MERIT RESEARCH JOURNALS
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Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 5(2) pp. 060-071, February, 2017
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Practical Genetic Study Confirming and Identifying the Horizontal Gene Transfer (HGT) of Bacterial DNA (*16S rDNA* Gene) Integrated in the DNA of Cancer Patients

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Abstract

Human DNA was extracted from 96 patients with hematological malignancies and from 26 healthy individuals, between 2014-2015. 72 (75%) bacterial *16S rDNA* genes were detected in the DNA of patients with hematological malignancies, but none were detected in the 26 healthy individuals. Thirty-one Gram-negative bacteria were responsible for the majority of bacterial DNA integrations (43.05%), 5 *Stenotrophomonas maltophilia* and 5 *Massilia timonae* were identified as more abundant (6.94% for each) followed by 4 *Methylobacterium lusitanum* (5.55%) and 2 *Methylobacterium rhodesianum* (2.77%). Only 12 gram-positive bacteria were identified (16.66%), 2 *Leuconostic lactis* and 2 *Bacillus endophyticus* (2.77% for each) were the most common species. However, the expression test (cDNA) showed no result for the integrated 50 *16S rDNA* genes compared with 50 positive for *IL-10-819* gene (100%). Interestingly, Random Amplified Polymorphic DNA for *Bacillus cereus* strains appeared only one case of cancer patient (1.38%) has the same strain integrated in the DNA and in his blood. This is the first practical study for detecting the bacterial DNA in the human DNA, Gram negative are the predominant bacteria including *Stenotrophomonas maltophilia*, *Massilia timonae* and *Methylobacterium lusitanum*. There was no expression of bacterial HGT in human.

Keywords: Bacteria, Cancer, HGT, Human, Integration

INTRODUCTION

Cancer is a growing health problem worldwide, the cause of many types of cancer is still ambiguous and the role of specific risk factors in certain cancers is unsolved across the world (Essa et al., 2007). Current dogma clarifies that cancer is a multigene, multistep disease arising from a solitary abnormal cell with an altered DNA sequence. Excessive proliferation of those abnormal cells is followed by a second mutation leading to a mild aberrant stage. Successive rounds of mutation and selective expansion of these cells results in the formation of a cancer mass (Hejmadi, 2010).

Many non-human eukaryotic chromosomes contain DNA of microbial origin that originated via horizontal gene transfer (Robinson et al., 2013). HGT is a one-way transfer of a limited amount of DNA from a donor cell to a single recipient cell (Nielsen and Daffonchio, 2010). HGT has been best studied for its capacity to transfer unprecedented genotypes between species (Riley et al., 2013). Since its discovery in the first half of the last century in archaea, prokaryotes and eukaryotes, moreover, the HGT can even occur among the three domains of biology (Atsmon-raz et al., 2015). HGT in human somatic cells will act as a mutagen, therefore, it will be important in bacteria-associated DNA damage ailments like cancer. Under this scenario, somatic HGT events would not allow adaptation to a new niche, but rather have the potential to be

disruptive to the normal gene function (Riley et al., 2013; Robinson et al., 2013).

Irrespective of extensive microbe-animal HGT in invertebrates, HGT from bacteria to humans and other mammals has rarely been described because the large size of microbial genomes made microbial integrations more difficult to detect prior to the widespread utility of whole genome sequencing, thus higher complexity of microbial genomes likely preclude identifying the integrations. Furthermore, sequencing reads that resemble bacterial DNA are sometimes removed from eukaryotic genome projects which further prevents identification of bacterial sequences in eukaryotic genomes (Robinson et al., 2013). Instead, the microbial contribution to carcinogenesis is generally thought to occur through increased inflammation leading to DNA damage and secretion of bacterial effector proteins like toxins (Chang and Parsonnet, 2010). However, only one theoretical study using sequencing data from The Cancer Genome Atlas (TCGA) by Riley et al. (2013) reported proof for integrations of Pseudomonas-like DNA into tumor suppressor and proto-oncogenes in stomach adenocarcinoma samples, and Acinetobacter-like DNA in acute myeloid leukemia samples. But it was not possible with this analysis to detect whether these integrations may be the cause of cancer or whether cancer cells may become more indulgent to mutations during carcinogenesis and thus receptive to HGT. Nonetheless, the clonal expansion of tumor cells containing HGTs facilitated this discovery and integrations of Pseudomonas-like DNA into proto-oncogenes may indicate direct carcinogenic potential (Robinson et al., 2013; Robinson and Dunning Hotopp, 2014). On the other hand, there are approximately 1014 more bacterial cells than human cells in the human body, therefore, somatic human cells can be bathed in bacteria and have the opportunity to be mutagenized by bacterial DNA via HGT. Such HGT will not become inherited by the progeny of the human, but may be proliferated over the individual's lifetime if the cell is able to undergo clonal expansion (Robinson et al., 2013).

The aim of this study was to investigate practically if the HGT from bacteria to the DNA of human somatic cells is associated with cancer compared with healthy individuals, identify the HGT by gene sequencing and screening if HGT is expressed. Moreover, is the bacteremia play a role as a donor for HGT?.

MATERIALS AND METHODS

Sample Collection

Ninety-six samples were obtained from patients with hematological malignancies including leukemia (n=63) and lymphoma (n=33) patients attended and /or admitted to The Center of Oncology and Hematology/Al-Saddar Educational Hospital in Basrah province from December 2014 to February 2015 who received several types of chemotherapy. Samples were obtained by informed consent of patients, with the permission of the center and Al-Basrah health directorate. In addition, 26 blood samples from healthy individuals were obtained for comparison. All samples were withdrawn under aseptic conditions using EDTA (Zhejiang Gongdong medical technology, China) tube and transported immediately to the laboratory.

