

## NEW REPORT OF SOME BACTERIAL SPECIES ISOLATED FROM HUMAN SPUTUM AMONG PATIENTS WITH RESPIRATORY TRACT INFECTIONS USING *16SRDNA* SEQUENCING

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### ABSTRACT

**Objective:** The objectives of the study were to identification of bacteria from sputum samples in patients suffering from respiratory tract infections (RTIs) and suspected with TB infection, by using *16SrDNA* sequencing.

**Methods:** The study was carried out to isolate and identify the common bacteria causing respiratory tract infections among 150 TB suspected patients attending to Advisory Clinic for Chest Diseases and Respiratory (ACCDR) in Basra city between March and December, 2013.

**Results:** During nucleotide sequences of *16SrDNA* gene thirteen (20%) of bacterial isolates were isolated from 150 sputum clinical specimens identical with reference strain these are *Sporosarcina aquimarina*, *Pseudomonas putida*, *Bacillus licheniformis*, *Lautropia mirabilis*, *Mycobacterium tuberculosis*, *Streptococcus pasteurianus*, *Sporomusa sphaeroides*, *Sphingomonas paucimobilis*, *Bacillus anthracis*, *Thermotalea metallivorans*, *Streptococcus oligofermentans*, *Enterococcus faecium*, *Bacillus subtilis*.

Our study showed *Sporosarcina aquimarina*, *Pseudomonas putida*, *Bacillus licheniformis*, *Streptococcus gallolyticus*, *Sphingomonas paucimobilis* *Enterococcus faecium*, *Bacillus subtilis*, *Streptococcus oligofermentans* were recorded that isolated in RTI at the first time in world and in Basra/Iraq patients.

**Conclusions:** isolated different bacterial species were recognized in the present study by using the PCR nucleotide sequences as the best test for diagnosing the bacterial species among the other assays.

**KEYWORDS:** Bacteria, Respiratory Tract Infections (RTI), TB, *16SrDNA*, Sequencing

### INTRODUCTION

Respiratory tract infections (RTIs) are some of the most important conditions affecting patients worldwide (Shah *et al.*, 2013). The human respiratory tract represents the major portal of entry for numerous airborne microorganisms, such as bacteria, fungi, or viruses (Delhaes *et al.*, 2012).

Tuberculosis (TB), a respiratory disease caused by *Mycobacterium tuberculosis*, It is one of the most widespread infectious diseases in the world. TB infected more than 1 billion persons annually, and More than two million people die each year (WHO, 2013). People with pulmonary TB bacteria can spread other bacteria, by talking, coughing, or sneezing, the spread of the pathogen through airborne particles that are inhaled by others (Botero *et al.*, 2014).

Sequence analysis of the 16S ribosomal RNA (rRNA) gene has been widely used to identify bacterial species and perform taxonomic studies (Petti *et al.*, 2005). Bacterial 16S rRNA genes generally contain nine “hypervariable regions” that demonstrate considerable sequence diversity among different bacterial species and can be used for species identification (Van de Peer *et al.*, 1996). Hyper variable regions are flanked by conserved stretches in most bacteria, enabling PCR amplification of target sequences using universal primers (Munson *et al.*, 2004). Numerous studies have identified 16S rRNA hyper variable region sequences that identify a single bacterial species or differentiate among a limited number of different species or genera (Stohr *et al.*, 2005).

## **MATERIALS AND METHODS**

### **Sample Collection**

This study includes 150 samples from patients admitted to the ACCDR, during nine months period (March and December, 2013). Samples of sputum were collected in sterile, screw-cap containers. The expectorated sputum was taken by asking the patient to cough deeply into the container, followed by immediate screwing on of the cap. Samples were transported to the laboratory within two hours and processed immediately or refrigerated at 4°C as soon as possible (SIREVA, 1998). All bacterial samples (n=30) were cultured on blood agar (Himedia) and incubated aerobically at 37°C and read within 12-24 h. Colonies grown after incubation were Gram stained and sub cultured onto Nutrient Agar (Talan *et al.*, 1989)

### **Genomic DNA Extraction**

DNA extraction was made according to the DNA PrepMat-M by the manufacturing company (Bioneer, Korea). The DNA was resuspended in 50 µl of Tris-EDTA buffer as stock. To check for DNA, the samples were loaded in 0.8% agarose gel 1 × TBE (54 g Tris-base, 0.5 M EDTA, 1- L distilled water, PH = 8, then diluted with 400 ml of distilled water) and electrophoresis at 60V for 30 min. (Sambrook and Russel, 2001).

