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Clinical Characteristics, Etiology and Phylogenetic Distribution of Bacteremia in Patients with Malignancies in Basrah Province

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Abstract: This is the first study in Basrah province/Iraq to detect the geographical distribution of adult oncology patients with bacteremia and the bacterial etiology. From 176 patients' samples, the highest incidence of cancer cases was recorded in central 62 (35.22%) followed by western 45 (25.56%), northern 34 (19.31%), southern 24 (13.63%) and eastern 11 (6.25%) regions. The majority of cancer with bacteremic episodes (64) were located in the central 23 (35.93%), western 16 (25%) and southern 13 (20.31%) regions, while they were less likely to occur in the northern 7 (10.93%) and eastern 5 (7.81%) regions. Some clinical characteristics, including age (30-60 year), leukemia (64.06%), healthcare exposure (73.43%), neutropenia (71.87%), non-antibiotic administration (81.25%) and fever (100%) were associated with bacteremic episodes. Sixty-three (98.43%) of these episodes were caused by Gram-positive pathogens, especially 48 (75 %) *Bacillus* spp., these were 21 *B. licheniformis*, 19 *B. cereus*, 3 *B. subtillus*, 3 *B. sonorensis*, 1 *B. aeruius* and 1 *B. toyonensis*. On the other hand, 3 *Staphylococcus epidermidis*, 1 isolate of *S. warneri*, *Lysinibacillus sphaericus*, *Lysinibacillus xylanilyticus*, *Enterobacter cancerogenous* and 9 other of Gram-positive bacteria (unidentified). Exclusively, this is the first study isolated Gr-ve *Enterobacter cancerogenous* from cancer patients with bacteremia. Three isolates *Bacillus licheniformis* "IRQBAS18", *Bacillus licheniformis* "IRQBAS19" and *Bacillus licheniformis* "IRQBAS20" were reported as new global bacterial strains. RAPD analysis showed four patients from different regions, each two (9.5% for each) had the same strain of *Bacillus licheniformis* and other four (19.04%) patients from different regions had closely related strains of *Bacillus licheniformis*, these results confirm that the bacteremic infections were coming from the same source.

Keywords: Bacteremia, *Bacillus, licheniformis*, cancer, RAPD

Introduction

Cancer is a growing problem over the world, the etiology of many types of cancer is still ambiguous and the role of specific risk factors in certain cancers is unsolved with across the world, Iraq as whole and the southern region including Basrah , has been exposed to tremendous environmental damage as a result of wars, economic siege and lack of resources to protect or reinstate secure environment, as a consequence, the health condition of the population was under high risk of different diseases such as cancer (Essa *et al*., 2007). The latest statistics presented by Iraqi Cancer Board / Cancer Registry Center for the year 2011 recorded 1420 cancer cases in Basrah province outperforming the rest of Iraq's provinces cases (CSO, 2014).

Blood stream infection (BSI) remains a serious cause of grievous complications in patients receiving anticancer chemotherapy, by leading to delay and reduce dosages of chemotherapeutics, by causing longer hospitalizations, it participates in suboptimal treatment, higher mortality rate and incremented health care costs to cancer patients (Montassier *et al*., 2013). BSIs include bacteremia indicating the presence of bacteria in the blood (Gopal Katherason *et al*., 2010). The most common risk factors reported for bacteremia are: younger age at onset of bacteremia, absolute neutrophil count (ANC) 500 cells/µl, bone marrow status, intravascular catheters, high temperature and more immunosuppressive anticancer regimens (Al-Mulla *et al*., 2014).

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Patients with malignancies have been characterized by a variable spectrum of pathogens as a cause of BSIs in the last three decades, at the star of the 80s, Gram-negative bacteria were responsible for approximately two- thirds of the infections in most clinical centers, at the end of the 80s the trend was differed, Gram-negative pathogens accounted for one-third, in 90s and in the 21st century, Gram-positive bacteria have dominated. (Åttman *et al*., 2015). Especially, Coagulase-negative *Staphylococci* (CoNS), *Staphylococcus aureus*, viridans group *Streptococci*, β-hemolytic *Streptococci*, *Streptococcus pneumoniae* and various organisms that colonize the human skin, such as *Bacillus* species and *Corynebacterium* species (Wisplinghoff *et al*., 2003; Rolston *et al*., 2006). For bacterial identification, *16S rDNA* sequencing is important in the case of bacteria with uncommon phenotypic profiles, rare bacteria, slow growing bacteria, uncultivable bacteria, culture-negative infections and new bacterial strains (Woo *et al*., 2008; Abd Al-Abbas and Chmagh 2014). Molecular characterization techniques are now widely used for ecological and epidemiological analyses of a wide range of bacterial species such as Randomly amplified polymorphic DNA (RAPD) which is a PCR-based technique, using arbitrary primers to detect changes in the DNA sequence at sites in the genome (Maiti *et al*., 2009). This technique is fast and inexpensive, can detect DNA variations even at a single-base level, hence, it has become a commonly used technique for exploring intraspecies and interspecies diversity, thus, detecting the epidemiology of bacterial species (El-Hamshary *et al*., 2012).

The aim of this study was to determine the phylogenetic relatedness of bacteremia among cancer patients in Basrah regions at the strain level and their frequencies according to some clinical characteristics.

