Polytechnic Journal. 2022. 12(2): 92-99
ISSN: 2313-5727
http://journals.epu.edu.iq/index.php/polytechnic

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. Othman[1], Fattma A. Ali[2]

[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

Received: 2 June 2022
Accepted: 28 July 2022
Published: 1 February. 2023

DOI10.25156/ptj.v12n2y2022.pp92-99

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*, *atp*D1, *atp*D2, *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 *atp*D1 from cancer patients were (52%), as well as the appearance among non-cancerous patients were (44%) isolates. The results of *atp*D2 among cancer patients were (52%). However, the average of *atp*D2 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*, *atp*D1 and *atp*D2 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).

SUBJECTS AND METHODS

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*, *atp*D1, *atp*D2, *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.

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

Name of Genes	Primer	Amplicon Size	Reference
tuf	F- GGGACGCCAACTATGTT R- AACGGTACGGCCGCCTTC	903	(Jawad, 2020)
***atp*D1**	F- TGAGGAGGAAGCTCATGGCCG R- CGGCCCCCGAGACGA	866	
***atp*D2**	F- CGCAAGGCATTGAGGAGAAG R- CGGCCCCCGGAGACGA	876	
***ms*-1**	F- GTAATCCTCAACCGCACCAGGC R- TGATGCGTTACCACACT	684	
***ms*-2**	F- CCTGAAGTGACCGTGAAACAG R-TGTATTGTCTGCGCTTCCAG	498	
***ms*-3**	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)
tuf	95/3	35	95 °C/3min	55°C /1 min	72°C /1min	72/7
atpD1	95/3	35	95 °C /3min	50°C /1min	72°C /1min	72/7
atpD2	95/3	35	95 °C /3min	50°C /1min	72°C /1min	72/7
ms-1	95/2	30	95°C /30sec	58.4°C /30sec	72°C /30sec	72/5
ms-2	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
***ms*-3**	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

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 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).

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*, *atp*D1, and *atp*D2 genes among Gram-negative bacteria

Distribution and detection of virulence genes (*tuf*, *atp*D1, and *atp*D2) 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 *atp*D1 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 *atp*D1 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 *atp*D2 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 a*tp*D2 among patients without cancer was 24 (48%). The most frequently isolated bacteria for *atp*D2 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 (t*uf*, *atp*D1, *atp*D2) between cancerous and non-cancerous patients (*p*-value = **0.9782**).

Table 6. Prevalence of *tuf*, *atp*D1, and *atp*D2 genes among bacterial isolates in different cancerous patients and non-cancerous patients.

Virulence genes					
Types of sample	**Bacterial isolates**	***tuf*** **N (%)**	***atp*D1** **N (%)**	***atp*D2** **N (%)**	***p*-value**
Cancerous patients	*E. coli (33)*	23 (66)	17 (65)	20 (77)	**0.9782**
	K. pneumoniae (11)	8 (22)	7 (27)	4 (15)	
	A. baumannii (3)	2 (6)	0	0	
	P. aeruginosa (1)	1 (3)	1 (4)	1 (4)	
	P. mirabilis (1)	0	0	0	
	Cedecea (1)	1 (3)	1 (4)	1 (4)	
	Total (50)	**35 (70)**	**26 (52)**	**26 (52)**	
Non-cancerous patients	*E. coli (17)*	11 (42)	7 (32)	8 (33)	
	K. pneumoniae (10)	5 (19)	5 (23)	5 (21)	
	A. baumannii (5)	1 (4)	2 (9)	3 (13)	
	P. aeruginosa (6)	2 (8)	1 (4)	1 (4)	
	P. mirabilis (12)	7 (27)	7 (32)	7 (29)	
	Total (50)	**26(52)**	**22(44)**	**24 (48)**	

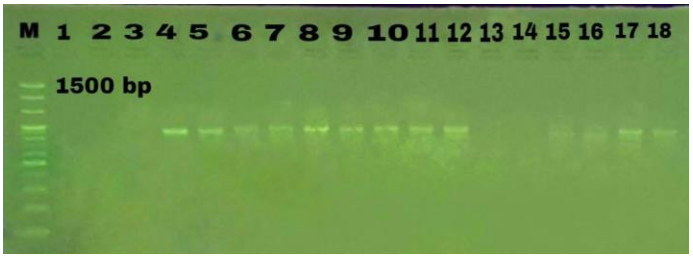

Figure 1: Agarose gel electrophoresis of *tuf* gene amplicon **(903 bp)** among cancerous cases. Lane **M**: DNA marker (100bp), Lane: **4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17,** and **18** positive results for amplification. Lane**: 1, 2, 3, 13,** and **14** negative results of amplification.