### **Primers (16SrDNA Gene) and PCR Amplification**

PCR method for amplification universal 16SrDNA gene were accomplished according to (Miyoshiy *et al.*, 2005) by PCR sprint thermal cycler (Thermo, USA), used the primers: B27 Forward 5'AGAGTTTGATCCTGGC-3' and U1492 Reverse 5'-GGTTACCT TGTTACGACTT-3'. The 25µl reaction mixture was containing; 12.5µl of go taq green master mix (Promega), 1µM of each of upstream and downstream primer, 5µl of template DNA sample and nuclease free water to complete the volume to 25µl. The PCR program involved initial denaturation at 95°C for 5 min, 30 cycle (denaturation at 95°C for 30sec, annealing at 51.8°C for 45 sec and extension at 72°C for 1.5 min) and final extension at 72°C for 5 min. The amplified product was then electrophoresed in 1% of a garose gel then stained with 0.5 µg/mL ethidium bromide at 60V for 1.5 h and visualized in an ultraviolet transilluminator. The band of 1500 bp was indicative to 16SrDNA gene.

### **16SrDNA Gene Sequences**

#### **Sending to NICEM Company**

The 16SrDNA products sequencing and its preparation was according to NICEM (USA) Sample Concentration [45 ng/ul] and sample volume [15 ul].

#### **Identification of Bacteria**

All bacterial species were identified (using the 16SrDNA sequencing products) in " BLAST " provided by the

National Center for Biotechnology Information Service (NCBI) <http://www.ncbi.nlm.nih.gov> after treatment and recorection (Kerbaui *et al.*, 2011)

## RESULTS

Of the 150 specimens, 30 specimens was extracted and examined by agarose gel electrophoresis and showed PCR products for the universal *16SrDNA* primers gave sharp bands on agarose gel at the position 1500bp when compared with standard molecular Figure 1 and Figure 2

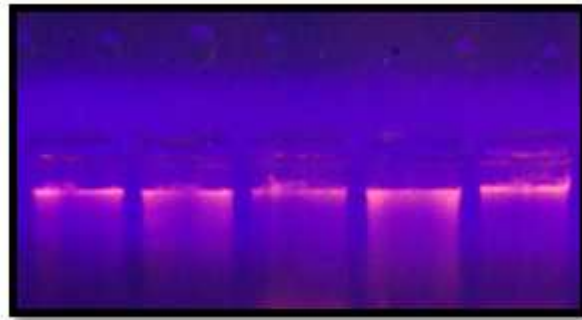


Figure 1: Agarose (0.8%) Gel Electrophoresis for DNA Bands from Bacterial Isolates under UV Transilluminator

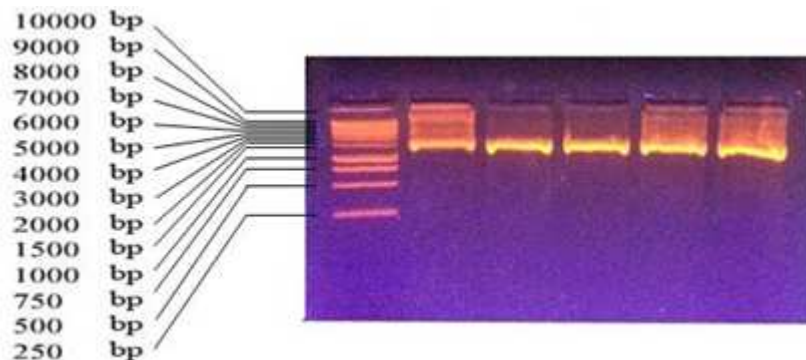


Figure 2: Agarose (0.8%) Gel Electrophoresis of Universal *16SrDNA* PCR Products for Bacterial Isolates under UV Transilluminator. Lane 1: (M): 1Kb (250 bp – 10000bp) DNA Ladder. Lane 2-6: (1to5): *16SrDNA* Bands (1500bp) for Bacterial Isolates

### Sequencing for Universal *16S rDNA* Gene

Out of 30 (20%) alignments *16SrDNA* gene sequences for all isolates, (n=13) species of bacterial isolates were identified (comparing with identical reference strain) from sputum: 1(3.3%) *Sporosarcina aquimarina*, 1(3.3%) *Pseudomonas putida*, 2(6.7%) *Bacillus licheniformis*, 1(3.3%) *Lautropia mirabilis*, 1(3.3%) *Mycobacterium tuberculosis*, 1(3.3%) *Streptococcus gallolyticus*, 2(6.7%) *Sporomusa sphaeroides*, 1 (3.3%) *Sphingomonas paucimobilis*, 1(3.3%) *Bacillus anthracis*, 1(3.3%) *Thermotalea metallivorans*, 1(3.3%) *Streptococcus oligofermentans*, 1(3.3%) *Enterococcus faecium*, 5(16.7%) *Bacillus subtilis*. other isolates (n=17) were fail sequencing but recognize only by morphological and gram's stain. As summarized in table 1.

