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Saman I. Othman

College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq, saman.othman@hmu.edu.krd

Fattma A. Ali

College of Health Sciences, Hawler Medical University, Erbil, Kurdistan Region, Iraq

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RESEARCH ARTICLE

Molecular Comparative Study of Virulence Factors Produced by Gram-Negative Bacteria Isolated from Several Cancer Cases and Non-Cancer Patients in Erbil Governorate, Iraq

Saman I. Othman¹, Fattma A. Ali²

¹ College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq

² College of Health Sciences, Hawler Medical University, Erbil, Kurdistan Region, Iraq

***Corresponding author:**

Saman I. Othman,
Department of
Pharmacognosy, College
of Pharmacy, Hawler
Medical University, Erbil,
Kurdistan Region, Iraq.

E-mail:

saman.othman@hmu.edu.krd

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ABSTRACT

Background and objectives: Gram-negative bacteria are one of the most prevalent complications among immunocompromised cancer sufferers and pose the greatest threat to these patients.

Our study aimed to conduct research on Gram-negative bacteria isolated from various clinical samples among cancerous patients and non-cancerous patients in Erbil city to analyze and compare some virulence genes among them such as *tuf*, *atpD1*, *atpD2*, *ms-1*, *ms-2* and *ms-3*.

Methods: The current study was carried out in Nanakaly hospital and other public health hospitals in Erbil-Kurdistan region at a period extending from August 2021 to March 2022. A total of 100 different clinical samples including urine, sputum, wound swab, stool, blood, have been collected from patients with different types of cancer (n=50) and non-cancerous patients (n=50), from both gender (male and female), without limited age group. Gram-negative bacterial isolates identified by microscopical, macroscopical analysis and Vitek II compact system.

Results: The results of amplification of *tuf* gene among the cancerous patients were (70%), while the results among the non-cancerous patients was (52%). The percentage of *atpD1* from cancer patients were (52%), as well as the appearance among non-cancerous patients were (44%) isolates. The results of *atpD2* among cancer patients were (52%). However, the average of *atpD2* among patients without cancer were (48%). The results of agarose gel electrophoresis of amplicons appeared that (32%) samples possess *ms-1* among cancer cases, while there have not any positive results for control patients. Also, the results of *ms-2* gene were (28%) from cancer patients, whereas, the prevalence of *ms-2* gene marker among patients without cancer was (6%). The distribution of *ms-3* gene was (22%) among cancer patients. Otherwise, the prevalence from samples of patients other than cancer were (20%). According to the statistical tests, the results showed no significant association between genes and type of bacteria in cancerous patients as well as in non-cancerous patients.

Conclusions: *tuf*, *atpD1* and *atpD2* genes sealed a discriminated gene as a virulence factor encoding genes for identification of gram-negative bacteria. To the public health and especially from patients with cancer there is a main distribution of *ms-1*, *ms-2* and *ms-3* genes among gram-negative bacteria.

Keywords: Gram-negative bacteria, cancerous patients, non-cancerous patients, virulence genes

INTRODUCTION

Cancer is an abnormal growth of cells caused by multiple expressions of genes that lead to a dysregulated balance between cell proliferation and cell death, eventually evolving into a cell population that can colonize tissues and spread to distant sites, causing significant morbidity and death of the host (Ashreen et al., 2019); (Ruddon, 2007). Ultimately, cancer is a disorder of abnormal gene expression (Parsa, 2012). Multiple mechanisms are involved in these changes in gene expression. These mechanisms can be triggered by a direct DNA insult, such as mutagenesis, translocation, amplification, deletion, or loss of heterozygosity, or by

abnormal gene transcription or translation (Ruddon, 2007).

