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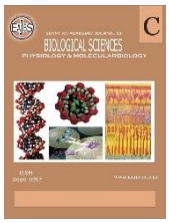

Molecular Detection of *IMP* Gene Isolated from *Stenotrophomonas maltophilia* Bacteria

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ABSTRACT

The *S. maltophilia* was resistant to multiple antibiotics, and its mechanisms also include intrinsic resistance and acquired resistance (Calvopina and Avison, 2018; Wang *et al.*, 2018).

It is a nosocomial bacterium that causes health-care-associated infections (HCAIs) by direct contact, ingestion, aspiration, or aerosolization of potable water, or healthcare workers' hands The non-fermentative, Gram-negative, rod-shaped bacteria *S. maltophilia* is abundant in the environment and has a wide geographical spread. Both in and out of clinical situations, this bacterium species has been isolated from aquatic sources.

For a study aimed at the isolation and identification *S. maltophilia* from different clinical specimens, using conventional methods, the VITEK-2 system and investigated the prevalence of MBLs (AIM) genes among *S. maltophilia*. Between November 2021 and November 2022, The specimens were cultured on MacConky and blood agar and incubated for 24 h, (200) 80% of specimens were given bacterial growth and (50) 20% showed no growth, from 200 bacterial isolates, (65) 32.5% of bacterial isolates recorded Gram-positive bacteria and (135) 67.5% of bacterial isolates recorded Gram-negative bacteria. Of 135 (Gram-negative), (43) 31.8% appeared lactose fermented bacteria (produce pink color colony) and (92) 68.2% were lactose non-fermented isolates (produce yellow color), in addition to biochemical tests, (45) 49.9% of the bacterial isolate were given oxidase negative, catalase positive, motile and produce alkaline/alkaline on kliglar, were suspected as *S. maltophilia.* Biochemical tests with VITEK-2 bacteria, *S. maltophilia* isolates during this period, isolated and identified depending on the primary methods of diagnosis, then the use of the VITEK-2 compact system. These were the outcomes: 20 additional bacterial isolates and 25 *S. maltophilia* isolates. Using PCR, it was discovered that 15 (60%) samples of *S. maltophilia* bacteria carried the IMB gene.

INTRODUCTION

Stenotrophomonas maltophilia is a gram-negative bacillus that is innately multi-drug resistant (MDR) and found in watery and humid environments in the environment, including plants, animals, and water sources (Gulcan *et al*., 2004; Yu *et al*., 2016; Han *et al.*, 2020). It grows well on MacConkey agar without forming colonies that are colored, is motile with polar flagella, non-capsulated, and non-sporulating, and is catalase-positive but oxidase-negative (which distinguishes it from most other members of the genus) Neela, *et al.*, (2014).

The bacterium *S. maltophilia* is naturally found in soil, plants, and aquatic settings.. In order to cause infections, the invasive pathogen must be able to manufacture a variety of virulence factors, and the amount of micronutrients present in the hospital environment greatly influences these factors Sritharan, *et al.*,(2006). *S. maltophilia* is known to exhibit its pathogenicity in these circumstances through pili/flagella/fimbrial/adhesins, which contribute to adhesion, auto-aggregation, decolonization of both biotic and abiotic surfaces, outer membrane lipopolysaccharide (LPS), which plays a role in biofilm formation and antimicrobial resistance as well as support cell trying to kill, and others. Quorum sensing, which in turn regulates peristalsis, extracellular enzyme production, LPS formulation, microcolony development, acceptability to antibiotics, and tolerance to heavy metal ions, depends on the secreted signal factor (DSF). For tissue invasion and evasion, extracellular enzymes including as elastase, gelatinase, hyaluronidase, proteases, lipases, DNase, RNase, and mucinase are generated by Brooke, *et al.*, (2012), *S. maltophilia* is a "newly emerging pathogen of concern" that is being isolated more frequently. The World Health Organization (WHO) also recognizes it as one of the underappreciated significant multi-drug resistant organisms in hospitals. British microbiologists ranked it as the ninth most important pathogen, and it is one of the most challenging pathogens in the infectious disease community and studies. It is also well-known as an opportunistic organism associated with Chang *et al.*,(2015). *S. maltophilia* is a new pathogen that is linked to serious diseases in patients who have cystic fibrosis, malignancy, and immunologically weakened individuals. The individual's adhesion to abiotic surfaces like catheters and medical devices poses a significant concern for hospitalized patients Alcaraz *et al.*,(2018).

MATERIALS AND METHODS

Specimen Collection and Bacterial Identification:

The colony appearance, microscopic inspection, and biochemical characteristics were used to identify bacterial isolates. 135 bacterial growth isolates showed Gram-negative bacteria growth. On MacConkey agar,(43) 31.8% of bacteria produced pink colonies, since the bacteria was lactose fermented growth on MaCconkey agar and produced pink colonies, and (92) 68.2% were lactose non-fermented isolates (produce yellow color), in addition to biochemical tests, that, (45) 49.9% of bacterial isolate were gave oxidase negative, catalase positive, motile and produce alkaline/alkaline on kliglar, were suspected as *S. maltophilia*. Biochemical tests with VITEK-2 compact system were used to identify the lactose non-fermented and oxidase-negative isolates, from suspected isolates (20) 44.4% of isolates were identified *Stenotrophomonas maltophilia*, (14) 31.2% of isolates appeared *Acinetobacter baumannii*, (3) 6.7% of isolates were *Providinicia* spp, and (8) 17.7% isolates were identified *Pseudomonas aeruginosae*.

DNA Extraction of Bacteria: By use (Genomic DNA promaga Kit).