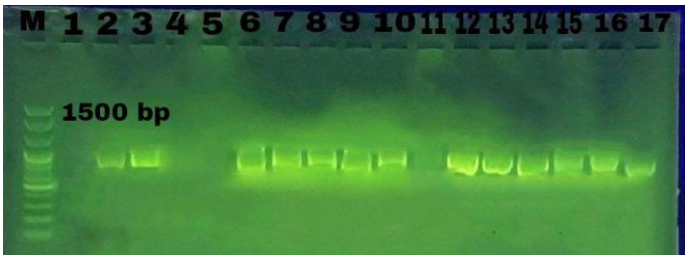

Figure 2: Illustrative gel electrophoresis of the PCR product for *tuf* gene among non-cancerous cases. **M**: DNA marker (100 bp), lane: **2, 3, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16,** and **17** represented *tuf* gene at **903 bp** band. Lane**: 1, 4, 5,** and **11** were negative results of amplification.

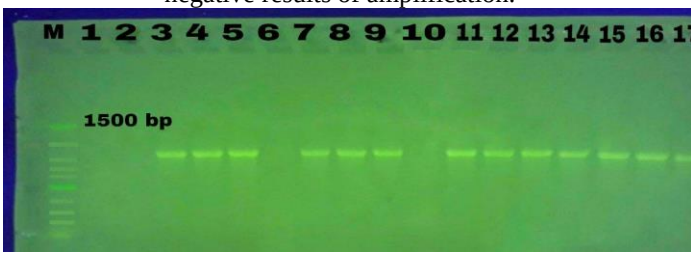

Figure 3: Agarose gel electrophoresis of *atp*D1 (**866 bp)** amplicon amplified by PCR approach for cancerous patients. Lane **M**: DNA marker (**100bp**). Lane **3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16,** and **17** positive results. Lane, **1, 2, 6,** and **10** were negative results.

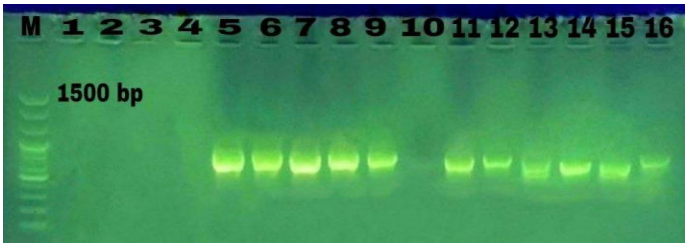

Figure 4: Agarose gel electrophoresis of *atp*D1 (**866 bp)** amplicon for non-cancerous cases Lane **M**: DNA marker **(100bp).** Lane **5, 6, 7, 8, 9, 11, 12, 13, 14, 15,** and **16** positive results. Lane, **1, 2, 3, 4, 10** were negative results of amplification.

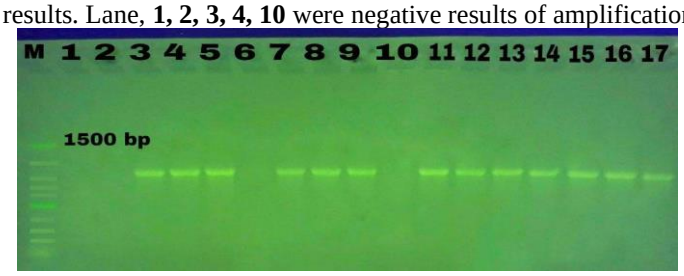

Figure 5: Agarose gel electrophoresis with PCR amplicons of the *atp*D2 gene for cancerous cases. Lane **M**: DNA marker (100 bp); lanes: **3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16,** and **17:** GNB *atp*D2 (**876 bp**) positive isolates; lanes **1, 2, 6:** negative for *atp*D2 gene.

