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SEQUENCES OF ADHERENCE GENES AMONG *S. AUREUS* AND *M. CATARRHALIS* ISOLATED FROM THROAT INFECTIONS, IRAQ

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Abstract

The thus study included two (204) samples were collected from patients with pharyngeal infections, who have been visited the Babylon General teaching Al-Hilla hospital and Private Clinic of Supervisor. The age range between 5-80 years old, at the period from February to December (2017), two swabs were collected one for culturing and the other for direct DNA extraction for swabs of *Moraxella catarrhalis*. Out of the (204) samples only 190 (93.1%) showed positive bacterial culture, whereas 14(6.9%) samples showed no bacterial growth. From the 190(100%) samples were shown that Gram-positive bacteria constitute 116/190 (61.10%) from the total isolates and Gram negative bacteria constitute 74/190 (38.90%). From the total of 116(100%) of Gram positive bacteria the *Staphylococcus aureus* constitute 38(20%), and out of the 74(100%) of Gram negative bacteria the *Moraxella catarrhalis* was the more predominant with percentage 44 (23.2%), the laboratory diagnosis done by biochemical test, vitek 2 system and molecular detection by specific primers. At Molecular level the *fnbA* find in the *S. aureus* showed that have 13(34%) for this virulence gene. Regarded to *mcaP* gene of *M. catarrhalis* were 44(100%). DNA sequencing were done for *fnbA* gene for *S. aureus* and *mcaP* gene for *M. catarrhalis* which showed some variation, then recorded in NCBI-gene sequencing as first described in Iraq. The DNA sequencing analysis of adherence genes *fnbA* of *S. aureus* as gram-positive bacteria and *mcaP* of *M. catarrhalis* as gram negative. The results revealed that *S. aureus* local number (NO. 111 and NO. 181) isolates were closely related to isolation of *S. aureus* recorded globally NCBI- BLAST *S. aureus* (AM749012.1) while the local isolates (NO.37 and NO. 95) were closely related to globally *S. aureus* (LC073768.1) and (LC 073762.1).

Key words: *Moraxella catarrhalis*, DNA sequencing, gram-positive bacteria, Molecular level the *fnbA*, *mcaP* of *M. catarrhalis*.

Introduction

A sore throat is usually from irritation or inflammation. The most common cause (80%) is acute viral pharyngitis, a viral infection of the throat, Other causes include other infections (such as Streptococcal pharyngitis), trauma, and tumors, Gastro esophageal (acid) reflux disease can cause stomach acid to back up into the throat and also cause the throat to become sore (Marx and John, 2010). In children, streptococcal pharyngitis is the cause of (37%) of sore throats (Elise *et al*., 2012). The main bacterial causative agents were Group A beta-hemolytic Streptococci, Groups B, C, G Streptococci, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Haemophilus para influenzae*, *Neisseria* species, and *Mycobacteria* species (Windfuhr *et al*., 2016). *Moraxella catarrhalis* is a fastidious, non- motile, Gram-negative, aerobic, oxidase-positive diplo-coccus that can cause infections of the respiratory system, middle ear, eye, central nervous system, and joints otitis media, bronchitis, sinusitis, and laryngitis of humans, it causes the infection of the host cell by sticking to the host cell using trimetric auto transporter adhesions. These bacteria are known to cause otitis media, bronchitis, sinusitis, and laryngitis. Elderly patients and long-term heavy smokers with chronic pulmonary disease should be aware that *M. catarrhalis* is associated with bronchopneumonia, as well as exacerbation of existing chronic obstructive pulmonary disease (Mawas *et al*., 2009). *S. aureus* is Gram-positive cocci accruing in grape formation, aerobic or facultative anaerobic, catalase positive (Garrity *et al*., 2005). The initial step in pathogenesis of infections is often cell adhesion, which is mediated by surface adhesions called MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrixn Molecules) (Cheng-Ching *et al*., 2012). The ability of *S. aureus* to establish a niche in the host is a crucial step in its pathogenesis. *S. aureus*