Materials and Methods

Sample collection

One-hundred and seventy-six samples were enrolled from patients with malignancies received various types of chemotherapy. There were two major types of malignancy: 1- hematological malignancy (102 samples) included leukemia (69) and lymphoma (33). 2- solid tumors (74) from breast, lung, osteosarcoma, prostate, ovary, gastrointestinal (pancreatic, stomach, colorectal and liver), bladder, uterus, kidney and cup cancers. The patients attended and /or admitted to The Center of Oncology and Hematology/Al-Saddar Educational Hospital in Basrah province from December 2013 to February 2014. Samples were gained by informed agreement of patients, with the permission of the center and Al-Basrah health directorate. All samples were withdrawn under aseptic conditions using EDTA tube and transported immediately to the Laboratory. All blood specimens were aseptically added to the sterilized tubes containing brain heart infusion broth (1:5 dilutions) and incubated aerobically at 37°C for 24h then subcultured on blood agar plates (Parikh *et al*., 2012). All isolates were Gram stained and undergone to next assays.

Genomic DNA extraction

Five ml of Brain Heart Infusion broth (BHIB) was inoculated with one tested bacterial colony and incubated at 37 °C for 18h. The grown bacteria were washed three times, in normal saline and centrifuged for 5 minutes at 13,000 x g (Japoni *et al*., 2004). Pure genomic DNA was extracted by *ExiPrep*™ 16 plus Genomic Bacterial DNA kit (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.

16S rDNA gene amplification

A molecular identification of the bacteria was performed by amplifying the *16S rDNA* using the universal oligonucleotide primers 27F (5´-AGAGTTTGATCC TGGCTCAG-3´) and 1492R (5´- GGTTACCTTGTTACGACTT-3´) according to Miyoshi *et al*. (2005). Gene amplification was performed in 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 by MyGenie ™ 96/384 thermal cycler (Bioneer, Korea). PCR product was separated on a 2 % agarose gel with 1% ethidium bromide, the *16S rDNA* bands (1500bp) were visualized under UV trans-illuminator and photographed by LG camera.

16S rDNA gene sequences

Polyethylene Glycol (PEG) Precipitation was used to clean up PCR products prior to sequence. Briefly, 60 µl of 30% polyethylene glycol $_{6000}$ (Applied Biosystem, USA) was added to 30 µl of PCR product (*16S rDNA* gene) and mixed by vortex (Fisher scientific, USA) then incubated at 4°C overnight after centrifugation (Eppendorf, Germany) at 1200 rpm for 20 min, the pelleted DNA was mixed with 0.5 ml of 70% chilled ethanol and centrifuged (this step repeated twice). The recentrifuged product was dried

in an eppendorf concentrator (Eppendorf, Germany) for 30 min. The pellet was dissolved in 30 µl of nuclease free water and left overnight at 4 ^{o}C, the purified PCR product was running out onto 2% agarose gel containing 1% ethidium bromide (Abd Al-Abbas and Chmagh, 2014). Purified PCR products were sequenced at MACROGEN Co. /Korea.

Identification of bacterial species

16S rDNA gene sequence was recorrected then analyzed using Basic Local Alignment Search Tool 'BLAST' to search for homologous sequences in the National Center for Biotechnology Information database (NCBI) http://www.blast.ncbi.nlm.nih.gov.

Detection of identical bacterial strains using Random Amplified Polymorphic DNA (RAPD) - PCR

RAPD – PCR was carried out according to Ronimus *et al*. (1997) for *Bacillus lechniformis* using five primers each comprised of 10 nucleotides (Table 1). Reaction mixtures of PCR amplification for each primer was prepared in a volume of 20 µl consisted 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 done using the following conditions: initial denaturation at 94^{o}C for 3 min and 45 s, 35 cycles include denaturation at 94^{o}C for 15s, annealing at 36 ^{o} C for 15s and extension at 72^{o}C for 2 min followed by a final extension at 72^{o}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.

Table 1: primers used in RAPD-PCR

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

RAPD –PCR 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 %, 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 was calculated first and from these matrices a combined matrix is calculated by average in the values, giving each independent analysis the same equal weight (Towner *et al*., 2008).

Statistical analyses

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

Results

Geographical distribution of bacteremic and non-bacteremic cancer patients

The distribution of 176 cancer cases in different geographical areas in Basrah province is illustrated in Table (2). The highest incidence was recorded in central regions 62 (35.22%) then in western (Al-Zubair district inclusive of Safwan and Um-Qasr) 45 (25.56%), Northern (Al-Hartha, Al Qurna and Al Medina) 34 (19.31%), southern (Abu Al-Khaseeb and Fao districts) 24 (13.63%) and eastern (Shatt Al-Arab district) 11(6.25%) regions, all at $P \leq 0.05$.

The majority of 64 bacteremic episodes was located in central, western and southern regions while they were less likely in eastern and northern regions (Table 3). The bacteremic episodes in central regions (35.93%) appeared that 7 (41.17%) of patients had a history of hospitalization, as for western regions, from 16 (25%) cases, 1 (5.88%) of patients had a history of hospitalization, in southern regions 13 (20.31 %), 4 (23.52%) of patients had a history of hospitalization, in Northern area 7 (10.93%), 2 (11.76) of patients had a history of hospitalization, in eastern regions 5(7.81 %), 3 (17.64%) of patients had a history of hospitalization. All those comparisons have a significant difference at $P \leq 0.05$, but between hospitalized and healthcare patients there was no significance in central and western regions. Generally, bacteremic episodes were more frequented in healthcare center patients' 47 (37.43%) compared with hospitalized cases 17 (26.56%).