**Table 1: Showed Nucleotide Sequencing of Bacterial Species Identical with Reference Strain**

Bacterial Species in	Nucleotide Sequence	Identical with Strain
<i>Sporomusa sphaeroides</i>	ATCGTCCACTTGGAGGCTGGCTCCTTTTAGGTTACCCACCGGCTTCGGGTGTT TCCAACCTTTCGTGGTGTGACGGGCGGTGTGTACAAGGCCGGGAACGTATTCA CCGCAGCATGCTGATCTGCGATTACTAGCGATTCCGACTTCACGCAGGCGAGT TGCAGCCTGCGATCCGAAGTGAAGCTTGTTTTTGGGGTTCGCTCCACCTCGCG GTCTTGCTTCCCTCTATTAAGCCATTGTAGTACGTGTGTAGCCAGGACATA AGGGGCATGATGACTTGACGTCATCCCCGCCTTCTCCGATTGTCTGCGGCAG TCTCCCATGAGTTCCCGACTTACTCGCTGGCAACATAGGATAAGGGTTGCGCT CGTTGCGGGACTTAACCCAACATCTCAGGACACGAGCTGACGACAGCCATGCA CCACCTGTTTTGTGTCTCCCGAAGGAGAGGCTCTAATCTTTAGACTTCACT CAATGTCAAGTCTTGTAAGGTTCTTCGCGTTGCGTCAATTAACACATACT CCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGCCGT ACTCCCCAGGCGGGTACTTATTGCGTTAACTCCGGCACAGAAGGGGTGCGATA CCGTACACCTAGTACCCATCGTTTACGGCCAGGATTACCGGGGTATCTAATC CCCTTCACTTCCCTGGCTTTCGCGCCTCAGCGTCAGACACCGTCCAGAAAGTCG CCTTCGCCACTGGTGTCTCCCAATATCTACGATTTACCCTACACTGGGA ATTCCACTTTCCTCTCCGGTACTCAAGATAAACAGTTTCCATTCATCACGGGG TTGAGCCCCGCACTTTTAAACAGACTTATCTACCGCCTACGCGCTTTTACG CCCAATAATTCCCGGACAACGCTCGCCACCTACGTATTACCGCGGCTGCTGGC ACGTAGTTAGCCGTGGCTTCTTCTTAGTACCGTCATATGACTCATTATTCA CAAAGTCACACATTCGCTTAGTGACAGAGCTTTACAATCCGAAGAACCTTCT CACTACGCGGGCTTGTCTCCGTCAGACTTTCGTTTATTGCGAGATTCCCCTGC TGCCTCCCGTAGAGTCTGGCGTTTCTCAGTCCCGGTGTGGCGATCATTCTTAC GACCGCCTAACGGATCGTGCCTGGTGAGCTTTACCTCTCACTACCTATCAGACG CGAAAACCTATCTCTCAAAG	DSM 2875
<i>Sphingomonas paucimobilis</i>	ACCCGGGGAACCCCGTGGTGCCTGCTTCTATTGCTAGGTTGGCGCAACGCCT TCGGGTGAATCCAACCTCCATGGTGTGACGGGCGGTGTGTACAAGGCCCTGGGA ACGTATTCACCGCGCATGCTGATCCGCGATTACTAGCGATTCCGCTTTCATGC TCTCGAGTTGCAGAGAACAATCCGAAGTGAAGCAACTTTTGGGGATTAGCTCG CCCTCGCAGGGTGCCTGCCCAGTGTAGTTGCCATTGTAGCACGTGTGTAGCCCA GCGCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCTCCGGCTTATCA CCGCGGTTCCCTTAGAGTACCAACCAAAATGATGGTAACTAAAGGCGAGGT TGCCTCGTTGCGGGACTTAACCCAACATCTCAGGACACGAGCTGACGACAGC CATGCAGCACCTGTGTAGGTCCCGAAGGGAAGAAATCCATCTCTGGAAGT CGTCTACCATGTCAAACGCTGGTAAGGTTCTGCGCGTTGCTTCAATTAACCC ACATGCTCCACCGCTTGTGACGGCCCGTCAATTCCTTTGAGTTTAAAGGTTG CGGCCGTACTCCCCAGGCGGATAACTTAATGCGTTAGCTGCGCCACCCAAGCA CCAAGTGCCTCGACAGCTAGTTATCATCGTTTACGGCGTGGACTACCAGGGTA TCTAATCCTGTTTGTCTCCACGCTTTCGCACCTCAGCGTCAATACCAGTCCAG TCAGCCGCTTCCGCACTGGTGTCTTCCGAATATCTACGAATTTCACTTAC ACTCGGAATTCAGCTGACCTTCTCTGGATTCAAGCGATGCACTCTCAAAGGCA GTTCTGGAGTTGAGCTCCAGGCTTTCACCTTACTGACTTACAAAGCCGCTACGTG CGCTTACGCCCAGTAATTCGAACAACGCTAGCCCTCTCCGTTATTACCGCGG CTGCTGGCACGGAGTAGCCGGGGCTTATCTCCCGTACAGTCAATATTCTTCCC GGGTAAAGAGCTTTACACCTAGGCTCATCACTACGCGCATTGCTGGATCAG GCTTCGCCCATGTCCAATATCCCAGTGTGCTCCCGTAGGAGTCTGGCGGTC TCAGTCCAGGTGTGACTGATCATCTCTCGACAGCTATGGATCGTCCGGCTTG GTAGGCCTACTACACATCTATCACGCGCTCATCTCAGCATAACCTTTGATCTC CGCCG	OAct 427
<i>Bacillus anthracis</i>	GGTTACCCACCGACTTCGGGTGTTACAACTCTCGTGGTGTGACGGGCGGTG TGTACAAGGCCCGGGAACGTATTACCGCGCATGCTGATCCGCGATTACTAG CGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAAGTGAACCG TTTTATGAGATTAGTCCACCTCGCGTCTTGCAGCTCTTTGTACCGTCCATTGT AGCAGTGTGTAGCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCA CCTTCTCCGGTTTGTACCGGCAGTCACTTAGAGTGCCTAAATGATGG CAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCAGCA CACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAG CCTATCTTAGGGTTGTGAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTT GCTTCAATTAACACATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTT TGAGTTTACGCTTTCGCGGCTACTCCCCAGGCGGAGTGCTTAATGCGTTAACT TCAGCACTAAAGGGCGAAACCTCTAACACTTAGCACTCATGTTTACGGCG TGGACTACCAGGATCTAATCCTGTTTGTCTCCCAAGCTTTCGCGCCTCAGTG TCAGTTACAGACCAGAAAGTGCCTTCGCCACTGGTGTCTCCATATCTCTAC GCATTTACCGCTACACATGGAATTCACCTTCTCTTCTGCACTCAAGTCTCC CAGTTTCCAATGACCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAG AAACCACTGCGCGCGCTTACGCCCAATAATTCGGGATAACGCTTGCACCT ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTCTGGTTAGGTAC CGTCAAGGTGCAGCTTATTCAACTAGCACTTGTCTTCCCTAACAAACAGAGTTT ACGACCGAAAAGCTTTCATCACTACGCGCGTGTCTGAGACTTCGTATGCGG AGATTCTACTGCTGC	B-48

