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PCR detection of Gene *Stmpr1, Stmpr2 and Stmpr3* encoding protease of *Stenotrophomonas malltophilia* isolated from different infection

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Abstract*---Stenotrophomonas maltophilia*** is an emerging opportunistic nosocomial pathogen, with many virulence factor and causing various infections. Therefore this study conducted to characterization and molecular investigation of some extracellular virulence factor particularly serin protease and detection of gene encoding this factor (*Stmpr1, Stmpr2* and *Stmpr3* genes) in order to determinants the role of this bacteria in causing infection in Al- Najaf province. Therefore 485 clinical specimens were collected from different infection 315 (64.9%) of specimens were collected from male and 170(35.1%) from female, the specimens were cultured on suitable media and cultivated at 37 C, The results showed that 373(76.9%) gave bacterial growth while 112(23.1%) appeared no growth after identification of bacterial isolate, 20 of isolate were identified *Stenotrophomonas maltophilia* recovered from the different infections, from those 12 of isolates were recovered from male specimens, while 8 from female, PCR were used to determinant the predominant of protease genes among *Stenotrophomona maltophili*a isolates, the results of PCR revealed that all isolate 20 (100%) were carried *stmpr1, stmpr2* and (85%) *Stmpr3 genes*, which appeared in positive bands with the correct product size.

***Keywords*---**PCR detection, *Stenotrophomonas malltophilia*, infections.

Introduction

Stenotrophomonas maltophilia is was first classified within the genus Pseudomonas in 1961, then as Xanthomonas in 1983 and finally as

Stenotrophomonas in 1993, S. maltophilia is the only species of Stenotrophomonas known to infect humans(Chawla *et al.*, 2014).it is glucose non-fermenting obligate aerobic, motile, and non capsulated gram-negative bacterium (Insuwanno *et al.*, 2020). widespread in many environmental sources, It is increasingly recognized as an opportunistic pathogen responsible for nosocomial infections in intensive care unit patients (such as ventilator associated pneumonia and sepsis), life-threatening diseases in immunocompromised patients with haematological malignancies and cancers, and chronic pulmonary infections in patients with cystic fibrosis (CF), This organism predominantly causes respiratory tract infections, and less commonly urinary tract infections, wound and soft tissue infections, bacteremia, sepsis, endocarditis, meningitis, ophthalmic infections, osteochondritis and peritonitis (Brooke & Brooke, 2012; Denton & Kerr, 1998; Scholte *et al.*, 2016; Trifonova & Strateva, 2018). which is still defined as an organism of limited pathogenicity, has risen prominently as a nosocomial pathogen. Despite the increase in the spectrum of clinical syndromes associated with *S. maltophilia*, very little is known about the extracellular enzymes profile, pigment production and motility patterns which may have potential roles in pathogenesis., Despite its clinical relevance, very little is known about the pathogenic mechanism of infections. Secretion of enzymes, namely proteinases, lecithinases, gelatinase, lipases, and DNase, biofilm formation, hemolysis, and motility are few properties that pathogenic microbes possess in order to establish an infection. These enzymes are considered virulent as they damage the host tissues making the host permissive for infection (Edberg *et al.*, 1996; Thomas *et al.*, 2014).

Materials and Methods

Sample collection and bacterial identification

A total of 485 samples were collected from patients with different infections admitted to the main hospitals and privates clinical laboratories in Najaf City during November 2021–March 2022. these specimens involved Urine(129),Burn(65),Blood(25),Wound swab(72),Tracheal aspirate(21),Throat swab(88),CSF(18) and sputum(67). specimens were cultured immediately onto blood agar and MacConkey (Merck, Germany) and incubated at 37 ± 0.5 ◦C for an overnight. Morphological characters and biochemical tests were initially performed to identify the suspected colonies of *S.maltophilia* ,were confirmed based on Vitek-2 system (Obaid *et al.*, 2021).

DNA extraction and PCR assay

Total genomic of 20 isolates of *Stenotrophomonas maltophilia*. were extracted using genomic DNA extraction mini kit (Favorgen, Biotech, Corp. Korea), the extraction was performed based on the guidance of manufacture corporation. The nucleic acid was conserved under -20°C state using the deep freezing device, the PCR technique was employed to examine and detect all the genes described in table (1). The gel document (Cleaver, United Kindom), was applied to migration of PCR products at 1% agarose (iNtRoN, Biotech. Inc., Korea), after dye of ethidium bromide at 0.5 µg/ml concentration was applied (Al-Hamdani and Tuwaij, 2020).