Gram-negative bacilli have become important pathogens in cancer patients (Montassier et al., 2013). Chronic bacterial infections are important since they enhance the immune response and increase oxidative stress in infected cells, causing the release of reactive oxygen species (ROS) as well as cell membrane and DNA leakage (Little et al., 2018). *E. coli* can be divided into four phylotypes (A, B1, B2, and D). This grouping detected by their multilocus enzyme electrophoresis patterns (MLEE), (Chaudhuri and Henderson, 2012). B2 *E. coli* strains have been shown to have cancer-causing properties (Raisch et al., 2014). *E. coli* frequently

colonize cancer lesions and adjacent epithelium, accumulating in such large numbers that they are sometimes the only cultivable organisms in close proximity to the diseased site (Nowrouzian and Oswald, 2012).

K. pneumoniae is one of the most predominant gram-negative bacteria among people with cancer and a significant complication for chemotherapy patients (Garg et al., 2019). *A. baumannii* is a Gram-negative coccobacillus that has emerged as a major nosocomial pathogen (Caricato et al., 2009). Cancerous individuals are at a high risk for contracting infections (Fukuta et al., 2013). *P. mirabilis* known as a gram-negative bacterium surrounded by flagella that is commonly found in water, soil, and human digestive environments. Sometimes, *P. mirabilis* is considered the causative pathogen in urinary catheter-associated hospital cross-infections (Gong et al., 2019). *P. aeruginosa* defined as an opportunistic pathogen that causes numerous infections (Vuotto et al., 2013). In immunocompromised hosts, such as those with chemotherapy-induced neutropenia, cystic fibrosis, or severe burns, as well as those receiving intensive care, Infections may be associated with a high rate of deaths (Markou and Apidianakis, 2014). *Cedecea* is a rare but increasingly significant pathogen, it is difficult to treat infections caused by *Cedecea* species that contain antibiotic resistance genes (Ahmad et al., 2021). The Centers for Disease Control and Prevention (CDC) identified *Cedecea* as a new genus in the family *Enterobacteriaceae*, formerly designated as CDC enteric group 15, and the organism was later named *Cedecea* from the letters CDC (Akinosoglou et al., 2012).

Virulence genes regulate various mechanisms such as the synthesis and release of many virulence factors, adherence and survival factors, proteins, toxins, enzymes, iron-binding compounds, and biofilm formations (Liu et al., 2019), host immune systems suppression; formation of lesions in intestinal epithelial cells urinary tract and bloodstream infection (Daga et al., 2019); adaptation for survival in the presence of intestinal microbiota; host-bacterial interaction; and determination of host specificity (Nimnoi and Pongsilp, 2020). These mechanisms are indispensable for habitation, survival, adaptation, and multiplication within hosts. Therefore, virulence genes are essential for bacterial infection and pathogenesis. Several factors, including nutrient starvation; concentrations of NaCl, oxygen, and glucose; temperature; growth phase; type of carbon source; pH; sodium glycocholate; and L-glutamine, have been observed to influence the expression of virulence genes (Joffre et al., 2019).

Sample collection

The current study was carried out in Nanakaly and other public health hospitals in Erbil- Kurdistan region at a period extending from August 2021 to March 2022. A total of 100 different clinical samples including urine, sputum, wound swab, stool, blood, have been collected from patients with different types of cancer (n=50) and non-cancerous patients (n=50), from both gender (male and female), without limited age group. All isolates were taken from the specimens were cultured on Blood and MacConkey agar and incubate at 37°C for 24 hrs. After that, gram staining was done to differentiate the gram-negative bacteria, and identification of all gram-negative bacteria by using Vitek II compact system.

Molecular detection

PCR approach used to 100 bacterial isolates taken from the urine, sputum, stool, wound samples, blood (50 isolates from cancerous patients and 50 isolates from non-cancerous patients). It was used to detect the presence of some virulence genes such as *tuf*, *atpD1*, *atpD2*, *ms-1*, *ms-2*, and *ms-3* among bacterial isolates.

Bacterial DNA extraction, primers and PCR amplification

The method that used for genomic extraction was performed by using PrimePrep™ plasmid DNA isolation kit according to the information of the supplying company. The synthesis of oligonucleotide sequences presented in table (1). Thermocycler of amplification condition was explained in tables (2) and (3).