DNA Extraction from Blood Samples

Pure genomic DNA was extracted from the blood samples of hematological malignancy patients and healthy individuals by *ExiPrep*™ 16 plus blood Genomic DNA kit Cat. No. K-4211 (Bioneer, Korea) using *ExiPrep*™ 16 plus automatic nucleic acid extraction instrument (Bioneer, Korea). DNA samples were electrophoresed in 0.8% agarose gel containing 1% ethidium bromide at 60V for 30 min. In an attempt to distinguish between bacterial and human DNA, a mixture of DNA aliquots from both sources was made and electrophoresed along with the separated bacterial and human DNA. This step was repeated for all blood samples.

Affirm the Blood DNA from Human by Amplification of *Interleukin 10-819* Gene

DNA isolated from the human blood samples was used as templates for PCR to amplify the *IL-10-819* gene according to Sun et al. (2010) by using the forward primer (5´-TCATTCTATGTGCTGGAGATGG-3´) and the reverse primer (5´-TGGGGGAAGTGGGTAAGA GT-3´). The PCR reaction mixture consisted of 5µl DNA template, 1µl forward primer (100 pmol), 1µl reverse primer (100pmol), 10µl *AccuPower*® PCR PreMix (Bioneer, Korea) and 23µl nuclease free water to bring the total volume to 40 µl. Amplification Parameters used in the PCR were as follows: denaturation at 94°Cfor 5min, 35 cycles each consisted of denaturation at 94 °C for 30s, annealing at 56.8 °C for 45s and extension at 72°C for 1 min followed by a final extension at 72°C for 10 min using Veriti ® thermal cycler (Applied Biosystem, USA). PCR product was separated on a 2 % agarose gel with 1% ethidium bromide. The *IL-10-819* gene bands (209bp) were visualized under UV trans-illuminator and photographed by LG camera.

16S rDNA Gene Amplification

The universal oligonucleotide primers 27F (5´-AGAGTTTGATCCTGGCTC AG-3´) and 1492R (5´-GGTTACCTTGTTACGACTT-3´) by Miyoshi et al. (2005) were used to look for the integrated bacterial *16S rDNA* gene in the human DNA. Gene amplification was

performed to a final volume of 50µl of reaction mixtures contained 1.5µl DNA template, 1.5µl forward primer (10pmol), 1.5µl reverse primer (10pmol), 11µl *AccuPower*® PCR PreMix (Bioneer, Korea) and 34.5µl nuclease-free water. The amplification conditions for PCR were as follows: initial denaturation at 92°C for 2 min followed by 30 cycles each consisted of denaturation at 94°C for 30s, annealing at 51.8 ° C for 45s and extension at 72°C for 1min and 30s, with a final extension at 72°C for 5 min using MyGenie ™ 96/384 thermal cycler (Bioneer, Korea). The success of the amplification of *16S rDNA* gene was ascertained by gel electrophoresis using a 2.0 % agarose gel containing 1% ethidium bromide The *16S rDNA* bands (1500bp) were visualized under UV trans-illuminator and photographed by LG camera.

16S rDNA Gene Sequences

AccuPrep® PCR and Gel Purification Kit Cat. No. K-3035 (Bioneer/Korea) were used to recover the band of interest (*16S rDNA* bands) from agarose gel prior to sequence following the manufacturer instructions. The purified PCR product was running out onto 2% agarose gel containing 1% ethidium bromide. Purified PCR products were sequenced at MACROGEN Co. /Korea.

Identification of Bacterial Species

16S rDNA gene sequence was identified using Basic Local Alignment Search Tool 'BLAST' to search for homologous sequences in the National Center for Biotechnology Information database (NCBI).

Investigation of the Expression of Bacterial Genes Integrated in Human DNA

RNA Extraction from Human Blood Samples

Total RNA was extracted from blood samples (n=50) of patients with hematological malignancies that had undergone Bacteria-human HGT using *AccuZol* ™ according to the manufacturer's instructions. RNA samples were electrophoresed in 0.8% agarose gel containing 1% ethidium bromide.

First-Strand cDNA Synthesis by Reverse Transcription

The isolated RNA was transcribed into cDNA using GoScript™ Reverse Transcription System kit Cat. No. A5000 (Promega/ USA) according to the manufacturer's instructions.

Amplification of *IL-10-819* Gene and the Integrated *16S rDNA* Gene by Conventional-PCR

PCR was carried out for both *16S rDNA* gene and *IL-10-819* gene. Negative control (Blank) was analyzed with every set of reaction mixtures to detect the unintended introduction of exogenous nucleic acids. The reaction mixtures and reaction steps for PCR were the same for *16S rDNA* gene amplification and *IL-10-819* gene amplification as previously described. PCR product was separated on a 2 % agarose gel with 1% ethidium bromide, visualized under UV trans-illuminator and photographed by LG camera.

Detection of Identical Bacterial Strains Using Random Amplified Polymorphic DNA (RAPD) – PCR

RAPD–PCR was carried out according to Ronimus et al. (1997). Strains pattern were accomplished among 19 bacteremial *Bacillus cereus* from cancer patients of previous related study of Abdul-Ridha and AbdAl-Abbas (2016) and a single bacterium DNA integrated into the DNA of patient case No.160. Using five primers each comprised of 10 nucleotides (Table 1). Reaction mixtures of PCR amplification for each primer were prepared in a volume of 20 µl consisting of 2.5µl of DNA templates, 1.5µl of primer (30pmol), 5 µl of *AccuPower*® PCR PreMix (Bioneer, Korea) and 11 µl of nuclease free water. PCR amplification was performed using the following conditions: initial denaturation at 94°C for 3 min and 45 s, 35 cycles including denaturation at 94°C for 15s, annealing at 36 ° C for 15s and extension at 72°C for 2 min followed by a final extension at 72°C for 4 min by Veriti ® thermal cycler (Applied Biosystem, USA). The RAPD patterns were detected by 2% agarose gel containing 1% ethidium bromide and photographed by LG camera. The amplification reactions were performed five times for each isolate/primer combination.