Table (1) Conditions and sequence of primers used in this study

Primer	Description	Primer Sequence 5' to 3'	Tm (◦C)	Product (bp)	Reference
StmPR1-F	StmPR1 protease	CAACGACTCGATGAATGTGG	52	174 bp	(Molloy *et al.*, 2019)
StmPR1-R		CAGACATAGCCGTTCGGATT	52		
StmPR2-F	StmPR2 protease	CAGGTCGAGAGCATCATCAA	55	168 bp	
StmPR2-R		GGTCACCGGTACGTTGTTCT	55		
StmPR3-F	StmPR3 protease	AGCGAAAACACGATTCGTTC	55	189	
StmPR3-R		ACGGTGATGACGTTGAACAG	55		

Results

The results of culture growth indicated that among 485 patients involved 315(64.9%) male and 170(35.1%) female were 373(76.9%) bacterial growth compared with 112(23.1%) no growth. the results listed in Table 2 indicated that although the number of bacterial growth in male was above that of female, the percentage of *Stenotrophomonas maltophilia* isolates in male were 12(4.9%) compared with 8(6.2) in male from a total of 20(5.0%) *S.maltophilia.* isolates.

Table (2) Distribution of *S.maltophilia* between gram negative according the gender

sex	specimens growth		Gram-negative		*Stenotrophomonas maltophilia*	
	number	%	number	%	number	%
Female	128	34.3	89	69.6	8	6.2
Male	245	65.7	169	69.0	12	4.9
Total	373	100	258	76.4	20	5.3

Data of the present study showed a heterogeneous distribution of *Stenotrophomonas maltophilia.* isolates among the different infections of the patients, whereas the results of microbial diagnosis showed that 20 isolates were returned to *Stenotrophomonas maltophiia.* The number of pathogens recorded according to the source of infection detailed in table 3.

Table (3) Distribution of *S.maltophilis* according the source of infection.

Sample source	Total sample	growth	Total *Stenotrophomonas maltophilia*
urine	129	110	8
Burn	65	55	3
Blood	25	11	3
Wound swab	72	57	0
Cerebro-Spinal fluid	18	7	2
Tracheal aspirate	21	15	3
Throat swab	88	65	0
Sputum	67	53	1
Total	485	373	20

PCR data was revealed that all *Stenotrophomonas maltophilia*. isolates in the current study were harbored 20(100%) *Stmpr1* , *Stmpr2* and (85 %) *Stmpr3* gene, which appeared in positive bands at the correct product size figure 1,2 and 3).

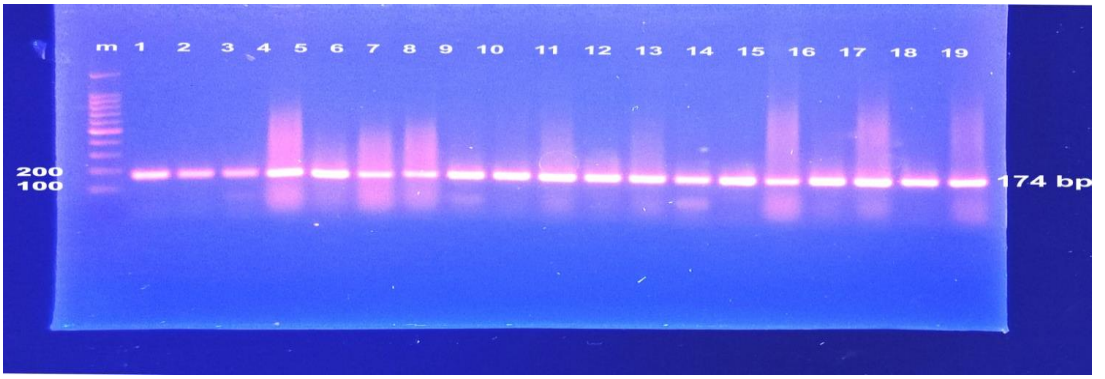

Figure (1): PCR amplification of *Stmpr*1 gene among *Stenotrophomonas maltophiia* isolates.