<p><i>Thermotalea metallivorans</i></p>	<p>TGGTATAGCCTACTCTCATGGGGTGACGGGGGGGGTGTACAAAAACACGGGAACGCATTCTCCGCGACATTTTGATTCTCGATTACTATCAACTCCACATTTTGCGGGCGGAGATGCGCCCTGCGATCCCAAATGGGATCGGGTTTTTTAGATTTCATCTTATCTTAGGGAGCTGCTCTTTGTACCCACCATTGTAGCACGTGTGTAGCCACAGATAAAAGGGGATGAGAATATGACACCATCCCCCTTCTCCCATATTTTCATC GGGAGTCCCTCTAGAGAGCCCACTTTATGCGGGGGACTAAAGGGGAGGGGGGCGCTCTTTGTGGGAGTTATACCAACATCTCACGACACAAGATGACAACAACC ATGCGCCACCTGTGTCCCCTGTGTCCCAAAGGATAAAAAACCCCTCTCTGATGCGGTCAGGGGCATGTCAAAGCCCTGGAAAGGTTCTTCGCGTTGCTTCAAAAATAAAA CACATGCTCCGCTGCGTGTGCGGGTCCCGTCAATTTCTTTGAGTTTCATACTT GCGTACATACTCCCAGGGGGAGTGTTTTATGTGTATCTGCGGCACCGAGGTT TGACCCCCACACCTAGCACTCATCGTTTACGGCGTGGAAATACCAGGGTATCT AATCCTGTGTGCTCCCCACGCTTCTCTTCTCAGCGTCAATATAAGTCAAAAA GTCCCCCTCCCCTGGTATTCTCCCAATATATACGCATTTCCCCTACACT AAGAATTTCCACTTTTCTCTCCCTATCTCAAGTCTTTCAGTTTCAAATGCTTAA CCACGGGGAGAGACGTGAGCTTTCATCATCTGACATAAAAAAACCCCTACAA CAACTTCTACGCACTAAATATTTGCGGAGCACGCTCGCCCCACTATATATA CACGCCGGCTGGTGGGGACAGTATATTAGCCGGGGTCTTTTCTTCTGGGGGTA CCGTATTATTCTGTCGCCCAAGGAGAAGAAATTTGAGACGCGGAAGGTTCT CATCTGATCACGGGGCGGTGCTACATCAGAGGTTTTCTCCAATTGTGCAATAT TC</p>	<p>B2-1</p>
<p><i>Bacillus subtilis</i></p>	<p>CCCGTTTCCTTTTGTGTGGCTCCTGGAGGCTACGTACCGACTTCGGGTGTGCAAACTCTCGTGGCGTGACGGGGCGGTGTGTACAAGGCCCGGGAACGTATTCA CCGCGGCATGTGATCCGCGATTACTAGCGATTCCAGCTTACGCGAGTCGAGTT GCAGACTGCGATCCGAACAGTGAACAGATTGTGGGATTGGCTTAACCTCGCG GTTTCGCTGCCCTTTGTTCTGTCCATTGTAGCACGTGTGTAGCCAGGTCATAA GGGGCATGATGATTTGACGTACATCCCACCTTCTCCGGTTTGTACCGGCAGT CACCTTAGAGTGCCTAACTGAATGTGGCAACTAAGATCAAGGGTTGCGCTCG TTGCGGGACTTAACCAACATCTCACGACACGAGCTGACGACAACCATGCACC ACCTGTACTCTGCCCCCAAGGGGACGTCTTATCTTAGGATTGTCAGAGGA TGTCAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAACCACATGCTCCA CCGTGTGTCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCAGCCGTACA CCCCAGGGGAGTGTAAATGCGTTAGCTGCAGCCTTAAGGGGGCGGACCCCC TAACACTTAGCACTCATCTTTCTGCGTGGACTACCAGGGTATCTATTTCTGTT GCTCCTCACGCTCTCG</p>	<p>95 (BP2)</p>
<p><i>Streptococcus gallolyticus</i></p>	<p>CGGGGGGCTCCGAACCTGGTTACCTCACCGACTTCGGGTGTTACGACTCTCGGG GCGTGTGGGGCGGAGTGACAAGGACCGTTTACGTATACGCCGCCCTGATAT TCCTCTGTATCCAAATATTAATCTAATAAGCATTGATTTACGTATCGATTGGA TCTCGATTAAAGCCTATAGGTAATGAGTGGTTTCATATTCCGAATTCATCAA TTCTTTGAACCGTCTTTGCTTCCGACGGTATTTAGGATGCCAGGCTGTCCGGG CGCTGTTCGCTGCGCGCCGTTGGATTTCGGTGTGATCAATTCGTTAACTCCT TGCCCTACCCGGGTATTGATTTCTCGGGA</p>	<p>GDYJ 2011</p>
<p><i>Lautropia mirabilis</i></p>	<p>CGCGCTCCTTGCGGTTAAGCTACCTACTTCTGGTAAAACCCACTCCCATGGTGT GACGGGGCGGTGTGTACAAGACCCGGGAACGTATTACCGCGGCATGCTGATCC GCGATTACTAGCGATTCCGACTTCATGCAGTCGAGTTGCAGACTGCAATCCGG ACTACGATCGGTTTTTTGGGATTGGCTCCACCTCGCGGCTTCGCAACCCCTCTGT ACCGACATTGTATGACGTGTGAAGCCCTACACATAAGGGCCATGAAGACTTG ACGTCATCCCCACCTTCTCCGGTTTGTACCGGCAGTCTCATTAAAGTGCCT TGCGTAACAATAATGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACAT CTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCCGGCTCTCTTT GAGCACCCCAATCTCTTTCAGGGTCCGGACATGTCAAGCGTAGGTAAGGTT TTTCGCTTGCATCGAATTAATCCACATCCACCGCTTGTGCGGGTCCCGCT CAATTCCTTTGAGTTTAACTTTCGCGACCGTACTCCCCAGGGGCTACTTTCAC GCGTTAGCTGCGTTACCAAGGAAATTAATCCCCGACAACAGTAGACATCGTT TAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGTCCCCACGCTTTCGTTG ATGAGCGTCAGTGTATCCAGGAGGCTGCCTTCGCCATCGGTGCTCCTCCGCA TATCTACGCATTTACTGCTACATGCGGAATCCACCTCCCTCTGACACACTCT AGCCTTGACAGTACCAATGCAGTTCACAGTTAAGCCCGGGGATTTACATCG GTCTTGAAAACCGCTGCGCACTCTTACGCTCAGTAATTCGGATTAACGCTT GCACCCTACGTATTACCGCGGCTGCTGGCACGTAGATAGCCGGTGCATTATTCTG CGGGTACCG</p>	<p>SSI AB 2188</p>
<p><i>Bacillus licheniformis</i></p>	<p>AAGGGTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGGGTGACGGGGCGG TGTTGTACAAGGCCCGGGAACGTATTACCGCGGCATGCTGATCCGCGATTACT AGCGATTCCAGCTTACGCGAGTCGAGTTGCAGACTGCGATCCGAACAGGAAAC AGATTTGTTGGGATTGGCTTACGCTCGCGGCTTCGCTGCCCCCTTTGTTCTGCCC TGTAGCACGTGTGTAGCCAGGTCATAAAGGGCATGATGATTTGACGTCATCC CCACCTTCTCCGGTTTTGTACCGGCAGTCACTTAGAGTGCCTAACTGAATGC TGGCAACTAAAATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA CGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCAAGGGG AAGCCCTATCTTAGGGTTGTGAGAGGATGTCAAGACCTGGTAAGGTTCTTCG CGTTGCTTCAATTAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATT CCTTTGAGTTTTCAGTCTTTCGCGACCGTACTCCCCAGGGGAGTGTAAATGCGT TTGCTGCACCACTAAAGGGCGGAAACCCCTAACACTTAACACTCATCGTTTAC</p>	<p>CG62</p>