Amplicon detection

I added 1.2g of agarose with 100 mL of 1X TAE into a conical flask, then melted in a microwave oven. After cooling added red safe dye, 5 µL into the mixture. The appropriate comb was inserted into the tray and the agarose poured slowly into the tank to a depth of about 1 cm. Carefully, remove the comb, and the gel is placed in the electrophoresis tank. The gel was covered with 1X TAE running buffer. Desired DNA samples were loaded and pipetting up 0.2 volumes of loading dye, then the sample and loading dye were mixed by filling and emptying the pipette a few times. The ready mixture is loaded into a well. The power source was switched on, then the voltage was increased to 75-100 volts for 1 hour. The progress of the gel is monitored by reference to the marker loading dye. The DNA bands were pictured by UV illumination at a (240–360 nm) wavelength on a UV transilluminator, the gel was photographed using a polaroid camera for photographic documentation.

SUBJECTS AND METHODS

Table 1. Presented the sequences of oligonucleotide, primer that used in the study

Name of Genes	Primer	Amplicon Size	Reference
<i>tuf</i>	F- GGGACGCCAACTATGTT R- AACGGTACGGCCGCCTTC	903	(Jawad, 2020)
<i>atpD1</i>	F- TGAGGAGGAAGCTCATGGCCG R- CGGCCCCCGAGACGA	866	
<i>atpD2</i>	F- CGCAAGGCATTGAGGAGAAG R- CGGCCCCCGAGACGA	876	
<i>ms-1</i>	F- GTAATCCTCAACCGCACCAGGC R- TGATGCGTTACCACACT	684	
<i>ms-2</i>	F- CCTGAAGTGACCGTGAAACAG R-TGTATTGTCTGCGCTTCCAG	498	
<i>ms-3</i>	F- CGCTGTAGCAGCTCATGCAG R- GACAACACTGACCGGATAATC	880	

Table 2. Presented the amplification condition program

Genes name	Initial Denaturation (C/min)	No of Cycle	Denaturation	Annealing	Extension	Final extension (C/min)
<i>tuf</i>	95/3	35	95 °C/3min	55°C /1 min	72°C /1min	72/7
<i>atpD1</i>	95/3	35	95 °C /3min	50°C /1min	72°C /1min	72/7
<i>atpD2</i>	95/3	35	95 °C /3min	50°C /1min	72°C /1min	72/7
<i>ms-1</i>	95/2	30	95°C /30sec	58.4°C /30sec	72°C /30sec	72/5
<i>ms-2</i>	95/2	30	95°C /3sec	58.4°C /30sec	72°C /50sec	72/5

Table 3. Touchdown PCR approach for amplification of *ms-3*

Gene	Thermocycler condition program
<i>ms-3</i>	95°C/2min 95°C /30sec 61.4°C decrease 0.5 per cycle,30sec 72°C /20sec, Repeat steps 2-4, 14 more time 95°C /30sec 56.4°C /30sec 72°C /90sec Repeat steps 5-7, 19 more time 72°C /5min

constituted 3 (6%), 3 (6%) respectively, finally blood specimen measured for the minority with only one specimen (2%), as shown in table (4). However, among the non-cancerous patients' different type of specimens was taken for examination. The growth of bacteria detected from urine samples which constituted (76%) of growths of all specimens, after that sputum samples which constituted (12%) of growths of all specimens, wound swab came in the third degree which constituted (8%), finally blood specimen measured for the minority with (4%), as mentioned in table (5).

RESULTS

Gram-negative bacterial isolates according to different site of isolation among cancerous patients and non-cancerous patients

Different type of specimens was taken for examination according to patient's complain, the growth of bacteria among cancerous patients could be easily detected from urine samples which constituted 37 (74%) of growths of all specimens after that sputum samples which constituted 6 (12%) of growths of all specimens, wound swab and stool samples came in the third degree which

Table 4. Distribution of clinical specimens according to the site of infection in cancer patients.