RAPD Data Analysis

Gel image analysis was performed using BioNumerics software v 7.6 (Applied Maths, Belgium). The digital images were inserted into the software and the bands were marked after standardization using a 100bp DNA ladder. The similarity was calculated using the number of different bands coefficient with an optimization of 1 % and a tolerance of 1 %. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) was used for clustering. In addition to the dendrograms obtained for each primer separately, a combined analysis was performed using the average from experiments. Then, the similarity matrices from each experiment were calculated first and from these matrices a combined matrix was calculated by averaging

Table 1. Primers used in RAPD-PCR

Primer	Primer Sequence 5´- 3´
1	TGCGGGTCCT
2	CACAGCTGCC
3	GGACGACAAG
4	GGACAACGAG
5	CTCTGCGCGT

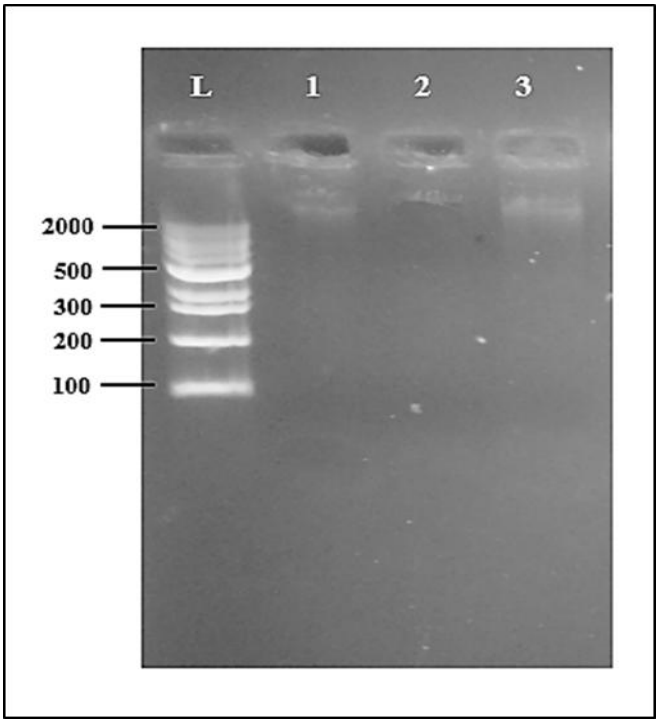


Figure 1. Agarose gel electrophoresis of human and bacterial DNA using a 0.8 % agarose gel containing 1% ethidium bromide. Lane L: 100bp molecular DNA ladder, Lane 1: a mixture of bacterial and human DNA bands, Lane 2: human DNA band and Lane 3: bacterial DNA band.

the values, giving each independent analysis the same equal weight (Towner *et al*., 2008).

Statistical Analyses

One-way ANOVA was performed to evaluate associations among the categorical data using IBM SPSS statistics 19 software, and $P \leq 0.05$ were considered statistically significant.

RESULTS

DNA from Human Blood Samples

Agarose gel electrophoresis analysis revealed a difference in size between the human and bacterial DNA which was sufficient to distinguish between the DNA from both sources (Figure 1).

Amplification of *Interleukin -10 -819* Gene

The amplicons of *IL-10-819* gene were detected in the DNA extracted from the 96 human blood samples (100%) but not in the 64 DNA samples extracted from the bacterial isolates (Figure 2). These results further asserted that the DNA extracted from the blood samples belong to human and not from bacterial source.

Integrations of *16S rDNA* Gene

Bacterial DNA fragments were detected in 72 (75%) of total 96 specimens of hematological malignancies

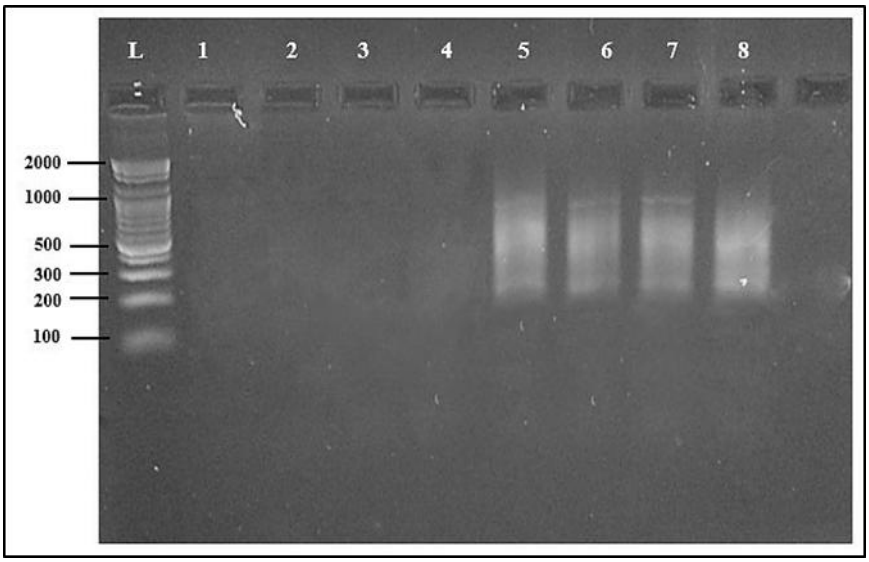


Figure 2. PCR assay of *IL-10-819* gene (209bp) using a 2 % agarose gel containing 1% ethidium bromide. Lane L: 100bp molecular weight DNA ladder, Lane 1-3: negative results of DNA from bacterial isolate, Lane 4: bacterial blank, Lane 5-8: positive results of DNA from human blood samples and Lane 9: human blank.