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produces a number of cell surface-localized binding proteins, including fibronectin binding proteins (FnBPs) (Flock *et al*., 2003; Signäs *et al*., 2009), a collagen binding protein (Cheng-Ching *et al*., 2012), fibrinogen binding proteins (FgBP) (Payne and Benninger, 2007), a vitronectin binding protein (Paulsson *et al*., 2009) and an elastin binding protein (Garrity *et al*., 2005). The sequencing of DNA is another PCR-based molecular method in which the nucleotide bases along a DNA strand are determined. The advent of rapid DNA sequencing methods has greatly accelerate biological and medical research and discovery (Ehsaniet *et al*., 2016). There are two different methods used in the sequence analysis chemical cleavage and dideoxy chain termination (Sanger sequencing method), Sanger sequencing was most frequently used which based on enzymatic DNA synthesis (Franca *et al*., 2002). Sequencing of the bacterial genomes has significantly improved our understanding about the biology of many bacterial pathogens as well as identification of novel antibiotic targets (Donkor, 2013). The advent of Sanger sequencing gave a boost to DNA sequencing in general and led to an even more rapid accumulation of sequence data for various genes and organisms. This increase in sequence data in the scientific literature also resulted in the establishment of the first DNA sequence repository by Walter Goad at Los Alamos National Laboratories in (1979). This repository has since become Gene Bank (Mount, 2001).

Aim of study:

1. To isolation of bacteria from throat infection, and identification by routine bacteriological and biochemical tests and Vitek 2 System.

2. Molecular detection of bacteria by using PCR technique by using specific primer (16SrRNA, Omp P4) diagnostic genes for *Moraxella*.

3. Study of some genes associated with important virulence factors such as adhesive by using specific primers (*fnbA*, *mcaP*).

Study of sequencing for *S. aureus* and *Moraxella catarrhalis*.

Materials and methods

Patients and clinical specimens:

This study involved (204) samples were collected from patients with throat infection. The samples include both direct swabs and culture swab from each female and male with different age admitted in to hospitals of Babylon Province, Al-Hillah General Teaching Hospital and private clinic of super-adviser, during the period from February to December (2017). The age of patients ranged from (5 to 75) years. The doctor of ENT (including inflammation, pus, and redness) diagnosed these patients.

Ethical Approval: A valid consent was achieved from hospitals administration and from patients before their inclusion in the study. For every patient, the procedure had been informed before the samples were collected, making sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects.

Identification of bacteria:

Colonial morphology and microscopic examination:

A single colony from each primary positive culture on blood, MacConkey and nutrient agar and identify it depending on its morphological properties (colony shape, size, color, borders, and texture) and exam it by light microscope after being stained with Gram's stain. After examination it, biochemical tests were done on each isolates to complete the final identification according to (Baron *et al*., 1994, Collee *et al*., 2006 and McFadden, 2000) and we used vitek2 System for identification of *Staphylococcus aureus*.

Identification of *Staphylococcus aureus* bacterial isolates with Vitek2 System:

In clinical microbiology Vitek 2 used as an auto instrument system for the identification (ID) However, the samples were achieved according to manufacture instructions as follows: a sterile plastic stick applier used to take pure colonies from culture media and transfer a sufficient number of them to plastic test tubes. All reagent and instrumentation required for process provided by Manufacturer Company. All isolates introduced to the computer before process and inoculated cards were processed within the instrument within 30 min of inoculation. GP cards were loaded (inoculated) with bacterial suspensions employing a vacuum chamber in machine. Check tubes containing the samples were placed into a cassette (special test tube rack) and therefore the identification card was placed within the neighboring place where as inserting the transfer tube into the corresponding suspension tube. The cassette might accommodate up to 10 test tubes. The filled cassette was placed into a vacuum chamber station inside the Vitek 2 instrument machine. The vacuum was applied then the air was recharged into the station, the bacterial suspension was forced through the transfer tube into micro channels that filled all the test wells. Inoculated cards were passed by a mechanism that stop the transfer tube and sealed the card before loading into the circular incubator. The incubator might

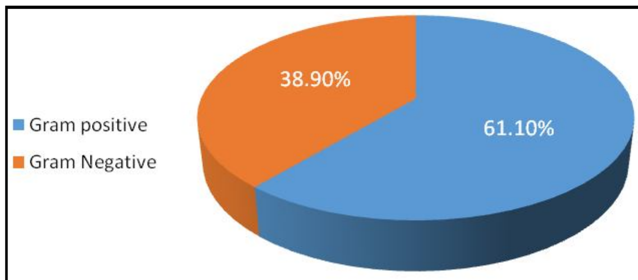

Fig. 1: The percentage of gram positive and gram negative among patients with tonsillitis and pharyngitis.

accommodate up to 30 cards. All card varieties were incubated at 35.5 ± 1°C. Every card was removed from the incubator once each 15 minutes, transported to the optical system for reaction readings, so came to the incubator until future read time. Information was collected at 15 min intervals throughout the whole incubation period.

