) (30 μg), ciprofloxacin (CIP, 5 μg), Norfloxacin (NOR, 10 μg), Meropenem (MEM) (30 μg), Linezolid (LZD, 30 μg), rifampicin (RD, 5 μg), Trimethoprim/sulphamethoxazole (SXT, 25 μg), Quinupristin/dalfopristin (QD, 15 μg), Cefazidime (CAZ (30 μg), Ertapenem (ETP) (30μg), Nalidixic acid (NAL) (30 μg) and Colistin (CST) (30 μg) were used. A bacterial suspension of 0.5 McFarland standard turbidity was first prepared using a 24 hrs old culture as described above. A sterile cotton swap was dipped into the bacterial suspension, and the swab was pressed and twisted against the inner surface of the test tube to remove excess fluid. The swab was streaked across a Mueller-Hinton agar (MHA) surface in a zigzag manner. The Mueller–Hinton (MHA) plate was turned 45° clockwise and streaked again using the same swab, and this step was repeated one more time so that the swab had been streaked across the agar a total of three times. The antibiotic disks were placed onto the agar using a pair of sterile forceps. Escherichia coli (ATCC 25922) was used as the control microorganism.

### Biofilm Formation Ability Test

For every *S. maltophilia* isolate, a loopful of bacteria was cultured in 10 mL of Tryptone Soya Broth (TSB) at 30°C for 24 h. A 2nd spherical of culturing become achieved by way of moving 0.1 mL of the TSB cultures into fresh TSB (10 mL) for incubation at 30°C for 24 h. The resultant cultures were diluted 1:100 times with the use of 10% TSB- , and 0.2 mL of every of the diluted TSB cultures was transferred into three different wells of a 96-well microtiter plate. Non-inoculated 10% TSB was used as a negative control. The microtiter plate was incubated at 30°C for 48 h prior to the size of biofilm-forming capability. After incubation, TSB was changed and removed from the wells. The wells are changed and then washed with 0.2 mL of Phosphate Buffered Saline (PBS) three times to be able to wash off planktonic bacterial cells previous to the air drying of the microtiter plate within the BSL-2 cupboard for 30 min. The cells had been stained with 0.1% crystal violet dye (0.1 mL in keeping with well) for 15 min. The dye was turned and eliminated and every well was changed and washed with 0.2 mL of deionized water thrice to put off any excess dye. The wells had been left to air dry within the BSL-2 cupboard for any other 30 min previous to the solubilization of the stained bacterial cells with 33% glacial acetic acid (0.2 mL in keeping with well). The microtiter plate was equilibrated at four °C for 15 min. The result in each well was homogenized by way of pipetting up and down before moving 0.125 mL into a new microtiter plate. Absorbance analysis was carried out at 595 nm using a microplate photometer. The biofilm-forming capability (measured by the full biofilm biomass) has been decided based on the criteria set up (Stepanović et al. (2007). The mean OD of each sample was compared to the optical density of the negative control, and the cut-off value (ODc) was calculated to be three times the standard deviation of the blank OD Mean plus the Mean of the blank OD. Bacteria strains were classified as non-biofilm formers (ODs < ODc); weak biofilm formers (ODc < ODs < 2ODc); moderate biofilm formers (2ODc < ODs < 4ODc) and strong biofilm formers (ODs > 4ODc) (O’Toole G. A. (2011).

### Statistical Analyses

Data for analysis were collected from the Gondar university microbiology laboratory. In order to determine the aerobic mesophilic count among the vegetable samples and vegetable markets, serial dilution and log_{10} CFU/gm of colony counting time dilution factor.
divided by total volume were used. Data analysis was done using SPSS version 16. For demographic data, descriptive analysis was used and results were represented as frequency (percentages). Continuous variables were expressed as mean ± standard deviation (SD). Biofilm production and antibiogram data were represented using frequency (percentages). All reported values were two-sided (α = 0.05) with a confidence interval of 95%. P value <0.05 was considered significant.