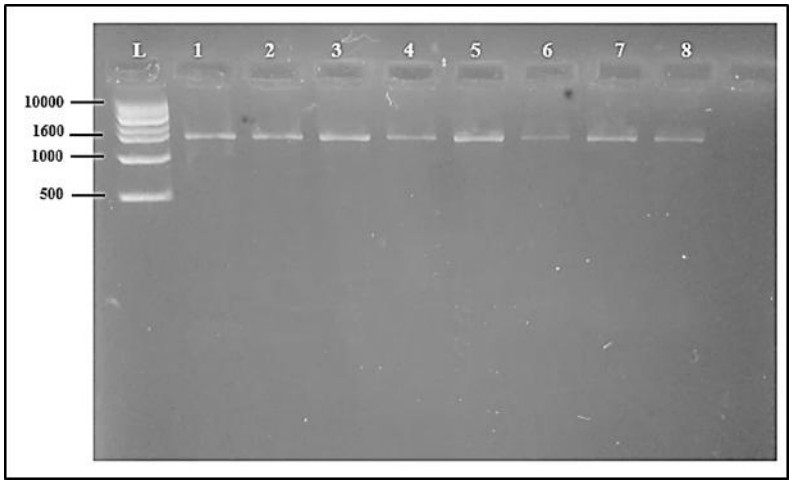


Figure 3. Bacterial *16S rDNA* gene band in the human blood DNA by agarose gel electrophoresis using a 2 % agarose gel containing 1% ethidium bromide: Lane L: 1kb molecular weight DNA ladder, Lane 1-8: bacterial *16S rDNA* gene bands in the blood DNA samples, Lane 9: Blank.

patients' genomes (Figure 3). None of the 26 healthy individuals had bacterial DNA within their genomes. These results indicate the occurrence of HGT from bacteria in humans. Moreover, statistical differences found that cancer patients had a significant rate ($P \leq 0.05$) of bacteria – human HGT compared with healthy individuals. Furthermore, there was no correlation between the date of hematological malignancy diagnosis and the rate of bacteria – human HGT (Table 2). However, 4-8 years after diagnosis with hematological malignancies appeared to be the best stage for identification of bacteria – human HGT.

Identification of the HGT

Only 43 of 72 isolates were successfully sequenced, aligned with BLAST and bacteria were identified to species level (Table 3). Twenty genera and 26 species have been identified. Gram-negative (n=31) bacteria were responsible for the majority of bacterial DNA integrations in the human DNA (43.05%) compared with Gram-positive (n=12) bacteria (16.66%). *Stenotrophomonas maltophilia* and *Massilia timonae* were the more frequent with 5 (6.94%) for each, followed by *Methylobacterium lusitanum*

Table 2. Date of diagnosis and its association with bacteria-human HGT in hematological malignancy patients

Characteristics		Patients with integrated bacterial DNA	Patients without integrated bacterial DNA
Date of diagnosis (years)	< 4	30 (41.66) [b]	11 (45.83)
	4-8	35 (48.61) [a]	13 (54.16)
	> 8	7 (9.72) [c]	0
Total		72 (75) [a]	24 (25)

$P \leq 0.05$.

Table 3. Bacterial species integrated into the human DNA

Bacterial species	Total n (%)	Hematological malignancy (%)	
		Leukemia	Lymphoma
Stenotrophomonas maltophilia	5 (6.94) [a]	4 (5.55)	1(1.38)
Stenotrophomonas pavanii	1 (1.38) [d]	1(1.38)	-
Stenotrophomonas sp.	1 (1.38) [d]	1(1.38)	-
Massilia timonae	5 (6.94) [a]	5 (6.94)	-
Massilia suwonensis	1 (1.38) [d]	1 (1.38)	-
Methylobacterium lusitanum	4 (5.55) [b]	2(2.77)	2(2.77)
Methylobacterium rhodesianum	2 (2.77) [c]	-	2(2.77)
Methylobacterium podarium	1 (1.38) [d]	1 (1.38)	-
Methylobacterium thiocyanatum	1 (1.38) [d]	-	1 (1.38)
Leuconostoc lactis	2 (2.77) [c]	2(2.77)	-
Thermithiobacillus tepidarius	1 (1.38) [d]	-	1 (1.38)
Acinetobacter calcaeceticus	1 (1.38) [d]	-	1 (1.38)
Acinetobacter sp.	1 (1.38) [d]	-	1 (1.38)
Naxibacter indica	1 (1.38) [d]	1 (1.38)	-
Nevskia ramosa	1 (1.38) [d]	1 (1.38)	-
Bacillus endophyticus	2(2.77) [c]	2(2.77)	-
Bacillus cereus	1 (1.38) [d]	1 (1.38)	-
Alcaligenes faecalis	1 (1.38) [d]	1 (1.38)	-
Marivirga tractuosa	1 (1.38) [d]	1 (1.38)	-
Thiothrix eikelboomii	1 (1.38) [d]	1 (1.38)	-
Lactobacillus paracasei	1 (1.38) [d]	1 (1.38)	--
Lactobacillus plantarum	1 (1.38) [d]	1 (1.38)	
Staphylococcus sp.	1 (1.38) [d]	-	1 (1.38)
Psychrobacter cibarius	1 (1.38) [d]	1 (1.38)	-
Clostridium algidixylanolyticum	1 (1.38) [d]	1 (1.38)	-
Tepidimonas taiwanesis	1 (1.38) [d]	1 (1.38)	-
Moorella humiferra	1 (1.38) [d]	1 (1.38)	-
Planococcus rifietoensis	1 (1.38) [d]	1 (1.38)	-
Flexibacter flexilis subsp. *pelliculosus*	1 (1.38) [d]	1 (1.38)	-
Other bacteria	29 (40.27)	20 (27.7)	9 (12.5)
Total number (%)	72 (100)	53 (73.61) [a]	19 (26.38) [b]

$P \leq 0.05$.