Table 2: Geographical distribution of cancer cases in Basrah province

Regions	Total n **(%)**
Western (Al-Zubair district, Safwan and Um-Qasr)	45 (25.56) [b]
Southern (Abu Al-Khaseeb and Fao districts)	24 (13.63) [d]
Central	62 (35.22) [a]
Northern (Al-Hartha, Al Qurna and Al Medina)	34 (19.31) [c]
Eastern (Shatt Al-Arab district)	11 (6.25)
Total	176 (100)

The mean difference is significant at $P \leq 0.05$.
[a, b, c, d] differences from high to low

Table 3: Geographical distribution of cancer patients with bacteremia in Basrah province

Regions	Hospitalized patients' n (%)	Healthcare patients' n (%)	Total n (%)
Western (Al-Zubair district, Safwan and Um-Qasr)	1 (5.88) [d]	15 (31.91) [a]	16 (25) [b]
Southern (Abu Al-Khaseeb and Fao districts)	4 (23.52) [b]	9 (19.14) [b]	13 (20.31) [c]
Central	7 (41.17) [a]	16 (34.04) [a]	23 (35.93) [a]
Northern (Al-Hartha, Al Qurna and Al Medina)	2 (11.76) [b]	5 (10.63) [c]	7 (10.93%) [d]
Eastern (Shatt Al-Arab district)	3 (17.64) [b, c]	2 (4.25) [d]	5(7.81)
Total	17 (26.56)	47 (73.43)	64 (100)

The mean difference is significant at $P \leq 0.05$.
[a, b, c, d] differences from high to low.

Clinical characteristics of bacteremic and non-bacteremic cancer patients

Sixty-four (36.3%) bacteremic episodes in 176 cancer patients were detected, statistically significant difference for some factors between bacteremic and non bacteremic cancer patients was observed at $P \leq 0.05$ (Table 4). The age group 30-60 years was more frequent among bacteremic 38 (59.25%) and non bacteremic 68 (60.71) cancer patients than other age groups. The bacteremic episodes among leukemic patients was observed to be significantly higher 41 (64.06 %) followed by solid tumors 15 (23.43%) while the majority of non bacteremic cancer patients was for solid tumor 61 (54.46%). The correlation between neutropenia and bacteremia was positive 46 (71.87%). The episodes of bacteremia were significantly higher among healthcare center patients 47 (73.43%), similarly, the number of bacteremic cancer patients who did not have a history of antibiotic intake 52 (81.25%) were higher than those who had a history of antibiotic intake. There were no significant differences between bacteremic and non bacteremic cancer patients associated with age, sex, healthcare exposure, surgery, neutropenia and antibiotic administration.

Identification of bacterial species

16S rDNA Gene was amplified in all 64 bacteremic samples analyzed (Figure 1). Only fifty-five isolates were successfully sequenced, aligned with BLAST and bacteria were identified to species level (Table 5). Four genera and eleven species have been identified, Gram-positive bacteria were more common 63 (98.43%) than Gram-negative bacteria 1 (1.56%), the most common Gram-positive pathogen was 21 (32. 81%) *Bacillus licheniformis* followed by 19 (29.68%) *Bacillus cereus*, 3 (4.68%) *Bacillus subtilis*, 3 (4.68) *Bacillus sonorensis* and 3 (4.68%) *Staphylococcus epidermidis*. Moreover, 1 (1.56%) isolate of *Bacillus aerius*, *Bacillus toyonensis*, *S. warneri*, *Lysinibacillus sphaericus*, *Lysinibacillus xylanilyticus* and other 9 Gram-positive bacteria (unidentified) were also isolated. The Gram-negative pathogen was only found in 1 (1.56%) which identified as *Enterobacter cancerogenous*. There was a significant correlation between the frequency of bacterial species isolated from the blood of cancer patients at $P \leq 0.05$, furthermore, the bacteremic episodes was significantly higher in leukemia patients with predominant of *B. cereus* 14 (21.87%) compared with lymphoma

Table 4: Clinical characteristics of bacteremic and non-bacteremic cancer patients

Characteristics		Bacteremia patients' n (%)	Non-bacteremia patients' n (%)
Age (years)	< 30	11 (17.18) [c]	20 (17.85)
	30-60	38 (59.25) [a]	68 (60.71)
	> 60	15 (23.56) [b]	24 (21.42)
Sex	Male	33 (51.56)	51 (45.53)
	Female	31 (48.43)	61 (54.46)
Malignancy	Leukemia	41 (64.06) [a]	28 (25) [b]
	Lymphoma	8 (12.5) [c]	25 (22.32)
	Solid tumor	15 (23.43) [b]	61(54.46)
Healtcare center patients		47 (73.43) [a]	96 (85.71)
Surgery		25 (39.06)	20 (17.85)
Neutropenia		46 (71.87) [a]	44 (39.28)
Non-antibiotic administration		52 (81.25) [a]	97(86.6)
Fever		64 (100)	109 (97.32)
Total number		64 (36.3) [b]	112 (63.6) [a]

The mean difference is significant at $P \leq 0.05$.
[a, b, c] differences from high to low.