Clinical specimens	Frequency	Percentage (%)
Urine	37	74
Sputum	6	12
Wound swab	3	6
Stool	3	6
Blood	1	2
Total	50	100

Table 5. Distribution of clinical specimens according to the site of infection in non-cancerous patients.

Clinical samples	Frequency	Percentage (%)
Urine	38	76
Sputum	6	12
Wound swab	4	8
Blood	2	4
Total	50	100

Prevalence of *tuf*, *atpD1*, and *atpD2* genes among Gram-negative bacteria

Distribution and detection of virulence genes (*tuf*, *atpD1*, and *atpD2*) were distinguished among all isolates of GNB as taken from cancerous and non-cancerous patients, as mentioned in table (6). The results of agarose gel electrophoresis of *tuf* gene among cancer patients shown that 35 isolates (70%) had appeared amplicon with molecular weight 903 bp. *E. coli* represents high level among the other isolated species which is 23 (66%), otherwise *P. aeruginosa* and *Cedecea* shows low level that accounts 1 (3%) for each of them.

Also, the appearance of *tuf* gene for non-cancerous patients was 26 (52%). *E. coli* seems high level 11 (42%) and *A. baumannii* represents the lowest level 1 (4%), as shown in figure (1), (2).

The frequency and percentage of *atpD1* with molecular weight of 876 bp as in figure (3, 4), from cancer patients were 26 (52%), as well as the majority of cases among *E. coli* in which 17 (65%), while, *P. aeruginosa* and *Cedecea* have only one case (4%) for each of them. The appearance of *atpD1* among non-cancerous patients were 22 (44%) isolates. *E. coli* and *P. mirabilis* which account 7 (32%) of the positive isolates for each of them. and only one isolate of *P. aeruginosa* represents (4%) among positive samples.

Also, agarose gel electrophoresis of amplicon *atpD2* was 26 (52%). Most of them, 20 (77%) isolates belong to *E. coli* by appearance of amplicon with molecular weight 884 bp, and 6 (23%) isolates belong to other GNB. The results of identification of *atpD2* among patients without cancer was 24 (48%). The most frequently isolated bacteria for *atpD2* among non-cancerous patients were *E. coli* 8 (33%), followed by *P. mirabilis* which accounts 7 (32%), while *P. aeruginosa* accounts for the minority with 1 (4%), as represented in table (6) and figure (5, 6). Depending on the obtained results that we get, there is no significance differences in the prevalence of the virulence genes (*tuf*, *atpD1*, *atpD2*) between cancerous and non-cancerous patients (p -value = **0.9782**).

Table 6. Prevalence of *tuf*, *atpD1*, and *atpD2* genes among bacterial isolates in different cancerous patients and non-cancerous patients.

Virulence genes					
Types of sample	Bacterial isolates	<i>tuf</i> N (%)	<i>atpD1</i> N (%)	<i>atpD2</i> N (%)	<i>p</i> -value
Cancerous patients	<i>E. coli</i> (33)	23 (66)	17 (65)	20 (77)	0.9782
	<i>K. pneumoniae</i> (11)	8 (22)	7 (27)	4 (15)	
	<i>A. baumannii</i> (3)	2 (6)	0	0	
	<i>P. aeruginosa</i> (1)	1 (3)	1 (4)	1 (4)	
	<i>P. mirabilis</i> (1)	0	0	0	
	<i>Cedecea</i> (1)	1 (3)	1 (4)	1 (4)	
	Total (50)	35 (70)	26 (52)	26 (52)	
Non-cancerous patients	<i>E. coli</i> (17)	11 (42)	7 (32)	8 (33)	
	<i>K. pneumoniae</i> (10)	5 (19)	5 (23)	5 (21)	
	<i>A. baumannii</i> (5)	1 (4)	2 (9)	3 (13)	
	<i>P. aeruginosa</i> (6)	2 (8)	1 (4)	1 (4)	
	<i>P. mirabilis</i> (12)	7 (27)	7 (32)	7 (29)	
	Total (50)	26(52)	22(44)	24 (48)	