4 (5.55%), *Methylobacterium rhodesianum* 2 (2.77). Among the Gram-positive bacterial integrations, *Leuconostic lactis* and *Bacillus endophyticus* were the most common 2 (2.77%) for each. There was a significant correlation between the frequency of bacterial isolates integrated into human DNA at $P \leq 0.05$. Bacterial DNA integrations were more frequent in patients with leukemia than in lymphoma patients. Furthermore, the sequence results showed the only one integrated bacterial DNA into the patient's DNA No. 160 was similar in identification (*B. cereus*) to the bacteria isolated from the blood of the same patient "Previous study" 1 (1.38%) suggesting that the bacteria isolated from the blood may be the donor of the *16S rDNA* gene that integrated into the patient's DNA via HGT. Nevertheless, the species *Stenotrophomonas pavanii*, *Massilia suwonensis*, *Thermithiobacillus tepidarius*, *Naxibacter indica*, *Nevskia ramose*, *Bacillus endophyticus*, *Marivirga tractuosa*, *Thiothrix eikelboomii*,

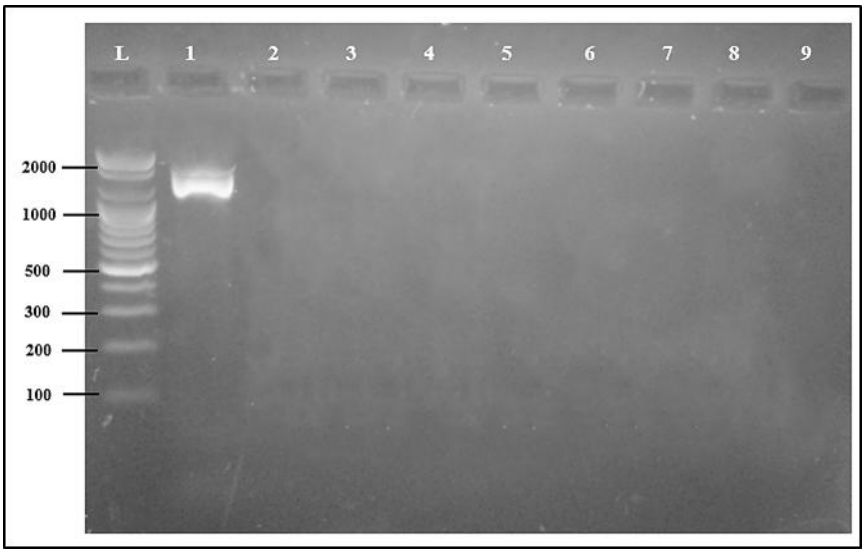


Figure 4. Agarose gel electrophoresis (2 %) containing 1% ethidium bromide for *16S rDNA* gene. Lane L: 100bp molecular weight DNA ladder, Lane 1: positive control (1500bp), Lane 2: negative control (Blank) and Lane 3-9: negative results of blood samples

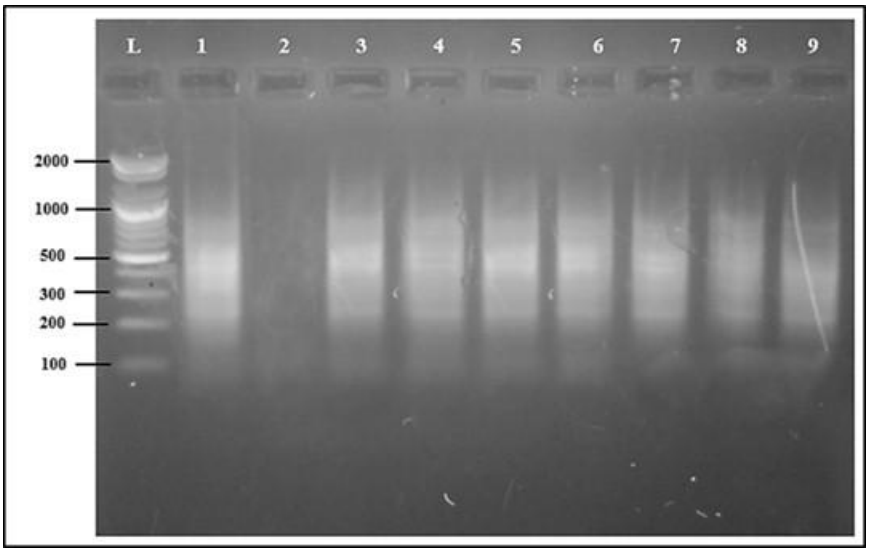


Figure 5. Agarose gel electrophoresis (2%) containing 1% ethidium bromide for *IL-10-819* gene. Lane L: 100bp molecular weight DNA ladder, Lane 1: positive control (209bp), Lane 2: negative control (Blank), Lane 3-9: positive results of blood samples.