Molecular study:

Genomic Bacterial DNA Extraction from Throat Swab Samples:

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA Favorgen Kit).

1. Transfer bacterial cells (up to 1 × 109) to a 1.5 ml microcentrifuge tube and Centrifuge for (1) min. at 14-16,000 × g then discard the supernatant.

2. Add (200) µl of FAGT Buffer then re-suspend the cell pellet by vortex or pipette. Incubate for 5 minute at room temperature.

3. Add (200) µl of FAGB Buffer to the sample and vortex for (5) seconds and incubated at (70ºC) or until the sample lysate is clear, and during the incubation, invert the tube every (3) min. At this time, pre-heat the required Elution Buffer (for step 5DNA Elution) in a (70ºC) water bath.

4. Add (200) µl of absolute ethanol (96-100%) to the sample and mixed by vortex for (10) seconds. (If precipitate is appears, break it up as much as possible with a pipette). Then place the FAGB Column in a (2) ml collection tube and transfer mixture (including any insoluble precipitate) to the FAGB column and centrifuge at (14000rpm or 10,000 xg). Discard the (2) ml Collection tube containing the flow-through and then place the FAGB Column in a new (2) ml collection tube.

5. Wash FAGB column with 400 µl of W1 Buffer. Centrifuge at (14000 rpm or 10000 × g for 30 seconds); discard the flow-through then place the FAGB column back in the (2) ml collection tube.

6. Add (600) µl of Wash Buffer (with ethanol) to the FAGB column then centrifuge at (14,000 rmp or 10000 × g) for 30 seconds, discard the flow-through. Place the FAGB Column back in the (2) ml collection tube and centrifuge again for (3) min. at (14,000 rmp or 10000 × g) to dry the column matrix.

Organism Origin	VITEK 2		
Selected Organism	98% Probability	Staphylococcus aureus	
	Bionumber: 010402060363021	Confidence:	Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			
Staphylococcus aureus	AGLU(79),		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig. 2: Biochemical testing of *S. aureus* isolates (No. 31) using Vitek2 system.

7. Place the dry FAGB Column to new (1.5) ml micro centrifuge tube, and add (100) µl of pre-heated Elution Buffer or TE to the membrane center of FAGB column. Stand FAGB column for 3-5 min or until the buffer is absorbed by the membrane and centrifuge at (14,000 rmp or 10000 × g) for (30) seconds to elute the purified DNA. Store the DNA fragment at (4ºC) or – (20ºC).

Measured DNA concentration and purity:

The extracted DNA was checked by using Nano drop spectrophotometer, which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260/280 nm) as following steps:

1. After opening up Nano drop software, chosen the appropriate application (Nucleic Acid, DNA).

2. A dry wipe was taken to clean instrument pedestals several times. Then carefully pipette 2 µl of ddH2O on to the surface of the lower measurement pedestals for blank system.

3. The sampling arm was lowered and clicked OK to initialized the Nano drop, then cleaning off the pedestals and 1 µl of extracted DNA carefully pipette onto the surface of the lowered measurement pedestals, then concentration and purity of extracted DNA was checked (Bunyan and Obais, 2018).

Polymerase Chain Reaction Assay:

Amplification of *mcaP* Genes by PCR:

All primer pairs used in the study, product size were listed below, and amplification condition was carried out by Polymerase Chain Reaction (PCR) were listed below, that was performed in a total volume of (25 µl) as mentioned below in Table (1).

Detection of Virulence Factors by PCR:

Nucleic acid (DNA) that extracted from bacterial cells, was used as a template in specific PCR for the detection of virulence genes listed in Table (2) (Bunyan and Obais, 2018, Bunyan *et al*., 2018). A single reaction mixture contained (2.5µl) of upstream primer, (2.5µl) of downstream primer, 5µl of extracted DNA, (12.5µl) of master mix and (2.5µl) of nuclease free water. The resulting PCR products were run in 1.5% agarose gel.

Gene Sequencing of *fnbA* gene and *mcaP* gene:

DNA sequencing method was performed for study of genetic changes and phylogenetic tree analysis of *fnbA* for *S. aureus* and *mcaP* for *M. catarrhalis* gene isolates by compared with NCBI-Gene Bank. The sequencing of the *fnbA* and *mcaP* gene were done after amplification by PCR method, then PCR products were purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). As the following steps:

1. The specific PCR products were excised from the gel by clean, sharp scalpel, then, transferred into a 1.5mL micro centrifuge tube.