3. RESULTS

Aerobic mesophilic count of vegetable isolates

Characterization of (N=166) total S. maltophilia isolates (n = 90; from clinical samples and n= 76; from leafy green samples) were identified. The mean average result of microbiological counts indicated the identification of S. maltophilia from leafy vegetables such as cauliflower, lettuce, spinach, and cabbage. S. maltophilia was found to be comparably higher in cauliflower(8.9cfu/gm) and lettuce (8.6cfu/gm) in all of the sampling locations, whereas lower in cabbage(7.3cfu/gm) (Table. 1). The CFU per gram fresh vegetables population counts of S. maltophilia isolates in cauliflower ranged from 5.5 × 10⁻¹ (CFU/gm) to 4.3 × 10⁻⁴ CFU/gm, fresh lettuce ranged from 5.5 × 10⁻¹ (CFU/gm) to 4.2 × 10⁻⁴ CFU/gm, fresh spinach ranged from 5.1 × 10⁻¹ (CFU/gm) to 3.4 × 10⁻⁴ CFU/gm and of cabbage ranged from 5.5 × 10⁻¹ (CFU/gm) to 3.6 × 10⁻⁴ CFU/gm (table 1). The reduction in population count by dilution of the vegetable extract was more in cabbage compared to other vegetables. A total of 76 S. maltophilia strains were isolated from four vegetables based on their distinguishable colony morphology (shape, size, color, and margin).

Identification of clinical isolates

Characterization of S. maltophilia isolates identified in clinical specimens from 2015 to 2016 was n = 35 (38.89% ± 6.33/year, while from 2016 to 2017 this number was n = 55 (61.1% ± 31.0/year). A sizable (P = .0008) increase was observed in the detection of S. maltophilia in the second part of the study period (2016-2017). Tracheal aspirates were the most common samples type (35.28%), followed by cerebrospinal fluid (CSF) (17.70%), sputum samples (15.20%), blood (16.82%), and urine samples (15.0%). The age distribution of patients was as follows: 16.03% 0 to 6 years, 5.84% 7 to 18 years, 8.51% 19 to 36 years, 11.20% 36 to 50 years, 22.23% 51 to 65 years, and 36.19% of patients were older than 65 years.

Analysis of antibiotic susceptibility

The antibiotic susceptibility of all the confirmed S. maltophilia isolates results indicated that both the vegetable and clinical isolates were highly resistant against the twenty-five antibiotics tested including the new generation beta-lactams and carbapenem drugs which implies S. maltophilia as an MDR bacteria (Table 2).

Table 1. Aerobic mesophilic counts of vegetable samples

<table>
<thead>
<tr>
<th>Type of Produce</th>
<th>No. of samples</th>
<th>Mean (CFU/gm)</th>
<th>Range (CFU/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauliflower</td>
<td>25</td>
<td>8.9</td>
<td>5.5x10⁻¹-4.3x10⁴</td>
</tr>
<tr>
<td>Lettuce</td>
<td>25</td>
<td>8.6</td>
<td>5.5x10⁻¹-4.2x10⁴</td>
</tr>
<tr>
<td>Spinach</td>
<td>24</td>
<td>7.6</td>
<td>5.1x10⁻¹-3.4x10⁴</td>
</tr>
<tr>
<td>Cabbage</td>
<td>24</td>
<td>7.3</td>
<td>5.1x10⁻¹-3.6x10⁴</td>
</tr>
</tbody>
</table>

Table 2. Antibiotic resistance of S. maltophilia isolates from vegetable and clinical isolates

<table>
<thead>
<tr>
<th>Class of antibiotics</th>
<th>Types of antibiotics</th>
<th>Vegetable isolates(N=76)</th>
<th>Clinical isolates(N=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive (%)</td>
<td>Resistant (%)</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Amoxyccillin (AMC)</td>
<td>3.94</td>
<td>96.05</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Ampicillin (AMP)</td>
<td>3.94</td>
<td>96.05</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Cefepime (FEP)</td>
<td>3.94</td>
<td>96.05</td>
</tr>
</tbody>
</table>
### Molecular determination of selected *S. maltophilia* isolates

The determination of selected *S. maltophilia* SM1 to SM17 was tested by performing individual PCRs with genomic DNA purified from *S. maltophilia* isolates using ATCC 13637 as a control gene with a forward primer of (GCTGGATTGGTTCTAGGAAAACGC) and reverse primer of (ACGCAGTCACTCCTTGCG) with a base pair of 278bp indicated all the randomly selected isolates are positive *S. maltophilia* detected (Figure 1).