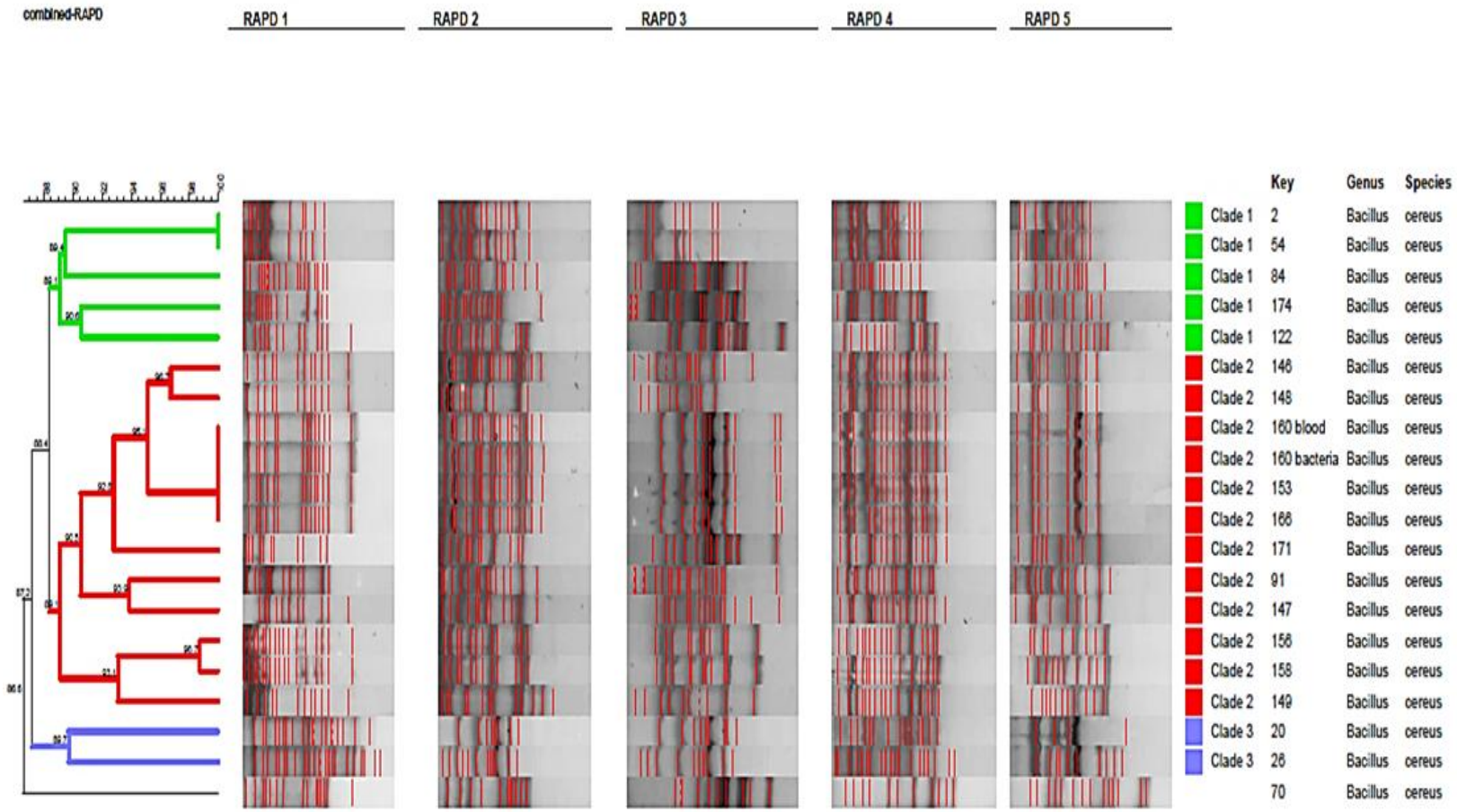
Psychrobacter cibarius, *Clostridium algidixylanolyticum*, *Tepidimonas taiwanesis*, *Moorella humiferra*, *Planococcus rifietoensis* and *Flexibacter flexilis* subsp. *pelliculosus* were isolated in the first time from human.

Expression of the HGT

There was no expression of the 50 integrated *16S rDNA* genes compared with positive for all 50 *IL-10-819* genes (100%) as Figure 4 and 5 show.

Detection of the Identical *Bacillus cereus* Strains

According to the dendrogram (Figure 6) in clade 2, the isolate that was found within the DNA of patient No.160 was identical to the bacteria isolated from the blood of the same patient with 100% similarity (Table 4).



:** Bacterial DNA integrated in patient's DNA No. 160. *:** Bacteria isolate from the blood of patient No. 160.

Figure 6. Dendrogram determined by RAPD-PCR fingerprints patterns of *Bacillus cereus* isolates recovered from the blood of cancer patients (Abdul-Ridha and Abd Al-Abbas,2016). The scale at the top of the figure shows the percentage similarity. The actual RAPD-PCR bands were given on the right of the dendrogram and the subsequent columns refer to data concerning the isolates.

