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PCR detection of Gene *lip* and *plcN1* encoding lipase and lecithinase of *Stenotrophomonas malltophilia* isolated from different infection

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Abstract*---Stenotrophomonas maltophilia*** is a low-virulence opportunistic pathogen that has been identified as an emerging hospital pathogen, Infections caused by *S. maltophilia* have increased over the years due to increased high-risk patients e.g. patients with immune suppression, hematological malignancies, ICU admission, It is an aerobic, gram- negative, bacilli, motile, non_capsulated and non_ferment glucose, has several virulence potentials for causing various infection. therefore this study conducted to characterization and molecular investigation of some extracellular virulence factor particularly lipase and lecithinase and detection of gene encoding this factors (*lip* and *plcN1*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 ℃, 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 *S. 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 lipase and lecithinase genes among *S. maltophilia* isolates, the results of PCR revealed that all isolate 20 (90%) were carried *lip* and *plcN1*genes, which appeared in positive bands with the correct product size.

***Keywords*---**PCR detection, *Stenotrophomonas maltophilia,* infection.

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).

Aim of the Study

Since, the newly isolation and identification of this bacteria and limitation of virulence factors of it, the study aims to determine the extra virulence factors (extracellular enzyme) of the bacteria and the predominant the genes encoding the extracellular enzymes among the clinical isolates from different infections, via the following steps:

1- Collecting the clinical specimens from different infection, included, urine from UTI, swabs (wound, burn, Throat) blood, CSF and sputum.
2- Isolating and identifying the bacterial isolates.
3- Phenotypically detecting the extracellular enzymes using specific media and specific tests.
4- Determining the genes encoding the extracellular enzymes among the clinical bacterial isolates

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 ℃ 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 *S. 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
lip-F	Lipase gene	CAGGCCTACAAGCTGCACTA	**60 ˚C**		
lip-R		TTGACGAGGTCGATGGCATT	**60 ˚C**	326 bp	NCBI
plcN1-F	Lecithinase gene	ATCGACCTCGTCAAAGCCAG	**60 ˚C**		
plcN1-R		AGGGTGGTCAGATAGGGGAC	**60 ˚C**	539 bp	

Result:

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 *S. 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 sex.

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 *S. maltophilia.* isolates among the different infections of the patients, whereas the results of

microbial diagnosis showed that 20 isolates were returned to *S. 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 *S. maltophilia* isolates in the current study were harbored 20(90%) *Lip* and *plcN1* gene, which appeared in positive bands at the correct product size figure 1and 2).

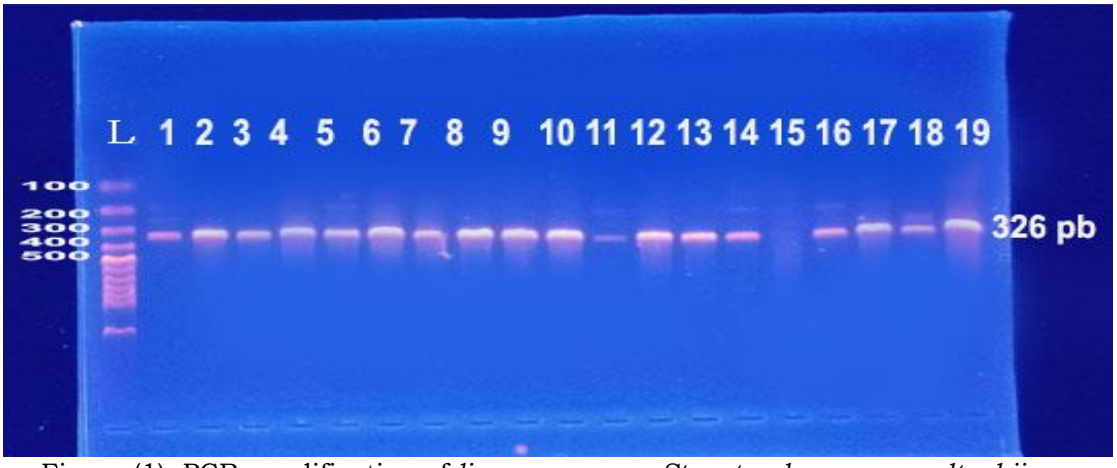

Figure (1): PCR amplification of *lip* gene among *Stenotrophomonas maltophiia* isolates.

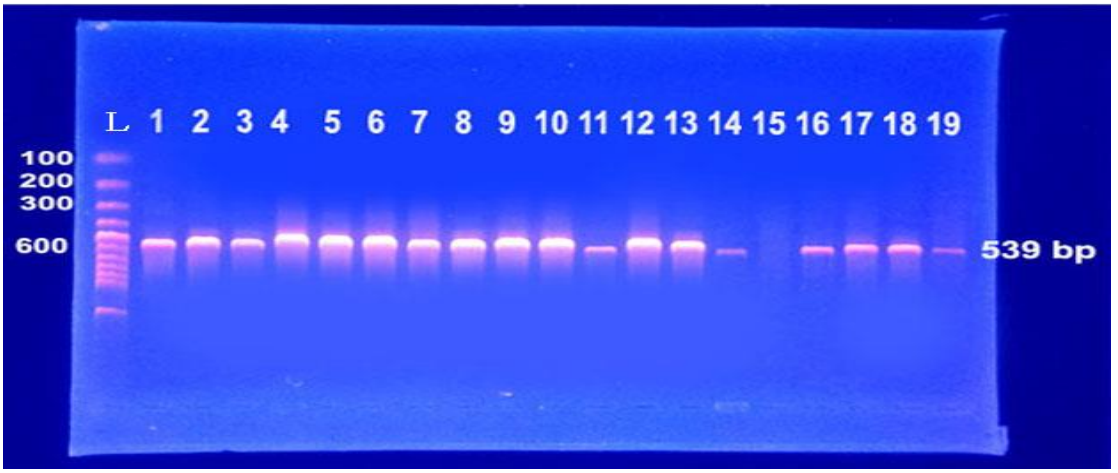

Figure (2): PCR amplification of *plcN1* gene among *Stenotrophomonas maltophiia* isolates

Discussion

In the study of Amoli *et al*(2016) found that (75%) bacterial isolates caring *(lip)* genes by using PCR amplified with specific primer. Meheissen *et al.*, (2022)reported in previous studies, that the result of lipase gene were detected in (57.1%) from all bacterial isolates, by using PCR amplification technique. Study in (2014) conducted by Adamek *et al.*, on different strains *of S. maltophilia*, showed that the all strains carried (*plcN1*) gene. Cruz-Córdova *et al.*, (2020) reported that (83.33%)from 30 strains of *S. maltophilia* isolated from different specimens carried the phospholipase C (Lecithinase) gene (*plcN1*), while study of Obaid *et al.*, (2021) exhibited that (84%) of 100 bacterial isolates from different infectious was carried *plcN1*gene encoding for phospholipase C enzyme .

Conclusion

1- Stenotrophomona maltophilia is newly important pathogen that causing different infection among Iraqi patients.
2- All bacterial isolates have neumerous virulence factors enable bacteria to invade human body.
3- Phenotypic detection revealed that all bacterial isolates have ability to produce major virulence factors like protease, DNase, lecithinase and haemolysin.
4- All bacterial isolates carrying genes encoding extracellular enzymes.

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