	GGCGTGGAC	
<i>Pseudomonas putida</i>	<p>AAAGGTTAGACTAGCTACTTCTGGTGAACCCACTCCATGGTGTGACGGGCG                  GTGTGTACAAGGCCCGGGAACGTATTACCGCGACATTCTGATTCCGCGATTAC                  TAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGAT                  CCGTTTTGTGAGATTAGTCCACCTCGCGGCTTGGCAACCCCTCTGTACCGACCA                  TTGTAGCACGTGTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATC                  CCCACCTTCTCCGGTTTGTACCCGGCAGTCTCCTTAGAGTGCCACCATAACG                  TGCTGGTAACAAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCAACATC                  TCACGACACGAGCTGACGACACGCCATGCAGCACCTGTGTGACAGTTCCCGAAG                  GCACCAATCCATCTCTGGAAAGTTCTCTGCATGTCAAGGCCTGGTAAGGTTCTT                  CGCGTTGCTTCGAATTAACACATGCTCCACCCTTGTGCGGGCCCCGTCAA                  TTCATTTGAGTTTTAACCTTGGCGGCGTACTCCCAGGCGGTCAACTTAATGCG                  TTAGCTGCGCCACTAAAATCTCAAGGATTCCAACGGCTAGTTGACATCGTTTAC                  GCGGTGGACTACCAGGGTATCTAATCTGTTTGTCTCCCACGCTTTCGCACCTC                  AGTGTCAGTATC                  AGTCCAGGTGGTTCGCCCTTCGCCACTGGTGTTCCTTCTATATCTACGATTCA                  CCGTACACAGGAAATTCACCACCCTTACCCTACTTAGCTTGCAGTTTTG                  GATGCAGTTCCAGGTTGAGCCCGGGGCTTTCACATCCAACCTTAACAAACCAC                  CTACGCGCGCTTACGCCAGTAATCCGATTAACGCTTGCACCCTCTGTATTA                  CCGCGGCTGCTGGCACAGAGTTAGCCG</p>	M-X15A
<i>Sporosarcina aquimarina</i>	<p>AGGGTTACCCCaCCGACTTCGGGTGTTACAACTCTCGGGGTGTGACGGGCGGT                  GTGTACAAGACCCGGGAACGTATTCCCGTGGCATGCTGATCCACGATTACTA                  GCGATTCCGGCTTCGTGCAGGGGATTTGCACCCTACAATCCAACTGGGAACG                  GTTTTCTGGGATTGGCTCCCCCTCGCGGGTTTGCAGCCCTCTGTACCGTCCAT                  GTAACACGTGTGTACCCAGGTCATAGGGGGCATGATGATTGACCTCTCC                  CACCTTCCCGGTTTGTACCCGGCAGTACCTTAGAGTGCCCAACTGAATGAT                  GGCAACTAAAATTAGGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCAC                  GACACGAGCTGACGACACCATGCACCACCTGTCACCCTGCCCCAGAAGGAA                  AAGGCATGTCTCCGTGCCGGTAAGAGGGATGTAAGAGACTGGGAAGGTTCTTC                  GCGTTGCTTCAAATTAACACATGCTCCACCCTTGGGCGGGTCCCCCTAT                  TCCTTTGAGTTTACGCTTTCGCGCCGACTCCCCGGGCGGAGTGCTTAGTGCCT                  TATCTGCATCAATAAGGGGGCGACACCCTAACACTTATCACTCATCGTTTAC                  GGCGTGGACTACCAATGTATCTAATCTGTTTGTCTCCCCTCTTTCGCGCCCT                  AGCGTCAGTTACAGACCAGAAAGCCGCTTCCCCTAGTGTTCCTCATATCT                  CTACGTATTACCCGCTACGCGTGAATTCCTTCTTCCCTTCTGTACTAACT                  CCCCCATTTACAATGACCCTCGGGGGGTGAGGCGTGGGCTTACACATCATATT                  AAAAGACCGCCTGCGCGTGTACGACTAATTATTCCAGACACTGCCTAGCCA                  CCTACGTAACCGCTGCCTGCTGCACGTAGTCAGCGTGTCTTCTGACGAGTAC                  CGACAGGCTACGTAATTAACACATGCTCCAGTACGTTTTCTCCTTACAAAGATTAACC                  ATCCAGAATCTTCTACTACCGGGATGCTCCACAATCTTCCCAATGGGAATTCC                  ATGGTGCCCCGTAAGATTGGCAGGTCCATCAGTGGCAATCCCTAGGCGACTTA                  CCTGTCTGAAGCTACATA</p>	ML9