Table 4. Similarity matrix between each isolate of *Bacillus cereus* from the blood of cancer patients and the bacterial DNA integration in patient No

strain	2	54	84	174	122	146	148	160Bl*	160Ba**	153	166	171	91	147	156	158	149	20	26	70
2	100																			
54	100	100																		
84	89.6	89.3	100																	
174	89.0	88.6	88.4	100																
122	90.1	88.5	89.8	90.6	100															
146	87.5	88.5	87.5	86.8	87.4	100														
148	90.1	90.6	88.6	86.4	88.8	96.7	100													
160Bl*	88.1	87.9	89.2	87.4	88.9	96.5	93.3	100												
160Ba**	88.2	87.9	88.8	85.6	88.0	96.8	93.7	100	100											
153	89.1	88.4	89.3	86.7	88.5	96.2	93.3	100	100	100										
166	88.7	88.1	89.4	88.4	89.2	96.6	94.6	100	100	100	100									
171	89.0	89.6	88.5	87.6	88.7	92.2	92.4	93.1	92.6	93.4	93.0	100								
91	89.8	89.3	87.8	88.1	88.3	90.1	88.7	88.8	88.8	88.8	88.6	89.6	100							
147	87.4	88.6	87.4	86.5	89.0	93.5	92.4	91.1	91.6	92.4	91.3	91.2	93.9	100						
156	88.6	88.5	88.7	87.7	91.7	89.1	89.6	88.4	88.4	88.9	88.9	89.7	88.5	89.5	100					
158	88.3	88.4	89.2	87.0	90.4	89.4	89.4	88.5	88.5	89.3	89.3	88.3	88.0	89.0	98.7	100				
149	87.0	87.5	89.0	88.1	88.1	91.8	92.3	89.4	88.8	89.1	89.5	89.2	86.4	88.2	93.6	92.5	100			
20	87.1	87.5	85.5	86.1	87.0	86.6	86.5	90.0	90.2	88.7	89.2	88.1	84.7	88.0	87.5	87.4	86.2	100		
26	87.2	86.8	85.5	85.3	87.9	86.8	87.4	87.2	86.9	86.7	88.6	85.2	86.0	85.9	87.8	87.7	87.9	89.7	100	
70	88.8	88.1	86.5	85.8	89.7	85.3	85.6	85.7	86.3	86.0	85.5	85.9	85.8	86.0	87.1	86.4	88.0	87.3	84.7	100

:** Bacterial DNA integrated in patient's DNA No. 160. *:** Bacteria isolate from the blood of patient No. 160.

DISCUSSION

Agarose gel electrophoresis was performed on a separate and/or a mixture run of bacterial and human DNA aliquot in order to gain a primitive discrimination between both sources. The results showed a difference in size between the extracted bacterial DNA and human DNA (Figure 1). Since, bacterial DNA like Escherichia coli DNA is of 1300µm in length, while the 46 chromosomes in human nucleus of 1.8m (Willey et al., 2008).

A comparison between the DNA extracted from the blood and that extracted from the bacterial isolates was made using IL-10-819 gene (as an indicator) amplifying all DNA from blood but not from bacteria (figure 2). This result confirmed that the origin of the DNA is from human only. IL-10 also known as human Cytokine Synthesis Inhibitory Factor "CSIF" (Oft, 2014). Its location on chromosome 1 and composed of five exons and four introns (Sun et al., 2010).

The majority of the 16S rDNA gene sequences was to identify the integrated DNA of the bacterial species in the human somatic DNA. But some nucleotides alignments were unable to analyze due to their poor quality, and the reason behind this, the occurrence of interference between the nucleotides during the integration process may affect the sequence quality, since the purification and sequencing were repeated twice for each

sample but showed the same result. So far, the only study that addressed this topic was conducted by Riley et al. (2013) using bioinformatics by computer only. They used publicly available sequence data from the Human Genome Projects, the 1000 Genomes Project and The Cancer Genome Atlas TCGA to examine bacterial DNA integration into the human somatic genome, particularly tumor genome. This revealed that bacterial DNA integrates into human somatic genomes more often in tumors than normal samples, since, Acinetobacter-like DNA in acute myeloid leukemia samples and Pseudomonas-like DNA in stomach adenocarcinoma samples were detected. The present study gave evidence on the occurrence of different bacterial DNA integrations into human somatic DNA in hematological malignancies samples. Twenty genera and twenty-six species have been identified. Gram-negative organisms were responsible for the majority of bacterial DNA integrations in the human DNA, especially, Stenotrophomonas maltophilia and Massilia timonae followed by Methylobacterium lusitanum. Since the 1980s, the occurrence of S. maltophilia hospital acquired infections has risen especially in immunocompromised patients. Most infection occurs 1- after a prolonged stay in critical care units. 2- subsequent to prolonged mechanical ventilation. 3- with exposure to broad spectrum antibiotics and 4- diarrhea (Safdar and Rolston 2007).

Massilia timonae was first suggested by La Scola et al. (1998) who described this new genus based on a fastidious, slowly growing bacterium isolated from a blood culture from a 25-year-old man with immunodeficiency. Therefore, the utilization of 16S rRNA sequence analysis has led to the identification of the second isolate of M. timonae from a surgical wound infection in an immunocompetent 36-year-old patient. Four additional strains of M. timonae were identified, all four strains were isolated from immunocompromised patients (Lindquist et al., 2003). The source of infection showed to be unclear in all former cases, however, there is a growing number of evidence that M. timonae is an environmental organism. Comparison of 16S rRNA gene sequences with those of closely related species observed that M. timonae is located within a cluster of soil-living bacteria (Van Craenenbroeck et al., 2011). Other Massilia strains have been isolated from air (Gallego et al., 2006) and drinking water (Weon et al., 2010) confirming the environmental nature of this organism.

Methylobacterium lusitanum, as well as, Methylobacterium rhodesianum, Methylobacterium thiocyanatum and Methylobacterium podarium were also identified. Similarly, Lai et al. (2011) described six patients' bacteremia caused by Methylobacterium species including M. thiocyanatum (2) and M. lusitanum (1). All patients had different underlying diseases. However, Methylobacterium spp. are opportunistic pathogens which have been continually isolated from tap water in hospitals, the capability of its tolerance to disinfecting agents, high temperatures, drying and to form biofilms may explicate the recurrent occurrence and colonization of Methylobacterium in the hospital environment (Kovaleva et al., 2014). Most Methylobacterium infections evolved in immunocompromised patients, such as patients with an organ transplant, HIV infection and renal failure (Lai et al., 2011).