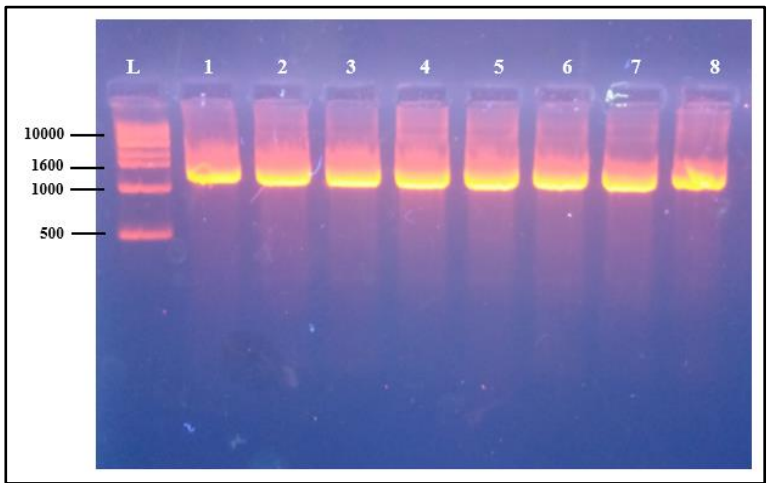

Figure 1: Amplification of *16S rDNA* gene form bacterial isolates showed by 2 % agarose gel containing ethidium bromide: Lane L: 1kb molecular weight DNA ladder, Lane 1-8: *16S rDNA* gene bands of bacterial isolates (1500bp).

and solid tumor in which *B. licheniformis* was the most common species 3 (4.68%) and 10 (15.62%) respectively.

Table 5: Bacterial species of bacteremia episodes in leukemia, lymphoma and solid tumor cases

Bacterial species	Total n (%)	Malignancy (%)		
		Leukemia	Lymphoma	Solid tumor
Bacillus licheniformis	21 (32.81) [a]	8 (12.5) [b]	3 (4.68) [a]	10 (15.62) [a]
Bacillus cereus	19 (29.68) [b]	14 (21.87) [a]	2 (3.12) [a]	3 (4.68) [b]
Bacillus subtilis	3 (4.68) [c]	2 (3.12) [c]	1 (1.56) [b]	-
Bacillus sonorensis	3 (4.68) [c]	1 (1.56) [d]	-	2 (3.12) [b]
Bacillus aeurius	1 (1.56) [c]	1 (1.56) [d]	-	-
Bacillus toyonensis	1 (1.56) [c]	1 (1.56) [d]	-	-
Staphylococcus epidermidis	3 (4.68) [c]	3 (4.68) [c]	-	-
Staphylococcus warneri	1 (1.56) [c]	1 (1.56) [d]	-	-
Lysinibacillus sphaericus	1 (1.56) [c]	1 (1.56) [d]	-	-
Lysinibacillus xylanilyticus	1 (1.56) [c]	1 (1.56) [d]	-	-
Enterobacter cancerogenus	1 (1.56) [c]	1 (1.56) [d]	-	-
Gram positive bacteria	9 (14.06)	7(10.93)	2(3.12)	-
Total number	64(100)	41 (64.06) [a]	8 (12.5) [c]	15 (23.43) [b]

The mean difference is significant at $P \leq 0.05$.
[a, b, c, d] differences from high to low

Identification of new bacterial strains

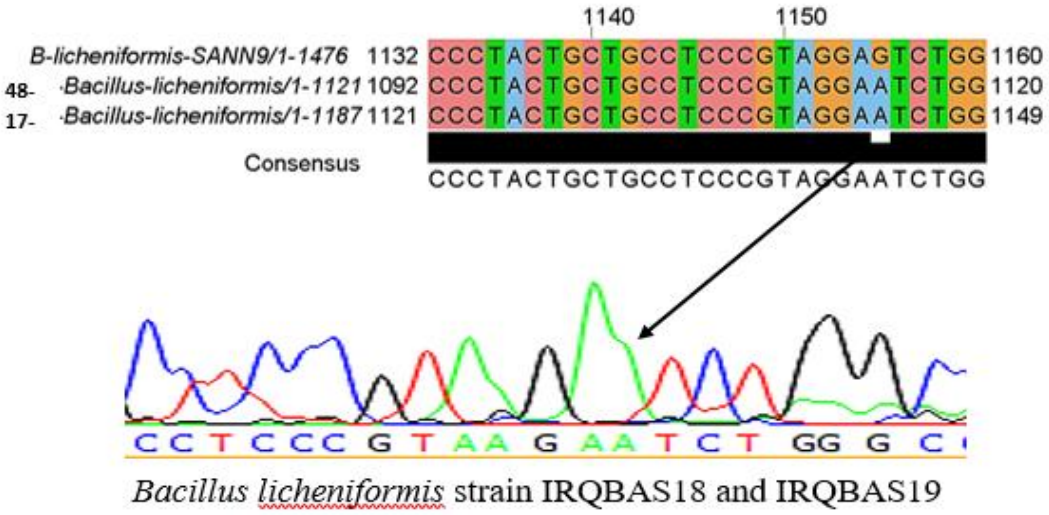

Figure 2: Comparison of *16S rDNA* nucleotide sequences to isolates No. 17 and 48 with their type strain SANN9 showing the transition point mutation (A instead of G) at 1155bp.