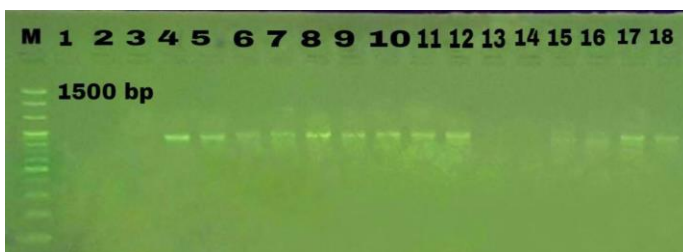


Figure 1: Agarose gel electrophoresis of *tuf* gene amplicon (903 bp) among cancerous cases. Lane M: DNA marker (100bp), Lane: 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17, and 18 positive results for amplification. Lane: 1, 2, 3, 13, and 14 negative results of amplification.

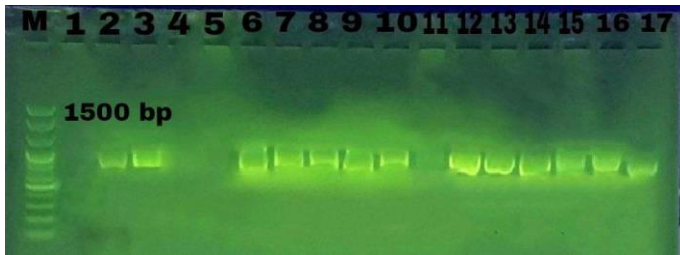


Figure 2: Illustrative gel electrophoresis of the PCR product for *tuf* gene among non-cancerous cases. **M:** DNA marker (100 bp), lane: 2, 3, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, and 17 represented *tuf* gene at 903 bp band. Lane: 1, 4, 5, and 11 were negative results of amplification.

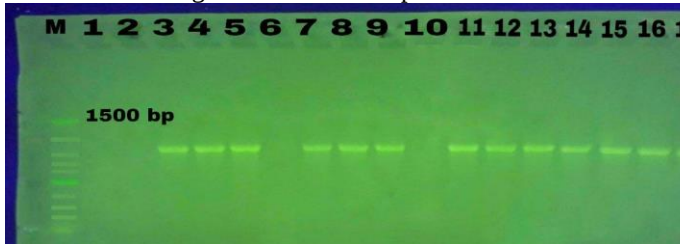
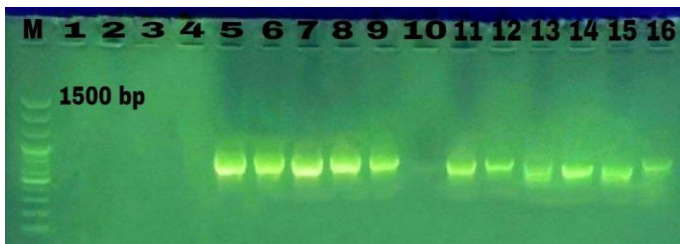


Figure 3: Agarose gel electrophoresis of *atpD1* (866 bp) amplicon amplified by PCR approach for cancerous patients. Lane **M:** DNA marker (100bp). Lane 3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, and 17 positive results. Lane, 1, 2, 6, and 10 were negative results.



Prevalence of *ms-1*, *ms-2*, and *ms-3* genes among Gram-negative bacteria

The genes *ms-1*, *ms-2*, *ms-3* were detected by PCR approach for 50 specimens from cancerous patients and 50 samples among non-cancerous patients, as shown in the table (7). The results appeared that 16 (32%) samples possess *ms-1* by the presence of amplicon with 684 bp among cancer cases, in which *E. coli* represents 13 (81%) isolates after that *K. pneumoniae* that 2 (13%) isolates, followed by *Cedecea* with 1 (6%) isolate, while, there have not any positive results for non-cancerous patients.