2. 400µl Binding Buffer II was added to gel fragment. Then, incubated at 60ºC for 10 minutes and shaked until the agarose gel is completely dissolved.

3. Add the above mixture to the EZ-10 column and let stand for 2 minutes. Centrifuge at 10,000 rpm for 2

Table 1: Detection primer sequence with their amplicon size Base pair (bp) and their condition of bacteria *M. catarrhalis*.

Bacteria	Genes	Primer sequence (5'-3)	Size (bp)	PCR condition	Reference
M. catarrhalis	16S rRNA	F-AGAGTTTGATCCTGGTTCAG	600	95°C 2min 1x	Bootsma *et al*., 2000
		R-CTTTACGCCCATTTAATCCG		72°C 5min 1x	

Table 2: Virulence factors primers sequences with their amplicon size Base pair (bp) and their condition.

Bacteria	Genes	Primer sequence (5'-3)	Size (bp)	PCR condition	Reference
M. catarrhalis	*McaP*	F-CGCAATAAAGA TCACCATGCTTG R-CGGGATCCCGCTGAC ACATTGCATTGA TAAA	120	95°C 2min 1x	Edward *et al*., 2005
				95°C 30sec 1x 64°C 30sec 14x 72°C 20sec 1x	
				95°C 30sec 1x 57°C 30sec 19x 72°C 20sec 1x	
				72°C 5min 1x	
S. aureus	*Fnb A*	F– GATACAAACCCAGGTGGTGG F- TGTGCTTGACCATGCTCTTC	191	94°C 3min 1x	Arciola *et al*., 2005.
				94°C 30sec 1x 55°C 45sec 30x 72°C 45sec 1x	
				72°C 5min 1x	

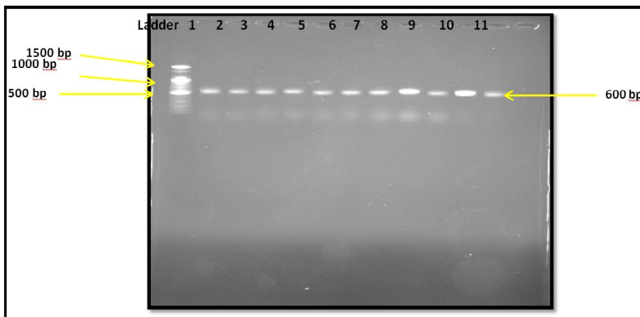

Fig. 3: 1% Agarose gel electrophoresis at 70 volt for 50 min for *16SrRNA* PCR products visualized under U.V light at 301 nm after staining with Ethidium bromide. L: 1500 bp ladder; lane (1-11) were positive for this gene, the size of product is 600 bp.

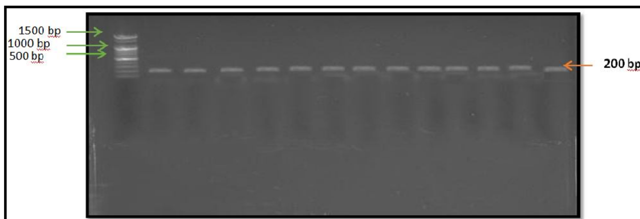

Fig. 4: 1% Agarose gel electrophoresis at 70 volt for 50 min for *fnbA gene* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-13) were positive for this gene, the size of product is 200 bp.

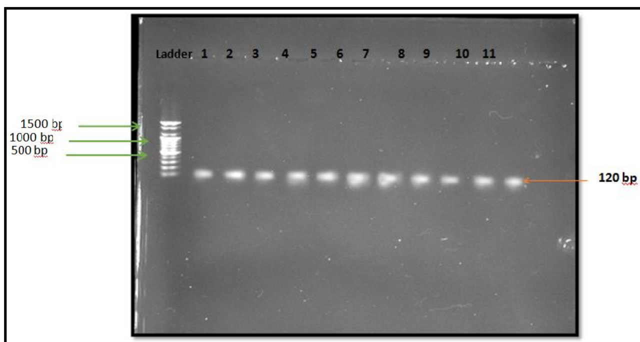

Fig. 5: 1% Agarose gel electrophoresis at 70 volt for 50 min for *McaP gene* PCR products visualized under U.V light at 301 nm after staining with ethidium bromide. L: 1500 bp ladder; lane (1-11) were positive for this gene, the size of product is 120 bp.

minutes and discard the flow-through in the tube.