Three bacterial isolates were identified as global new strains which vary from their type strains in some nucleotide positions. These new bacterial isolates were recorded in the National Center for Biotechnology Information (NCBI) by European Nucleotide Archive (ENA) and GenBank for DNA sequences. First isolate No. 17 *Bacillus licheniformis* strain IRQBAS18 "LN871449"and the second No. 48 *Bacillus licheniformis* strain IRQBAS19 "LN871450" were closely related (99%) to *Bacillus licheniformis* strain SANN9 but with a transition point mutation (A instead of G) at 1155bp for both strains (Figure 2). The third bacterial isolate No.15 *Bacillus licheniformis* strain IRQBAS20 "LN871451" was closely related (99%) to *Bacillus licheniformis* strain SANN7 with a transversion point mutation (C instead of A) at 1043bp (Figure 3).

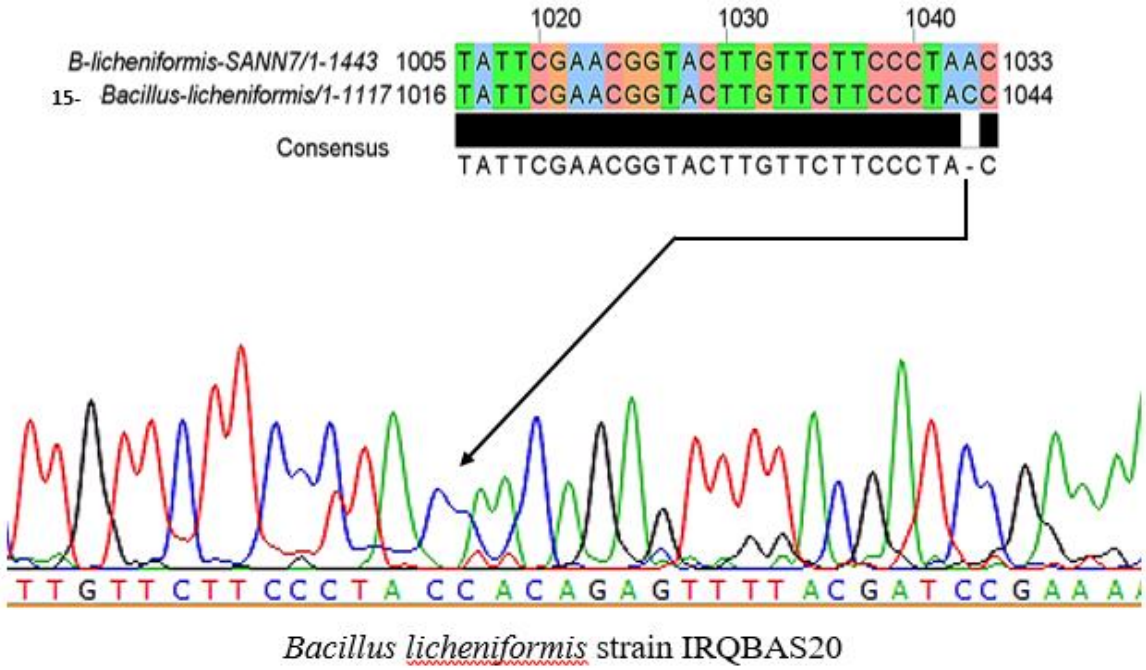

Figure 3: Comparison of *16S rDNA* nucleotide sequences to isolate No. 15 and its type strain SANN7 showing the transversion point mutation (C instead of A) at 1043bp.

Detection of identical *Bacillus licheniformis* strains using Random Amplified Polymorphic DNA (RAPD) - PCR

The RAPD technique was used for *Bacillus licheniformis* to generate strain - specific profiles for the tracing of those species among cancer patients. According to the dendrogram (Figure 4) *Bacillus licheniformis* was divided into five clades, four of 21 patients had identical strains. But, No. 1 from Abu Al-Khaseeb and No. 16 from Al-Hartha within clade 1 had the same strain of *Bacillus licheniformis* and No. 136 from Nathran and 161 from Al-Ddir within clade 4 had the other same strain of *Bacillus licheniformis*. However, the closely related strains were appeared in clade 1 which contained strain 18, 48 and 100 with $\geq$ 95.6% similarity (Table 6), clade 2 contained strains 1, 11, 16, 17, 63, 37 with $\geq$ 95.9% similarity and clade 3 contained strains 13, 15, 74, 77, 78, 112 and 123 with $\geq$ 94.4% similarity. Clade 5 contained strains 10 and 12 with $\geq$ 90.8% similarity which were considered to be relative.