<i>Mycobacterium tuberculosis</i>	<p>CTCCCGAGGGTTAGGCCACTGGCTTCGGGTGTTACCGACTTTCATGACGTGACG                  GGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCAGCGTTGCTGATCTGCG                  ATTACTAGCGACTCCGACTTCACGGGGTCGAGTTGCAGACCCCGATCCGA                  GAGACCGGCTTTTAAAGGATTGCTTAACTTCGCGGCATCGCAGCCCTTTGTACC                  GGCCATTGATGATGTGTGAAGCCCTGGACATAAGGGGCATGATGACTGACG                  TCATCCCCACCTTCTCCGAGTTGACCCCGGCACTCTCTACGAGTCCCCACCA                  TTACGTGCTGGCAACATGAGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCA                  ACATCTCAGCACGAGCTGACGACAGCCATGCACCACCTGCACACAGGGCCAC                  AAGGGAACGCCTATCTAGACGCGTCTGTGCATGTCAAACCCAGGTAAGGT                  TCTTCGCGTTGCATCGAATTAATCCACATGCTCCGCCCTTGTGCGGGCCCGG                  TCAATTCCTTTGAGTTTTAGCCTTGGCGGCGTACTCCCCAGGCGGGGACTTAA                  TGCGTTAGCTACGGCACGGATCCCAGGAAGGAAACCCACACCTAGTACCCAC                  CGTTTACGGCGTGGACTACCAGGGTATCTAATCTGTTGCTCCCCACGCTTTC                  GCTCCTCAGCGTCAGTTACTGCCAGAGACCCGCTTCCACCAGCGGTGTTCTC                  CTGATATCTGCGCATTCCACCCTACACCAGGAATTCCAGTCTCCCCTGCAGTA                  CTCTAGTCTGCCCCGATCGCCCGCACGCTCACAGTTAAGCCGTGAGATTTACAG                  AACAACGCGACAAACCCTACGAGCTCTTACGCCAGTAATTCGGACACG                  CTCGACCCCTACGTAATACCGCGGCTGCTGGCACGTAGTTGGCCGGTGTCTTCT                  CTCACCTACCGTCAATCCGAGAGA</p>	58MT
<i>Streptococcus oligofermentans</i>	<p>GTGGCGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTACCGCAGTATG                  CTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACA                  ATCCGAAGTGAAGAAAGGGTTTACAGATTGCTCGGCCCTCCCGGGCTTGTACT                  CCTTATTACCTCCCATGGTAGAAGCGGGGTACCCCGGCCATAAGGGGAATG                  ATAATTTGCTCTCCACCTTCTCCGGGTTTGTACCGGCAGTCCCCTTATA                  ATGCCCCACTAAATGATGGGAATAAAATGAGGGGTGTGGTCTTGTGGGAC                  TTAACCCACATCTCCAGACAAGAATGACAACAACCATGCACCCACTGTGGT                  TTCTTTCCGAAAGAAATAAGATCTTCTAAAGGTCTTAAGGAAGTGAAGGAAC                  GGGGAGGGTCTTCTCGGTGCATCCAAAAAACACATGGTCCAACGCTTGTGGG                  GGGCCCCCAATCTTTGAGTTTTAACTTGTGGCGTACTCCCCCGGGGGG                  GGGGTTTATTCTGTAGCTGCCGCACTAAACACGGGAAAAAGGCCCCACCCTA                  ACAATCTCCTTTTTTGGGGGGGAAAAACAGGGGAGTTAATTCGTGCTTGT</p>	ChDC B689