### Assessment of biofilm formation and its relationship with antibiotic resistance

According to the average optical density (OD) values most of the strains studied were biofilm producers (97%). From 166 bacterial isolates tested for biofilm formation, 140 (84.33%) were categorized as strong producers, 16 (9.6%) were categorized as moderate producers, 9 (5.42%) were categorized as weak producers, and 1 (0.602%) was categorized as a non-biofilm producer.

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**Table**

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>MIC (mg/L)</th>
<th>Resistance %</th>
<th>Surviving Bacteria %</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams</td>
<td>Cefotixin</td>
<td>5.26</td>
<td>94.7</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Cefazolin</td>
<td>1.32</td>
<td>98.7</td>
<td>8.89</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Ceftazidime</td>
<td>1.32</td>
<td>98.7</td>
<td>7.77</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin</td>
<td>6.58</td>
<td>93.42</td>
<td>13.33</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamicin</td>
<td>2.63</td>
<td>97.4</td>
<td>12.22</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Kanamycin</td>
<td>6.58</td>
<td>93.42</td>
<td>13.33</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Cefaclor</td>
<td>1.32</td>
<td>98.7</td>
<td>5.55</td>
</tr>
<tr>
<td>β-Lactams</td>
<td>Cefotaxime</td>
<td>2.63</td>
<td>97.4</td>
<td>11.1</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Clindamycin</td>
<td>2.63</td>
<td>97.4</td>
<td>6.7</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Doripenem</td>
<td>1.32</td>
<td>98.7</td>
<td>2.23</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Imipenem</td>
<td>1.32</td>
<td>98.7</td>
<td>1.12</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxac</td>
<td>13.15</td>
<td>86.84</td>
<td>11.1</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Norfloxacin</td>
<td>6.58</td>
<td>93.42</td>
<td>2.23</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Meropenem</td>
<td>1.32</td>
<td>98.7</td>
<td>1.12</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>Linezolid</td>
<td>13.15</td>
<td>86.84</td>
<td>11.1</td>
</tr>
<tr>
<td>Ansamycins</td>
<td>Rifampicin</td>
<td>14.47</td>
<td>85.53</td>
<td>2.23</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>Trimethoprim</td>
<td>11.84</td>
<td>88.15</td>
<td>5.55</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Quinupristin</td>
<td>10.53</td>
<td>89.47</td>
<td>14.44</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftazidime</td>
<td>14.47</td>
<td>85.53</td>
<td>13.33</td>
</tr>
<tr>
<td>Carbapenem</td>
<td>Ertapenem</td>
<td>1.32</td>
<td>98.7</td>
<td>1.12</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>Colistin</td>
<td>10.53</td>
<td>89.47</td>
<td>8.89</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Nalidixic acid</td>
<td>11.84</td>
<td>88.15</td>
<td>6.7</td>
</tr>
</tbody>
</table>
Regarding the relationships between antimicrobial resistance and biofilm formation, our analysis showed that multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial isolates (resistance to three or more antibiotic families and resistance to all but one or two agents, respectively) tended to be more biofilm forming than those isolates susceptible to all antibiotics studied or resistant to one or two antimicrobials (Figure 2). However, the correlation between antimicrobial resistance and biofilm formation was not statistically significant.

![Fig 2. Relationship of antimicrobial resistance and biofilm formation of S. maltophilia from vegetables and clinical samples R1, resistance against one antimicrobial; R2, resistance against two antimicrobials; R3, resistance against three antimicrobials; R4, resistance against four antimicrobials; R5, resistance against five or more antimicrobials.](image)

4. DISCUSSION

*Stenotrophomonas maltophilia* is an important nosocomial pathogen and an emerging multiple-drug-resistant bacteria, which has previously been found both in the environment and hospital and clinical settings, including on plants and animals and in water treatment and distribution systems (Said MS, et al., 2022). In the current study, *S. maltophilia* was isolated from 76 of 98 leafy green samples collected. The mean aerobic mesophilic count of cauliflower, lettuce, spinach, and cabbage was 8.9 cfu/g, 8.6 cfu/g, 7.7 cfu/g, and 7.3 cfu/g, respectively, while the ranges were, cauliflower (5.5-4.3 cfu/g), lettuce (5.5-4.2 cfu/g) spinach (5.1-3.4 cfu/g) and cabbage (5.1-3.6 cfu/g). The current study matches previous reports (Qureshi et al. (2005). The higher bacterial load in this study may be partly due to the use of contaminated irrigation water and organic fertilizers in the farms, coupled with the poor hygienic environment from harvesting to transportation and dusty farm market environments.