Discussion

The highest incidence of cancer cases was in central regions followed by western, northern, southern and eastern regions (Table1). Similarly, Hussain (2015) studied cancer in Basrah during 2010-2012 and reported the highest incidence of cancer cases was in the center while the lowest incidence was in southern and eastern regions, but the cases were also higher in northern and western regions. In the present study, this graduation in cases according to regions were identical (in most regions) to that cases of bacterial infection disease, which may be due to number and their economic capacity of populations to diagnosis then registration those cases in the healthy center (Table 2). 73.43% of cancer patients with bacteremia had a history of healthcare exposure and 26.56% of patients had a history of hospitalization suggesting that bacteremia is a healthcare associated rather than hospital acquired. Bacteremia has been traditionally classified as either community acquired (CA) or hospital acquired (HA), however, a third category Healthcare-associated BSI (HCA) community onset has been recognized and identified by recent hospital admission or exposure to a significant medical care in a community or outpatient setting (Lenz *et al*., 2012). Community-onset bacteremia were categorized as being healthcare associated if one or more of the subsequent criteria were present: outpatient treatment, hemodialysis, intravenous chemotherapy during the past 30 days, hospitalization for at least 2 days during the past year, home intravenous therapy, wound care during the past 30 days or residence in a long-term care facility

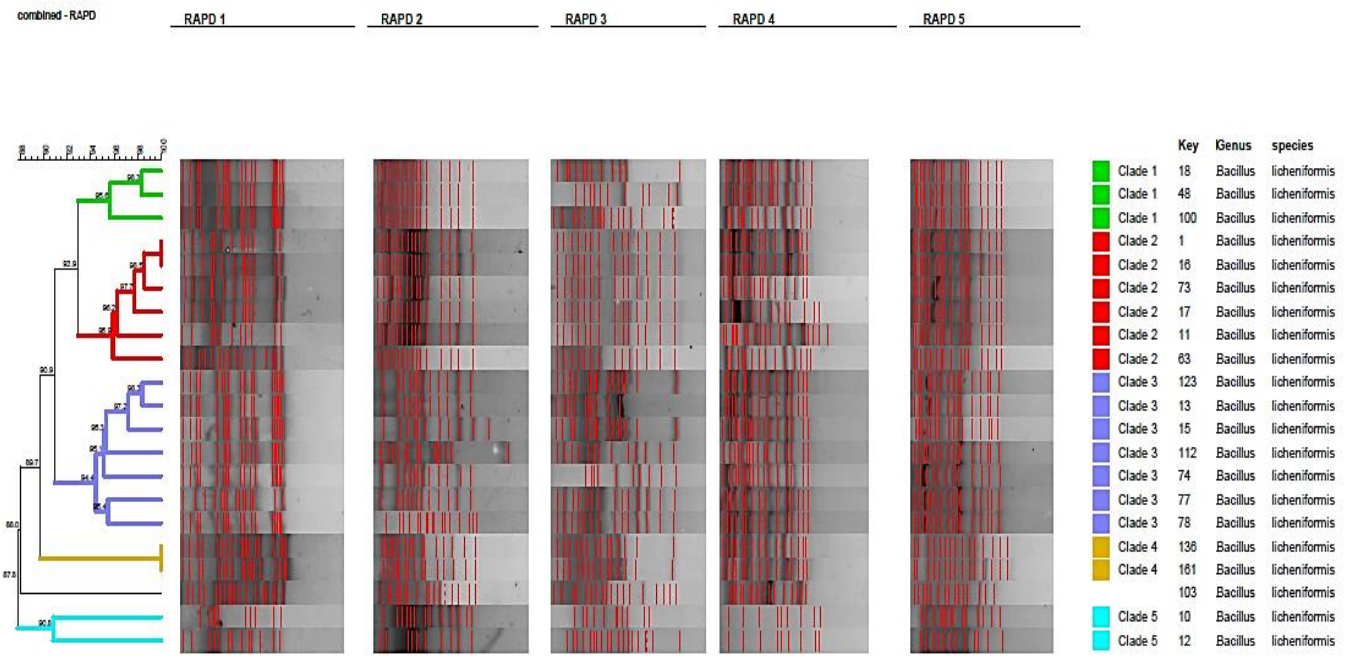

Figure 4: Dendrogram determined by RAPD-PCR fingerprints patterns of *Bacillus licheniformis* isolates recovered from the blood of cancer patients. The scale at the top of the figure shows the percentage similarity. The actual RAPD-PCR fingerprints were given on the right of the dendrogram and the subsequent columns refer to data concerning the isolates.

Table 6: Similarity matrix between each isolate of *Bacillus licheniformis* from the blood of cancer patients