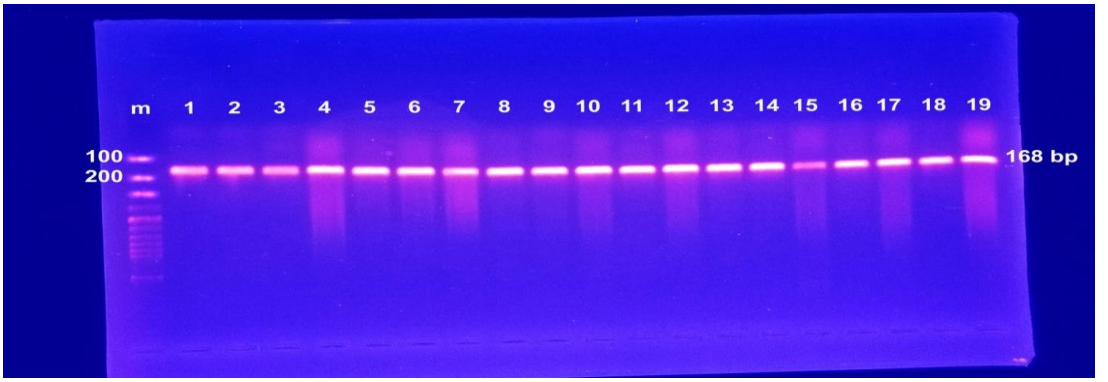

Figure (2): PCR amplification of *Stmpr*2 gene among *Stenotrophomonas maltophiia* isolates.

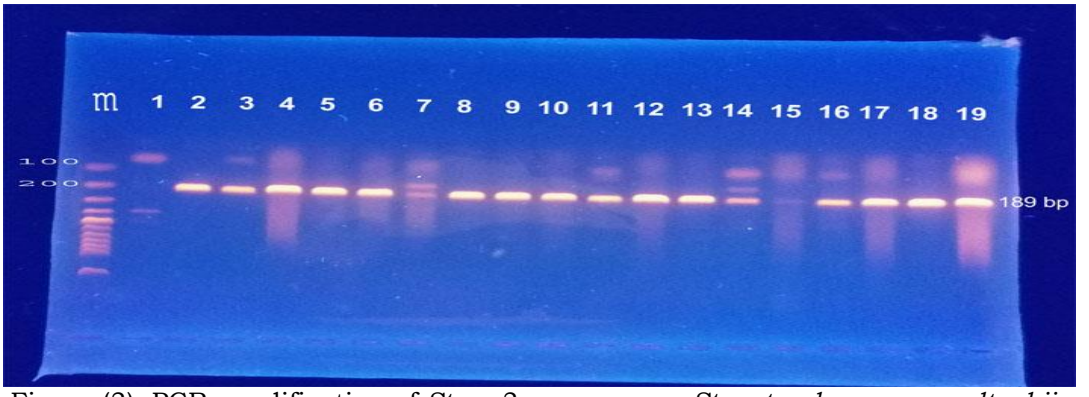

Figure (3): PCR amplification of *Stmpr*3 gene among *Stenotrophomonas maltophiia* isolates

Discussion

Kim *et al.*,(2018) reported in their study that the result of PCR technique for *stmpr1* and *stmpr2*geneswere detected in (93.8 %) and (96.8%) of bacterial isolates , in the same time ,Alcaraz *et al.*, (2018)found that the PCR amplification of virulence gene *Stmpr1* gave positive results in (82.5%) of all bacterial isolates. Duan *et al.*,(2020)reported in results of their study that the virulence gene represented by *Stmpr1and Stmpr2*were detected in (79.6%) and (91.4%) of *S maltophilia* strain .Eliana *et al.*,(2020) showed that the positive result from total 63 isolates was (82.5 %). similar finding was reported in a S. maltophilia study by Ibrahim (2020) that the virulence gene *Stmpr2* was found (100 %) in all bacterial isolates.

Fluit *et al.*,(2021) recorded in their study that the positive results of PCR amplification for virulence gene *Stmpr1* was found in *all S maltophilia* isolates (100 %) ,while, Elbaradei & Yakout(2022) reported that the serine proteases genes *StmPr1* (1621 bp), *StmPr2*(868 bp) was found in (70%) and(15%.) of bacterial strain.

Fluit *et al.*,(2021) reported that the result of detection virulence gene *Stmpr3* was (98.5 %) from all *S.maltophilia* isolates ,Ibrahim (2020) detected that all *S.maltophilia* isolates produced virulence gene Fluit *et al.*,(2021) reported that the result of detection virulence gene *Stmpr3* was (98.5 %) from all *S.maltophilia* isolates (64/65).Ibrahim (2020) detected that all *S.maltophilia* isolates produced virulence gene *Stmpr3* at rate (100 %).

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