	<p>CCCCCCTGTTTTTTAGCCCCACCAGATAATAACCAAAAAACGCTTTCCT          CCCCAGGGGGTTCCTTTTTTTTTTCCCATTTTTCCCCCCACATGGAAATTTT          CCTCTTCTTTCTTCAATTAAGTTTTAAAGGGTTTTAAAATCGCCATTATGGGG          GAAACCACGCCCTTTTTTTTTTAAACTTATTGAAACCGCCCTGCCCGCCGCTT          TTCTCCCATAAAAATCGGAACGACGCTCGGGACCTACCTTTTTACCACCGGCGG          GTGGGGGGGAATTAACCGCCGTTTTTTGTTAAAAAACCGCCCCACATTCATC          TFACTATTCCAAAATT</p>	
<p><i>Enterococcus faecium</i></p>	<p>TCGTTCAATACAGTGGGGCCGTTATCTTGTA AAAACATATCCACACGGGCTAG          ACCTCTACAGCCGAGCGCTTTATATATTTTTTTGCCGTTTCCTGTATCCGTCCT          CGCTCCTCTGCTGAAAGGTCTGCGGGAACGGTTATAACTGCGTTTTTCAGAGCCT          TTTCCGGCTCGACTTCCTGATGAATACGAAAGATTCCGTACTGCAGCCTGATT          TGGTCCACCTCGCCAACAATAACGCGGCACTGTTTCCCAATACCGCACAAACC          GACCTCACAGCCCGAAACAGCCTGCTCAATTAAGATTTTGTCTGCATATTGTCT          TGCCGATTCAATTGCGTAGTCCAATTGCTCCGCGCTATTGACTTTTTTCACACC          GAAGGATGAGCCTGAACGCGCCGGCTTAACAAAAACAGGATAGGTAAACGTA          CCTGCCACCGGCCTATCATCTTTATTAATAACCAAAAAGGCGGGAGTAGCTAT          CCCACATTTTTTCGCAACGATGTATGTCAACGATTTGTCATACAAATTGTGA          GCTTTGAATATCGCAGCCTACAAAAGGGATACCGGACAATTCAAAACAGACCTT          GTATGGATCCATCTTCACTGACTTGCCATGCAAAGTGAAAATGCTACATCA          ACATGGTTGATTCATATTCATGGTTCTTTTTACAAGTAATCCGTGCATTTTTTT          TATCCGCGGAGAGTACAGTGAATAGCAATTGTCGTTTTCCCATTCGCGGCA          AGGTTTTTTCGCACATTTCCATACACCCAGATTTTCGTAATTCCCAATGT</p>	<p>64/3xUW2774</p>