Molecular Identification:

The P.C.R assay was used to identify *S. maltophilia* as shown in Table by identifying the IMB gene (2). These primers were created by the Canadian Alpha DNA, as shown in Table (1). 1% agarose gel electrophoresis is used to magnify results in order to determine the size of the PCR. The gel was stained for roughly 1.5 hours at 80 volts using 4 mL of ethidium bromide. The Mwut. of amplified products was measured using a 100bp ladder (Bionaer, Korea).

Table 1: Primers utilized in this study.

Prime	sequence	Amplicon si	
IMP	F: 5'GGA ATAGAGTGG CTTAACTCTC 3' R: 5' CGA ATG CGC ACC AG 3'	232	Adam and Elhag (2018).

Table 2: PCR program of shv primer that was applied in the thermocycler.

Gene	Primary denaturation	No.of cycles	Denaturation	Annealing	Extension	Final extension
IMP	94\4 min.	30	94˚C / 30sec	55˚C/30sec	72˚C/30sec	72˚C/5min

RESULTS AND DISCUSSION

The total number of (200) 80% specimens. The results revealed that (65) 32.5% of bacterial isolates recorded Gram-positive specimens gave up a positive culture and (135) 67.5% of bacterial isolates recorded Gram-negative bacteria. A culture analysis based on morphological and biochemical tests revealed a high incidence of Gram-negative bacteria. 65 G+ bacteria and 135 G- bacteria showed growth during this time period; *S. maltophilia* isolates were isolated and identified using the VITEK-2 compact system after 135 Gram-negative bacteria showed growth. These were the outcomes: 100 additional bacterial isolates and 35 *S. maltophilia* isolates.

Molecular Detection of IMB gene of *Stenotrophomonas maltophilia:*

The Ambler class B MBL(IMB) gene-specific primers were used to conduct a conventional PCR screening for possible MBL gene determinants on all 35 *S. maltophili* isolates, only 15 isolated positive results for this gene with a percentage (60%) as shown in Figure (1) Even though multiple stable medications and inhibitor combinations are in different phases of development, they elude all recently approved -lactam—lactamase inhibitor combinations.

S. maltophilia is an emerging nosocomial pathogen that causes a variety of diseases, according to a study by Saleh *et al.*, (2021)in Iraq published in. No specimens were affected by ceftazidime or chloramphenicol. Furthermore, all of them (100%) and 43% of them (100%) respectively acquired extended spectrum -lactamases (ESBLs) and carbapenemases. Currently, 33 of the 51 known IMP variations are classified as IMP-like enzymes. The percentage of amino acid similarity among these subgroups ranges from 90% to 99%, indicating that these subgroups have extremely comparable hydrolytic activity Sachdeva *et al.*,(2017). This enzyme, which is a member of subclass B3, was the first of its kind to be discovered on a mobile genetic element from the serious human disease *S. maltophilia* as opposed to coming from an outside source Bahr *et al.*, (2021).

AIM-1 differs in sequencing from other B3 MBLs (*Stenotrophomonas maltophilia* L1, Janthinobacterium lividum THIN-B, Chryseobacterium meningosepticum GOB, Legionella gormanii FEZ-1, Caulobacter crescentus CAU-1, Bradyrhizobium sp.) by only having 23% identity to FEZ-1, 29% identical to BJP-1 Khorvash *et al.*,(2017).

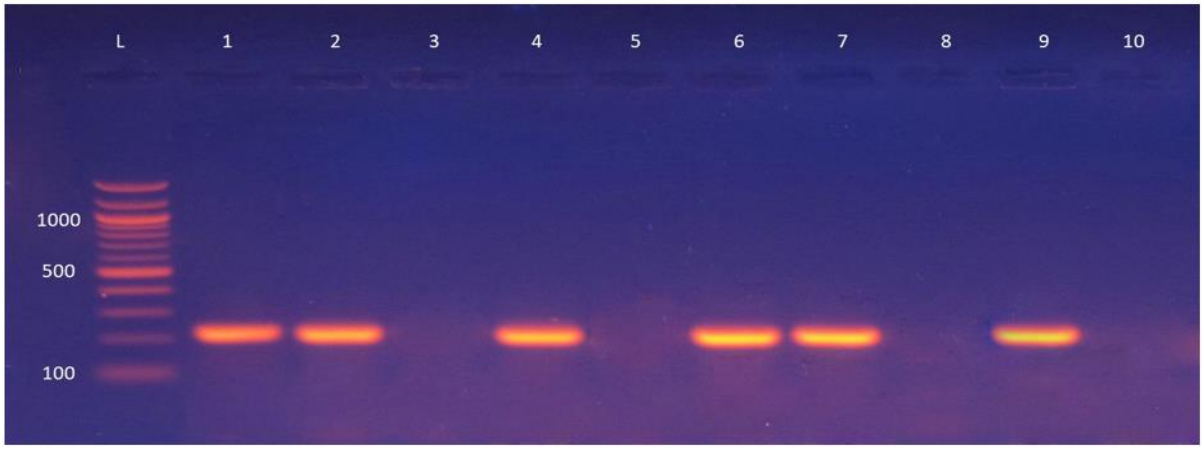

Fig. 1: PCR products of *Stenotrophomonas maltophilia* isolates amplified with AIM gene primers, having a product size of 232 bp. Lane (L), DNA molecular size marker (1000-bp ladder), Lanes (1,2,4,5,6,7,9) indicate AIM gene positive findings.

Conclusions:

The outcomes of this study revealed a great spread of *S. maltophilia* isolates in Najaf hospitals that produce MBLs enzymes and are resistant to many antibiotics, The Molecular study for the detection of MBLs genetic factor of *S. maltophilia* microbes, was as 15 bla-IMB with percentages (60%).

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