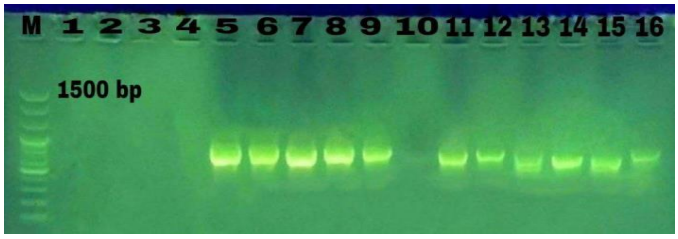

Figure 6: Agarose gel electrophoresis of *atp*D2 (**876 bp)** amplicon for non-cancerous patients. Lane **M**: DNA marker **(100bp).** Lane **5, 6, 7, 8, 9, 11, 12, 13, 14, 15** and **16** positive results. Lane**, 1, 2, 3, 4, a**nd **10** were negative results of amplification.

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, 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*-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.

Virulence genes					
Types of samples	**Bacterial isolates**	***ms*-1 N (%)**	***ms*-2 N (%)**	***ms*-3 N (%)**	***p*-value**

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

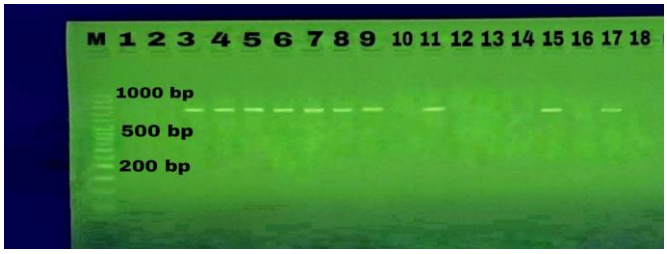

Figure 7: Agarose gel electrophoresis with PCR amplicons of *ms*-1 gene among cancer patients. Lane **M** DNA marker (**50 bp**). Lanes **3, 4, 5, 6, 7, 8, 9, 11, 15,** and **17**: *ms*-1 (**684 bp**) positive among cancer isolates, lane **1, 2, 10, 12, 13, 14, 16**, and **18** were negatives.

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.

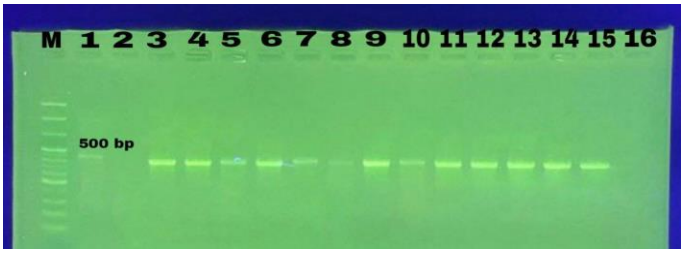

Figure 9: Agarose gel electrophoresis with PCR amplicons of the *ms*-2 gene. Lane **M**: DNA marker (50 bp); lanes **1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,** and **15**: *ms*-2 (**498 bp**) positive isolates, lanes **2,** and **16**: negative in cancerous patients.

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.

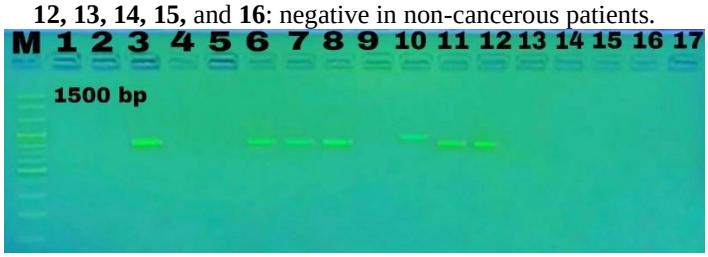

Figure 11: Electrophoresis in 1.2% agarose gel PCR products for the *ms*-3 gene in clinical isolates. Lane **M**: molecular weight marker (100 bp); lanes **3, 6, 7, 8, 10, 11,** and **12**: positive for *ms*-3 gene (**880 bp**); lanes **1, 2, 4, 5, 9, 13, 14, 15, 16,** and **17** were negative results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