**DISCUSSIONS**

In the present study, used to amplify the 16SrRNA gene with primer B24F and U1492R for all bacteria species to prevent losing of any species. Conventional biochemical tests and commercial identification system as well as phenotypic variants are not included in the level of subspecies and often miss identified (Seifert *et al.*, 2003). In contrast, the high-quality of 16SrDNA sequence database provides excellent identification at the species and subspecies levels; furthermore, it can lead to the recognition of novel pathogens and non-cultured bacteria (Mellmann *et al.*, 2006).

*Sporosarcina aquimarina* had been isolated from seawater in Korea (Yoon *et al.*, 2001) Spore formation which belongs to the family Bacillaceae (Kluyver and van Neil, 1936), may be transmission spores and ingested through the mouth and stability in respiratory system .

*Pseudomonas putida* cause soft rot disease in potato (Janse *et al.*, 1992) may be transmission these bacteria during eat potato and stability in respiratory tract infection.

*Bacillus licheniformis* spore-forming bacterium widely distributed as a saprophytic organism in the environment, as the species generally persists in soil as endospores (Alexander., 1977), may be inhalation of these spores.

*Streptococcus gallolyticus* now named *Streptococcus bovis* (biotype I) is a normal inhabitant of the gastrointestinal tract, isolated from endocarditic patient with chronic liver disease (Klein *et al.*, 1977; Zarkin *et al.*, 1990; Gonzalez *et al.*, 2001) may be transmission through germ blood from these bacteria into respiratory tract.

*Sphingomonas paucimobilis* recorded the presence from diverse sources in the hospital environment, including hospital water system, respiratory therapy equipment, and laboratory instruments, it has been nosocomial out breaks of *Sphingomonas paucimobilis* have been reported and considered to originate from contaminated hospital environment and equipment (Bourigault *et al.*, 2007; Han *et al.*, 2011), presence in respiratory therapy equipment *and other* instruments this reinforces the presence in RTI patient.

*Enterococcus faecium* found in the oral cavity (Donelli *et al.*, 2004) may be transmission from oral cavity into RTI. *Bacillus subtilis* is non-pathogenic, they can contaminate food and caused food poisoning (Perez, 2000) these bacteria may be entire with food through the eating into RTI patient.

However, *Sporosarcina aquimarina*, *Pseudomonas putida*, *Bacillus licheniformis*, *Streptococcus gallolyticus*, *Sphingomonas paucimobilis* *Enterococcus faecium*, *Bacillus subtilis* were recorded that isolated in RTI at the first time in world and in Basra/Iraq patients.

*Lautropia mirabilis* isolate from RTI was in agreement with Gerner *et al*, (1994) it was isolated from oral and upper respiratory sites, and isolate these bacteria in Basra patients at the first time. *Mycobacterium tuberculosis* is isolated from RTI was in agreement with Chiang and Kim (2009).

*Bacillus anthracis* isolated from RTI agreement with Kyriacou *et al*, (2007) because of this bacteria characterized more common causes of respiratory illness.

*Streptococcus oligofermentans* isolated at first time in oral from caries-free humans by Tong *et al.*, (2003), Oral streptococci comprise part of the normal microbial flora of the oral cavity and upper respiratory tract of humans (Hardie & Marsh, 1978), presence these bacteria in RTI patient at first time in the world and in Basra/Iraq patient after recording yare 2003 in the oral.

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