Also, the results of *ms-2* gene showed that 14 (28%) of isolates have *ms-2* gene with band size 498 bp from cancer patients, among them *E. coli* had 9 (64%), and *P. mirabilis*, *Cedecea* had 1 (7%) for each one. Whereas,

Figure 4: Agarose gel electrophoresis of *atpD1* (866 bp) amplicon for non-cancerous cases Lane **M:** DNA marker (100bp). Lane 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, and 16 positive results. Lane, 1, 2, 3, 4, 10 were negative results of amplification.

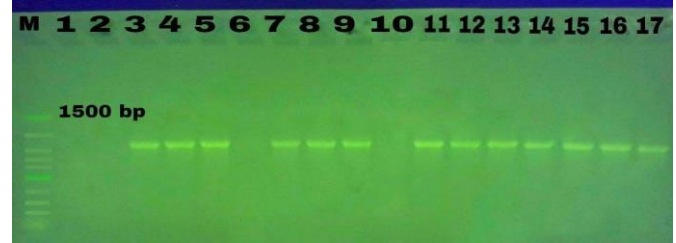


Figure 5: Agarose gel electrophoresis with PCR amplicons of the *atpD2* gene for cancerous cases. Lane **M:** DNA marker (100 bp); lanes: 3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, and 17: GNB *atpD2* (876 bp) positive isolates; lanes 1, 2, 6: negative for *atpD2* gene.

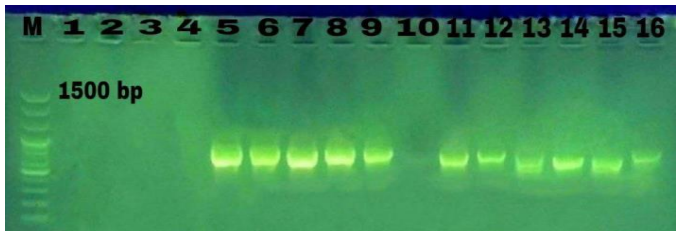


Figure 6: Agarose gel electrophoresis of *atpD2* (876 bp) amplicon for non-cancerous patients. Lane **M:** DNA marker (100bp). Lane 5, 6, 7, 8, 9, 11, 12, 13, 14, 15 and 16 positive results. Lane, 1, 2, 3, 4, and 10 were negative results of amplification.

the prevalence of *ms-2* gene marker among patients without cancer was 3 (6%), as mentioned in table (7) and figure (9, 10).

The distribution of *ms-3* virulence gene with band size 880 bp among bacterial isolates of cancer patients was 11 (22%), the majority of cases among *E. coli* with 8 (73%) isolates, and *K. pneumoniae*, *A. baumannii*, *P. mirabilis* have (9%) for each of them as in table (7) and figure (11). Otherwise, the prevalence from samples of non-cancerous cases was 10 (20%), *E. coli* and *K. pneumoniae* show 4 (40%) isolates for each one. After that, *P. mirabilis* represent 2 (20%), as mentioned in figure (12). based on the results that we obtained, there is no significance differences in the prevalence of the virulence genes (*ms-1*, *ms-2*, *ms-3*) between cancerous and non-cancerous patients ($p\text{-value} = 0.9621$).

Table 7. Prevalence of *ms-1*, *ms-2*, and *ms-3* genes among bacterial isolates in different cancerous patients and non-cancerous patients.