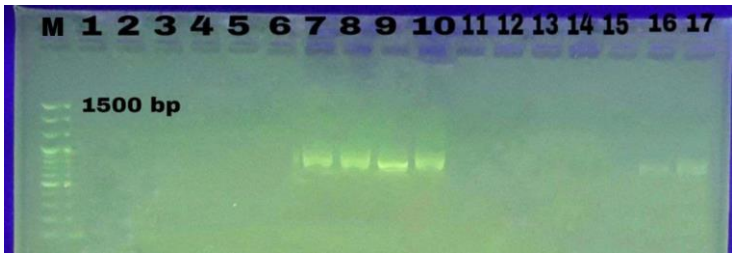

Figure 12: Electrophoresis in 1.2% agarose gel PCR products for the *ms*-3 gene for non-cancerous isolates. Lane **M**: molecular weight marker (100 bp); lanes **7, 8, 9, 10, 16, and 17**: positive for *ms*-3gene (**880 bp**); lanes **1, 2, 3, 4, 5, 6, 11, 12, 13, 14,** and **15**: negative.

DISCUSSION

Prevalence of *tuf*, *atp*D1, and *atp*D2 among GNB

Elongation factor Tu is involved in peptide chain formation because *tuf* and *atp*D 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 Cl_3 among virulent seropathotype shrines 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*, *atp*D1 and *atp*D2 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.

REFERENCES

AHMAD, H., MASROOR, T., PARMAR, S. A. 2021. Urinary tract infection by a rare pathogen *Cedecea neteri* in a pregnant female with Polyhydramnios: rare case report from UAE. BMC Infectious Diseases, 21**,** 1-6.

AKINOSOGLOU, K., PERPERIS, A., SIAGRIS, D., GOUTOU, P., SPILIOPOULOU, I., GOGOS, C. 2012. Bacteraemia due to *Cedecea davisae* in a patient with sigmoid colon cancer: a case report and brief review of the literature. Diagnostic Microbiology and Infectious Disease, 74**,** 303-306.

ASHREEN, S., AHMED, A., HASAN, N., 2019. Isolation and Identification of Gram-Negative Bacteria from Oral Cancer Site Infections and Study of Their Antibiotic Resistance Pattern. Bangladesh Journal of Microbiology, 36**,** 85-90.

CARICATO, A., MONTINI, L., BELLO, G., MICHETTI, V., MAVIGLIA, R., BOCCI, M. G., MERCURIO, G., 2009. Risk factors and outcome of *Acinetobacter baumanii* infection in severe trauma patients. Intensive Care Medicine, 35**,** 1964-1969.

CHAUDHURI, R. 2012. The evolution of the *Escherichia coli* phylogeny. Infection, Genetics and Evolution, 12**,** 214-226.

DAGA, A. P., KOGA, V. L., SONCINI, J. G. M., DE MATOS, C. M., PERUGINI, M. R. E., PELISSON, M., KOBAYASHI, R. 2019. *Escherichia coli* bloodstream infections in patients at a university hospital: virulence factors and clinical characteristics. Frontiers in Cellular and Infection Microbiology, 9**,** 191.

DRANCOURT, M., BOLLET, C., CARLIOZ, A., MARTELIN, R., GAYRAL, J.-P. & RAOULT, D. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. Journal of Clinical Microbiology, 38**,** 3623-3630.

FUKUTA, Y., MUDER, R. R., AGHA, M. E., CLARKE, L. G., WAGENER, M. M., HENSLER, A. M. 2013. Risk factors for acquisition of multidrug-resistant *Acinetobacter baumannii* among cancer patients. American Journal of Infection Control, 41**,** 1249-1252.

GARG, V. K., MISHRA, S., GUPTA, N., GARG, R., SACHIDANAND, B., VINOD, K., GAUTAM, H., KAPIL, A. & BHATNAGAR, S. 2019. Microbial and antibiotic susceptibility profile among isolates of clinical samples of cancer patients admitted in the intensive care unit at regional tertiary care cancer center: a retrospective observational study. Indian Journal of Critical Care Medicine: Peer-reviewed, Official Publication of Indian Society of Critical Care Medicine, 23**,** 67.