Strain	18	48	100	1	16	73	17	11	63	123	13	15	112	74	77	78	136	161	103	10	12
18	100																				
48	98.3b	100																			
100	95.9b	95.4b	100																		
1	92.7	93.0	92.8	100																	
16	92.7	92.6	92.8	100a	100																
73	94.1	93.6	92.6	98.5b	98.5b	100															
17	92.6	92.5	91.9	97.9b	97.9b	97.3b	100														
11	92.6	91.8	90.9	95.7b	96.1b	96.5b	96.6b	100													
63	93.7	93.9	94.8b	96.3b	96.3b	96.5b	95.5b	94.6b	100												
123	92.5	91.0	92.2	92.0	91.8	90.0	89.7	88.1	91.2	100											
13	93.6	92.0	91.5	90.0	90.3	89.0	88.5	87.0	89.4	98.3b	100										
15	93.6	92.4	91.4	89.4	89.8	90.0	88.8	89.9	89.2	97.9b	96.4b	100									
112	91.5	90.6	91.3	90.9	91.0	89.6	88.7	89.5	89.9	94.9b	95.2b	95.7b	100								
74	92.0	92.8	92.0	89.7	89.2	90.2	89.3	89.4	89.3	94.7b	95.6b	95.2b	95.0b	100							
77	90.3	89.3	90.9	94.6	95.3	92.7	92.4	91.5	92.7	95.8b	95.2b	94.0	93.7	93.3b	100						
78	91.2	91.4	91.6	93.0	93.0	91.6	90.9	90.6	92.0	95.3b	94.9b	93.7	94.8b	93.1	95.4b	100					
136	90.0	90.0	89.7	90.8	90.0	88.3	87.8	87.1	88.6	91.1	91.1	89.9	90.2	90.3	90.3	90.0	100				
161	89.7	88.8	89.2	90.0	90.4	89.0	89.0	86.7	88.9	91.5	90.7	89.5	90.6	89.1	89.9	90.3	100a	100			
103	87.2	87.9	90.6	88.8	88.4	88.2	87.4	86.5	90.0	87.8	88.1	88.5	87.6	88.1	87.3	86.8	87.5	87.5	100		
10	88.3	88.4	88.9	89.1	89.4	88.1	89.9	88.5	89.3	87.7	88.3	86.4	86.8	87.7	88.5	87.8	87.5	87.0	86.4	100	
12	88.8	87.8	88.4	88.6	89.1	87.7	89.2	88.1	88.4	86.7	87.0	86.1	85.6	86.9	87.1	86.3	87.6	86.7	85.3	90.8c	100

a: Identical, b: Closely related, c: related

a: Identical, b: Closely related, c: related

(Marschall *et al*., 2009; Tissot *et al*., 2014). Nosocomial infections or healthcare associated infections in immunocompromised patients with cancer may be due to equipment and material used in the hospital or healthcare facility often become contaminated with bacterial strains that may be transferred to immunocompromised patients (Abdallah *et al*., 2008).Bacteremia is among the main causes of infections in cancer patients and had been correlated with decreased survival compared with cancer patients without bacteremia (Samonis *et al*., 2013) . This is the first study in Basrah province to detect the geographical distribution of adult oncology patients with bacteremia and the bacterial etiology. The number of patients with bacteremia was 64 (36.3%), which is within other reported values of 27-38%. (Pereira *et al*., 2013; Al-Mulla *et al*.,2014). Although, all age groups were statistically insignificant among bacteremic and non bacteremic cancer patients (Table 3) suggesting all age groups of cancer patients are prone to bacterial infections, but the bacteremic episodes were significantly higher 59.25% in the age group 30-60 years which reflects the high incidence of cancer cases among this age group, the results are in agreement with Hussain (2015). Furthermore, 51% of the patients involved in the episodes were males and 48% were female which fall within the reported range of 48-52% respectively (Kelly *et al*., 2010; Al-Mulla *et al*., 2014; Moghnieh *et al*., 2015). The bacteremic episodes among all leukemic patients was observed to be significantly higher (64.06%) compared with lymphomas and solid tumors. This is in agreement with the results of Asturias *et al*.(2010); Al-Mulla *et al*. (2014); Moghnieh *et al*. (2015), indicating that patients with hematological malignancies, in particular, leukemic patients, are more prone to BSIs. Patients with hematologic malignancies may have normal or even high neutrophil accounts, but the risk is in infection as a result of defects in neutrophil function including a significant decrease in phagocytosis, reduction in bactericidal and fungicidal activity, reduction in production of superoxide anions and defects in granulocyte movement (Nesher and Rolston 2014) . On the other hand, the percentage of solid tumor was significantly higher among non-bacteremic cancer patients, because in such patients profound neutropenia is generally short lived and neutrophil function is usually normal (Nesher and Rolston 2014). This also may suggest that bacteremia may be involved in the occurrence of leukemia while solid tumor resulted from different causes other than bacteremia. The presence of neutropenia was statistically insignificant among bacteremic and non bacteremic cancer patients, while 71.87% of bacteremic episodes were neutropenic and 28.12% of episodes did not have a neutropenia at the time of diagnosis. This is in consistent with results from other groups who found BSI in neutropenic and non-neutropenic cancer patients (Gupta *et al*., 2010; Samonis *et al*., 2013; Al-Mulla *et al*., 2014; Bartholomew *et al*., 2015). Regarding that, neutropenia makes cancer patients at higher risk for serious infections and complications, since, the relationship between fever, neutropenia and bacteremia has been well known for more than 40 years. (Gaur *et al*., 2001; Asturias *et al*., 2010). Bacteremic episodes among healthcare center's cancer patients as well as among those who did not have a history of antibiotic intake were high and the difference was not statistically significant among bacteremic and non bacteremic cancer patients suggesting that the occurrence of bacteremia may have been an indirect outcome of the greater immunosuppression in these patients. Moghnieh *et al*. (2015) reported no statistical relationship between bacteremia and hospitalization in cancer patients. Similarly, there was a negative association between submission to surgery and the occurrence of bacteremia among cancer patients suggesting the former factor was not a risk factor.