Leuconostoc spp. are found in the environment such as on vegetables, in dairy and other fermented products, however, they are sometimes known to cause infection in humans. Following 1985, Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides and Leuconostoc lactis have been reported as BSIs, furthermore, these bacteria are also a cause of meningitis, endocarditis, pneumonia, osteomyelitis, pleural empyema and urinary tract infections (Deng et al., 2012).

Lactobacillus paracasei, L. plantarum, Alcaligenes faecalis and Acinetobacter calcoeceticus have been recognized as opportunistic pathogens causing serious infections such as bacteremia, meningitis in newborn, corneal ulcer, pancreatic abscess and respiratory infections (Salminen et al., 2004; Lai et al., 2012; Mordi et al., 2013; Lee et al., 2015). Nevertheless, A. calcoeceticus was also identified as being integrated into human DNA (Riley et al., 2013).

Importantly, the present study showed Bacillus cereus that caused bacteremia in the cancer patient No. 160 had integrated into the same patient's DNA via HGT.

The remaining 14 bacterial species that identified as being integrated into human DNA, were considered environmental organisms and widely distributed in different environments such as soil, waters and food products (Pladdies et al.,2004; Lee et al., 2012; Rajput et al., 2013). The presence of these bacteria in the bloodstream of cancer patients may be due to wound infection (e.g. surgery wounds) or the bacteria translocated from the gastrointestinal tract. The means by which bacterial translocation (BT) is thought to occur by two distinct routes of gastrointestinal permeability: (1) transcellular through the enterocytes and (2) paracellular utilizing tight junctions, there are also two major processes by which bacterial components gain access to systemic circulation: (1) through the enteric venous system to the portal vein and (2) following lymphatic drainage. BT have been discovered in a wide group of diseases such as malignancy, hemorrhagic shock, acute pancreatitis, cirrhosis, heart failure and abdominal surgery (Balzan et al., 2007). For instance, Cannon et al. (2005) found that one of the leading underlying conditions increasing patients' vulnerability to BT associated bacteremia was immunosuppression. Experimental and clinical studies have revealed both indigenous and non-indigenous bacteria within the mesenteric lymph node which is the primary pathway of translocation (Cummins and Tangney, 2013).

Pathogenic microorganisms can undergo lysis due to the host immune system or the antibiotic treatment of infections. Thomas and Nielsen (2005); Nielsen et al.

(2007) have been detected that between 95% and 100% of the bacterial DNA is liberated after contact with the immune system, the majority of this DNA is probably degraded by DNases that exist in human serum and plasma within a few minutes, however, several studies reported longer persistence times for chromosomal and plasmid DNA in serum, these studies indicate that bacterial DNA can persist in the blood long enough for transformation to occur, Therefore, DNA liberated by commensal and pathogenic bacteria which present in the intestinal tract can enter the bloodstream and reach various organs in mammals.

Robinson et al. (2013) hypothesized that the bacterial DNA integrations into the somatic cell had a potential to become transcribed and produce a protein which plays a crucial role in carcinogenesis, but no data has been presented to demonstrate this occurs. The present study sought to test this hypothesis and the results were demonstrated no expression for the integrated 16S rDNA in comparison with IL-10-819 gene. Thus, in turn, might refer to the integration of bacterial DNA is more likely to disrupt gene function than to result in expression in the new host. The integrity of the total RNA and the complementary DNA (cDNA) was checked by electrophoresis which showed only one visible band of cDNA. Since, blood can be stored in EDTA tube up to 30 days at -20C, however, this causes RNA to be partially degraded, thus reducing the full molecules length of cDNA produced (Rainen et al., 2002; Gallego Romero et al., 2014). Moreover, the very small amount of RNA resulting bands of cDNA is not easy to see, nevertheless, the successful subsequent amplification of IL-10-819 (positive indicator) was sufficient to confirm the presence of RNA and cDNA.

Interestingly, Bacillus cereus strain integrated into the patient's DNA No. 160 was same to that strain isolated from the blood of the same patient identified by Abdul-Ridha and Abd Al-Abbas (2016), This confirms that the Bacillus cereus strain isolated from the blood was the donor to the bacterial genes integrated into the human somatic DNA through HGT. Since, the two species were the same strain isolated from the same patient, it also suggested that different bacterial genes may be transferred to the human DNA because the RAPD-PCR is designed to screen out along all of the DNA. Although, the percentage of the conformity for bacteremia as the causative agent to integrated bacterial DNA was low (1.38 %) but this may be due to several reasons including 29 isolates underwent sequence were failed, the presence of other sources (not bacteremia) as a donor of bacterial DNA or the patients underwent previous bacteremia has been cured by antibiotic administration resulting the presence of bacterial DNA as an integrated case only.

CONCLUSION

Bacterial DNA integrations into the human somatic genome were detected more frequently in tumors than normal individuals. Most of leukemia patients have HGT from bacteria. Thus Bacterial DNA integration may be a mutagen associated with non-inherited genetic diseases, like cancer. Gram-negative bacteria were responsible for the majority of bacterial DNA integrations in the human DNA, particularly, *Stenotrophomonas maltophilia*, *Massilia timonae* and *Methylobacterium lusitanum*. However, the integrated *16S rDNA* gene was not expressed. Finally, the detection of strains reveals that bacteremia could be the source of bacterial DNA integrated into human somatic DNA.

ACKNOWLEDGEMENT

Authors are thankful to The Center of Oncology and Hematology staff in Al-Saddar Educational Hospital in Basrah province for sampling.

Conflicts of interest

There are no conflicts of interest.

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