Types of samples	Bacterial isolates	Virulence genes			<i>p</i> -value
		<i>ms-1</i> N (%)	<i>ms-2</i> N (%)	<i>ms-3</i> N (%)	

Cancerous patients	<i>E. coli</i> (33)	13 (81)	9 (64)	8 (73)	0.9621
	<i>K. pneumoniae</i> (11)	2 (13)	3 (22)	1 (9)	
	<i>A. baumannii</i> (3)	0	0	1 (9)	
	<i>P. aeruginosa</i> (1)	0	0	0	
	<i>P. mirabilis</i> (1)	0	1 (7)	1 (9)	
	<i>Cedecea</i> (1)	1 (6)	1 (7)	0	
	Total (50)	16 (32)	14 (28)	11 (22)	
Non-cancerous patients	<i>E. coli</i> (17)	0	0	4 (40)	
	<i>K. pneumoniae</i> (10)	0	2 (67)	4 (40)	
	<i>A. baumannii</i> (5)	0	0	0	
	<i>P. aeruginosa</i> (6)	0	1 (33)	0	
	<i>P. mirabilis</i> (12)	0	0	2 (20)	
	Total (50)	0	3 (6)	10 (20)	



Figure 7: Agarose gel electrophoresis with PCR amplicons of *ms-1* gene among cancer patients. Lane **M** DNA marker (50 bp). Lanes 3, 4, 5, 6, 7, 8, 9, 11, 15, and 17: *ms-1* (684 bp) positive among cancer isolates, lane 1, 2, 10, 12, 13, 14, 16, and 18 were negatives.



Figure 8: The gel electrophoresis of *ms-1* gene. Lane **M** DNA marker (100 bp). Negative result for all *ms-1* gene among non-cancerous cases.

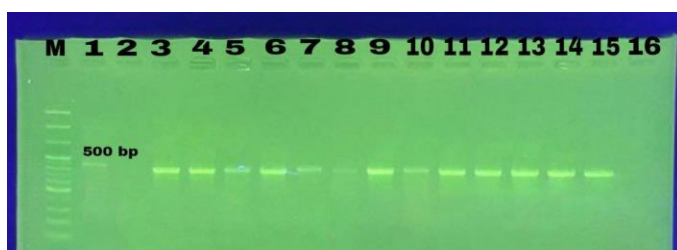


Figure 9: Agarose gel electrophoresis with PCR amplicons of the *ms-2* gene. Lane **M**: DNA marker (50 bp); lanes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15: *ms-2* (498 bp) positive isolates, lanes 2, and 16: negative in cancerous patients.



Figure 10: Agarose gel electrophoresis with PCR amplicons of the *ms-2* gene. Lane **M**: DNA marker (100 bp); lanes 2, and 5: *ms-2* (498 bp) positive isolates; lanes 1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16: negative in non-cancerous patients.

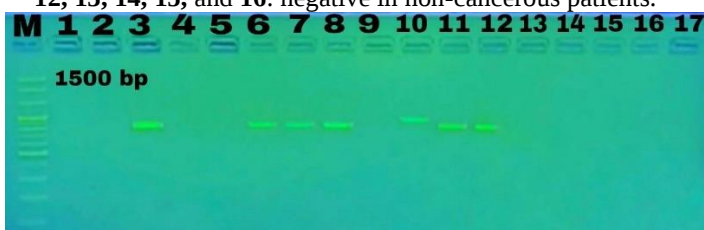


Figure 11: Electrophoresis in 1.2% agarose gel PCR products for the *ms-3* gene in clinical isolates. Lane **M**: molecular weight marker (100 bp); lanes 3, 6, 7, 8, 10, 11, and 12: positive for *ms-3* gene (880 bp); lanes 1, 2, 4, 5, 9, 13, 14, 15, 16, and 17 were negative results in cancerous patients.

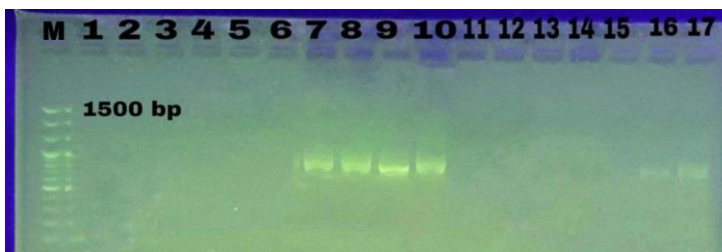


Figure 12: Electrophoresis in 1.2% agarose gel PCR products for the *ms-3* gene for non-cancerous isolates. Lane **M**: molecular weight marker (100 bp); lanes 7, 8, 9, 10, 16, and 17: positive for *ms-3* gene (880 bp); lanes 1, 2, 3, 4, 5, 6, 11, 12, 13, 14, and 15: negative.