GIRARDEAU, J. P., DALMASSO, A., BERTIN, Y., DUCROT, C., BORD, S., LIVRELLI, V., etal, 2005. Association of virulence genotype with phylogenetic background in comparison to different seropathotypes of Shiga toxin-producing *Escherichia coli* isolates. Journal of Clinical Microbiology, 43**,** 6098-6107.

GONG, Z., SHI, X., BAI, F., HE, X., ZHANG, H., LI, Y., etal, 2019. Characterization of a Novel Diarrheagenic Strain of *Proteus mirabilis* Associated With Food Poisoning in China. Frontiers in Microbiology, 10.

JAWAD, S. 2020. Molecular Study of *Enterobacteriaceae* Associated With Colorectal Cancer A Theses Submitted to the Council of the Faculty Education for Girls / The University of Kufa, in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology. Kufa.

JOFFRE, E., NICKLASSON, M., ÁLVAREZ-CARRETERO, S., XIAO, X., SUN, L., NOOKAEW, I., etal, 2019. The bile salt glycocholate induces global changes in gene and protein expression and activates virulence in enterotoxigenic *Escherichia coli.* Scientific Reports, 9**,** 1-14.

KAUR, K., TARASSOVA, O., DANGETI, R. V., AZMI, S., WISHART, D., MCMULLEN, L. 2016. Characterization of a highly potent antimicrobial peptide microcin N from uropathogenic *Escherichia coli.* FEMS Microbiology Letters, 363**,** fnw095.

LITTLE, M. S., PELLOCK, S. J., WALTON, W. G., TRIPATHY, A. 2018. Structural basis for the regulation of β-glucuronidase expression by human gut *Enterobacteriaceae.* Proceedings of the National Academy of Sciences, 115**,** E152-E161.

LIU, Y., LIU, B., YANG, P., WANG, T., CHANG, Z., WANG, J., WANG, etal, 2019. LysR-type transcriptional regulator OvrB encoded in O island 9 drives enterohemorrhagic *Escherichia coli* O157: H7 virulence. Virulence, 10**,** 783-792.

MARKOU, P. 2014. Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer. Frontiers in Cellular and Infection Microbiology, 3.

MONTASSIER, E., BATARD, E., GASTINNE, T., POTEL, G. 2013. Recent changes in bacteremia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. European Journal of Clinical Microbiology & Infectious Diseases, 32**,** 841-850.

NIMNOI, P. 2020. Distribution and expression of virulence genes in potentially pathogenic bacteria isolated from seafood in Thailand. CyTA-Journal of Food, 18**,** 753-763.

NOWROUZIAN, F. L. 2012. *Escherichia coli* strains with the capacity for long-term persistence in the bowel microbiota carry the potentially genotoxic pks island. Microbial pathogenesis, 53**,** 180-182.

PARSA, N. 2012. Environmental factors inducing human cancers. Iranian Journal of Public Health, 41**,** 1.

PATERSON, D. L. 2006. Resistance in gram-negative bacteria: *Enterobacteriaceae*. American Journal of Infection Control, 34**,** S20-S28.

POEY, M. E., AZPIROZ, M. F. 2006. Comparative analysis of chromosome-encoded microcins. Antimicrobial Agents and Chemotherapy, 50**,** 1411-1418.

RAISCH, J., BUC, E., BONNET, M., SAUVANET, P., VAZEILLE, E., DE VALLÉE, A., etal, 2014. Colon cancer-associated B2 *Escherichia coli* colonize gut mucosa and promote cell proliferation. World Journal of Gastroenterology: WJG, 20**,** 6560.

RUDDON, R. W. 2007. Cancer biology, University of Michigan Medical School, Oxford University Press.

VUOTTO, F., BERTHON, C., LEMAITRE, N., DUHAMEL, A., BALKARAN, S., LE RAY, E., MICOL, J. B., FAURE, K. 2013. Risk factors, clinical features, and outcome of *Pseudomonas aeruginosa* bacteremia in patients with hematologic malignancies: a case-control study. American Journal of Infection Control, 41**,** 527-530.