The clinical isolates were identified between the two study periods of 2015 to 2016 and 2016 to 2017 based on the study there is a significant amount of increase in *S. maltophilia* isolates in the second study year which correlates with the previous study (Gajdács, M., & Urbán, E. 2019). A high number of isolates were identified from tracheal aspirates followed by cerebrospinal fluid (CSF), blood, sputum, and urine sources (Bostanghadiri, N., et al., 2021). Among all clinical samples, the frequency of occurrence of *S. maltophilia* from blood and CSF was relatively more contaminated. It is because blood and CSF compared to urine are more enriched and complex. Therefore, they are very suitable media for the reproduction of *S. maltophilia*. There are many factors are involved in the transmission of *S. maltophilia* to susceptible individuals. These risk factors are host immune status, pathogen status, and the ability of the pathogen to cause infection (Brooke JS, et al. 2012). Therefore, patients with cancer, chronic respiratory disease, immunocompromised host, and long-term hospitalization or ICU stay are at risk of *S. maltophilia* infection (Nseir, S., et al., 2006; Wang, N., et al., 2022). Overall, in our study *S. maltophilia* can be considered as the common nosocomial agent in Gondar referral hospital. On the other hand, the isolates were identified from different age groups of 0 to higher than 65. According to a previous study, the isolation of *S. maltophilia* in different age groups indicated that two age groups of greater than one and less than sixty-five were relatively more infected. This result clearly illustrated that infection by *S. maltophilia* is generally related to human
Immunity systems. It means that persons with an immunocompromised system are more at risk of infection with opportunistic pathogens such as S. maltophilia (Mojica, M. F., et al., 2022). In this study, multiple drug-resistant (MDR) S. maltophilia isolates have been recovered from both vegetable and clinical samples. Almost all S. maltophilia isolates demonstrated high resistance (>95%) to newer-generation beta-lactams, cephalosporins, and carbapenems. Our study correlates with previous studies (Gibb, J., & Wong, D. W. (2021). On the other hand, studies suggest that S. maltophilia is a biofilm former (Di Bonaventura G, et al., 2004). S. maltophilia is able to adhere to abiotic surfaces: both clinical and environmental isolates have been reported to adhere to glass and to several types of plastic materials including an intravenous cannula, polyvinyl chloride, and Teflon (Jucker, et al., 1996; Elvers, et al., 2001). Biofilms, products of bacterial adherence, are structured communities of bacterial cells enclosed in a self-produced exopolysaccharide matrix and adherent to an inert or living surface (Abdallah, M., et al., 2014). The establishment of a biofilm is the prelude to the development of various chronic, intractable infections, such as biomaterial-associated infections and pulmonary infections in patients with cystic fibrosis (Koch, C., and N. Hoiby. 1993). In the current study, almost all the isolates of S. maltophilia are biofilm producers. Our report matches previous studies. On the other hand, the current study showed that multidrug-resistant (MDR) and extensively drug-resistant (XDR) S. maltophilia isolates (resistant to three or more antibiotic families and resistant to all agents, respectively) tended to be more biofilm-forming than those isolates susceptible to all antibiotics studied or resistant to one or two antimicrobials.

5. CONCLUSION
Recently, S. maltophilia has been detected as an emerging opportunistic pathogen. The bacteria are frequently isolated from both the water environments and clinical settings and are able to colonize moist surfaces. Studies indicated that S. maltophilia has been resistant to many antibiotics, including those used to treat the infections the bacteria cause. It is therefore important that new antibiotic targets be detected, and the appearance of resistance during treatment be predicted. In addition, other important measures of prevention must continue to be used by healthcare personnel in efforts to reduce the transmission of this serious pathogen. Measures include hand washing with soap and ensuring appropriate cleaning and disinfection of medical devices used with patients. Health professionals must avoid using hospital tap water to wash patient wounds and avoid disposing of potentially contaminated antimicrobial solutions at sites that can come into contact with susceptible individuals.

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CONFLICT OF INTEREST
There is no conflict of interest with any party in this research work.

REFERENCES