Four genera and eleven species have been identified, the Gram-positive organisms were found to be the most common organisms with 63 (98.43%). A similar trend has been documented in several reports including Ozkocaman *et al*. (2006) examined bacteremic isolates among hematologic malignancies patients throughout 2000–2005, 65.1% of bacteremic episodes were caused by Gram-positive pathogens. Moreover, a study involving cancer patients with febrile neutropenia adduced 62.7% of patients with Gram-positive bacteremia (Feld, 2008). Kjellander *et al*. (2012) examined the trends in the epidemiology of bacteremia occurred during chemotherapy-induced neutropenia in hematologic malignancy patients receiving no antibiotic from 2002 to 2008, the proportion of Gram-positive isolates was 53.1 %. Gram-positive bacteremias were mostly due to *Bacillus* species. *Bacillus licheniformis* alone outnumbered other species and accounted 32.81% of bacteremic episodes followed by *Bacillus cereus* (29. 68%) as Table (5). Several cases of *Bacillus* spp. bacteremia in cancer patients have been documented. Gaur *et al*. (2001) found twelve cases of *Bacillus cereus* septicemia in pediatric cancer patients. Ginsburg *et al*. (2003) reported a case of fatal *B. cereus* sepsis in a patient with newly diagnosed acute leukemia. In Ozkocaman *et al*. (2006) study, *Bacillus* bacteremias constituted 12 (3.4%) of the bacteremic episodes occurred among hematologic malignancy patients including 7 *Bacillus licheniformis*, 3 *Bacillus cereus* and 2 *Bacillus pumilus*. Chou *et al*. (2013) identified a *Bacillus cereus* septicemia in a 15-year-old patient with B-cell acute lymphoblastic leukemia. In a study conducted by Kalantar *et al*. (2014) in

patients with cancer, 20 % of bacteremia episodes were due to *Bacillus cereus*. For aforesaid reasons, *Bacillus* spp. isolated from the blood culture of patients with malignancies and fever should not be viewed routinely as a contaminant, but assessed as a potential pathogen (Ozkocaman *et al*., 2006). However, coagulase-negative *staphylococci* (CoNS) comprised 6.24% of bacteremic episodes in cancer patients and caused by *S. epidermidis* and *S. warneri*. Coagulase negative *staphylococci* (CoNS) are isolated most often from cancer patients, especially, *Staphylococcus epidermidis*, *S. haemolyticus* and *S. hominis*, these pathogens are of low virulence and rarely cause life-threatening infections even in intensely neutropenic patients, the BSI are the most usual infections caused by CoNS (Nesher and Rolston 2014). *Lysinibacillus* spp. were also identified in the present study which is usually regarded as contaminants if isolated in the laboratory foremost due to their ubiquitous nature and lack of pathogenicity, despite this, they have been known to cause severe infections in humans, furthermore, bacteremia has been the most usual presentation of systemic infections due to these species (Wenzler *et al*., 2015). The largest case report of *L. sphaericus* bacteremia represented 12 cases over a 10 year period at a children's cancer hospital in Italy (Castagnola *et al*., 2001). *Enterobacter cancerogenus* was also isolated and it is one of the new species identified (Garazzino *et al*., 2005). Several cases reported *E. cancerogenus* associated with human infections (Rubinstien *et al*., 1993; Abbott and Janda 1997; Tena *et al*., 2015). To date, no confirmed cases of bacteremia associated with *E. cancerogenus* in cancer patients have been reported.

Three isolates of *Bacillus licheniformis* No. 15,17 and 48 (1% difference in *16S rDNA* sequence) from the blood of cancer patients were reported as new strains, because the accumulative results from a several studies suggested a range of about a 0.5% to 1% difference (99.5 to 99% similarity) is often utilized for classification (Abd Al-Abbas, 2012). These new strains may be resulted from a mutation occurred during their transmission from one environment to another.

According to its number, the diversity of *Bacillus licheniformis* was further illustrated in their RAPD patterns. The present study recognized four strains each two were identical and isolated from two different patients (Figure 4). Interestingly, the four patients were from various regions in the province and all had a similar history of healthcare exposure or a history of hospitalization, the presence of similar strains within different patients could be due to the localization of the patients in the same wards of the hospital or healthcare facility but at different times which may indicate particular problems in these areas. Furthermore, 14 patients had closely related strains of *Bacillus licheniformis* with 94.4-98.3% similarity among them, these isolates might have evolved from the same origin (same strain), but, lost or acquired several nucleotides during the dissemination in the new environment. Furthermore, 2 patients had related strains of 90.8% similarity, since isolates that produced fingerprints with $\geq$ 80% similarity were considered as related (Hadi, 2015). However, with the exception of 100% for identical relatedness, there is no a precise percentage to detect the closely or related relationship among the organisms, because all these relatedness is depended on the number of organisms that using in the test.

Conclusion

The frequency of oncology patients with or without bacteremia was not equal among the regions of Basrah province. Bacteremial infections can consider as indicators for oncology treat especially leukemia, with interest by some clinical factors including age, healthcare exposure, non-antibiotic administration, neutropenia and fever. The pattern of bacteremia is tending towards Gram-positive organisms with a predominant of *Bacillus licheniformis* and *Bacillus cereus*. However, the isolation of Gr-ve *Enterobacter cancerogenus* from BSI of cancer patients is reported for the first time. Moreover, three new global bacterial strains from bacteremia were recorded. Finally, four cases (each two had the same strain of B. licheniformis) from different regions and another 16 cases from different patients had a closely related or related strains of *B. licheniformis*, these results confirm that they were either HCA or HA.

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