DISCUSSION

Prevalence of *tuf*, *atpD1*, and *atpD2* among GNB

Elongation factor Tu is involved in peptide chain formation because *tuf* and *atpD* encode elongation factor Tu and the F-ATPase b-subunit, respectively. There are two copies of the *tuf* gene in enterobacteria. Elongation factor Tu and F-ATPase have been highly conserved and functionally stable throughout evolution. The phylogenies derived from the protein sequences of elongation factor Tu and the F-ATPase b-subunit agree well with each other and with the rRNA gene sequence data (Poey et al., 2006).

Most *Enterobacteriaceae* are opportunistic pathogens, such as *Escherichia coli*, *Klebsiella* spp., are associated with morbidity and mortality. These bacteria can cause different types of infections (Paterson, 2006). Drancourt et al. (2000) showed that *tuf* and *atpD* distances provide higher discriminating power at the species level. They found that the distribution of both *tuf* and *atpD* genes among *Enterobacteriaceae* family members was varied. The highest prevalence was among *E. coli*, followed by *K. pneumoniae*.

Prevalence of *ms-1*, *ms-2*, and *ms-3* genes among Gram-negative bacteria

The *ms-1*, *ms-2*, and *ms-3* marker genes were detected in 50 isolates. The results amplicons showed that 16 (32%) isolates possess *ms-1* by the appearance of the amplicon with band size 684 bp. In comparison, 14 (28%) isolate and 11 (22%) isolates possess *ms-2* and *ms-3* genes by the appearance of the amplicon with molecular size 498 bp and 880 bp, respectively. Jawad (2020) detect *ms-1*, *ms-2*, and *ms-3* marker genes among 60 different clinical isolates of gram-negative bacteria. The results of amplicons revealed that 10 (16.6%) isolates possess *ms-1* by the appearance of the amplicon with band size 684 bp. In comparison, 13 (21.6%) isolates and 11 (18.3%) isolates include *ms-2* and *ms-3* genes by the appearance of the amplicon with molecular sizes 498 bp and 880 bp, respectively. In addition, they detected the pattern of three genes distribution among gram-negative bacteria. The results of the *ms-1*, *ms-2*, and *ms-3* patterns showed that isolates possessed all three genes were 26 isolates (74.2%) even though eight isolates (22.8%) included both *ms-1* and *ms-2* genes; on the other hand, only 17 isolates (48.5%) and ten isolates (28.5%) possessed both *ms-1* and *ms-2*, both *ms-2* and *ms-3* respectively (Jawad, 2020). Shiga toxin-producing *E. coli* (STEC) causes a spectrum of human illnesses such as hemorrhagic colitis and hemolytic-uremic syndrome. The results of our study showed a high percentage of the distribution of *ms-1*, *ms-2*, and *ms-3* sequence markers for PAIEL3 among *Enterobacteriaceae*, which is related to the increased pathogenicity of these strains. Many results demonstrated a significantly higher prevalence of

PAII Cl₃ among virulent seropathotype strains than in non-virulent seropathotypes (Kaur et al., 2016). However, the LEE seems to confer enhanced virulence. LEE-negative STEC strains are also associated with severe human diseases (Girardeau et al., 2005).

CONCLUSION

In the recent study, we have arrived that *tuf*, *atpD1* and *atpD2* as a discriminated gene for detection of GNB especially *Enterobacteriaceae* family. There is a main distribution of *ms-1*, *ms-2* and *ms-3* among gram-negative bacteria with cancer patients.

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