4. 750µl Wash Solution was added to each tube and centrifuged at 10000 rpm for one minute. Then, solution discarded.

5. After that, the step 4 was repeated, and then centrifuged at 10000 rpm for an additional minute to remove any residual wash Buffer.

6. The column was placed in a clean 1.5ml micro centrifuge tube and added 30µl of Elution Buffer to the center of the column and incubated at room temperature for 2 minutes. Then, the tube was centrifuged at 10000 rpm for 2 minutes to elute PCR product and store at -20ºC.

After that, the purified PCR products samples were sent to Macrogen Company in Korea for performed the DNA sequencing by AB DNA sequencing system. The genetic changes, phylogenetic tree analysis, multiple sequence alignment analysis was performed based on NCBI-Blast Alignment identification (Tamura *et al*., 2013). ORF analysis by using ExPASy (SIB Bioinformatics Resource Portal online).

Results and Discussion

Isolation of pathogenic bacteria:

Among (204) clinical samples, only 190 (93.1%) positive culture, whereas 14(6.9%) samples showed no bacterial growth, which may be treated with antibiotics or the presence another types of causative agents, that might need special technique for their detection such as viruses and fungus. From this results, it was shown that Gram-positive bacteria constitute 116/190 (61.1%) from the total isolates and were considered the predominant an etiological agents to Gram-negative bacteria which constitute 74/190 (38.9%). As shown in Fig. 1. *M. catarrhalis* was bacteria that isolated from throat infections that rate 44 (23.2%) this result were agree with (Itzhak and Alan, 2006), which found from (548) patient there 69 (32%) have *M. catarrhalis* and with (Ellie *et al*., 2009), which mentioned that the *Moraxella* responsible about (20% to 30%) on the infections of upper respiratory tract. (Melinda *et al*., 2008), which found that (63.1%) of *Moraxella* cause upper respiratory tract infection, The recognition of *M. catarrhalis* as an important human respiratory tract pathogen, together with another types of bacteria increase prevalence of β-lactamase producing strains, which confer resistance against β-lactam antibiotics. The isolation rate of *M. catarrhalis* from the respiratory tract in study published by Anita *et al*., 2011 was (9.8%), whereas Al-Turfei, (2002), showed that the isolation rate was about (3.6%). The rates of *M. catarrhalis* carriage in children and adults differ considerably, about two-thirds where most children have colonized within the first year of life by these bacteria (Faden, 2001). Whereas, it was estimated to be represented about (10-15%) in adults (Murphy *et al*., 2005) or may be more as in the case of (Almalki, 2011) who reported that the prevalence of *M. catarrhalis* in respiratory tract of adults has been detected in (15-32%) of the samples. The carriage of this organism is probably due to bacterium antibiotics resistance properties and there is a correlation of *M. catarrhalis* carriage with seasonal fluctuations (Anita *et al*., 2011). *S. aureus* was

bacteria isolated from throat infection 38 (20%). This frequency may be due to firstly, it may enter the throat from nasal canal as a normal flora and by reflux OM when the tympanic membrane was not intact, and secondly, *S. aureus* also contain teichoic acid and lipoteichoic acid, capsular material, which facilitates the adherence of these bacteria to epithelium. Our result agree with many studies mentioned by (Almalki, 2011) from Thiqar (Iraq) and (AD' hlah *et al*., 2006) from Baghdad (Iraq). (Raju *et al*., 2012) they showed that *S. aureus* was the most common bacteria isolated from tonsillitis (83%) which is higher than recent study. (Lee *et al*., 2011) from New York isolated (42.7%) of *S. aureus* from throat swabs in prison population, (Zautner *et al*., 2010) from Germany also demonstrated that intracellular residing *S. aureus* is the most common cause of recurrent tonsillitis. (Islam *et al*., 2011) from Pakistan showed that *S. aureus* was isolated from throat swabs that taken from patients reach to (20.13%) and (Sadoh *et al*., 2008) reported that (16.83%) of isolates from pharyngitis and tonsillitis were *S. aureus*.

Identification of *S. aureus* by Vitek 2 System:

To confirm the isolates of *S. aureus* was used automated VITEK 2 system using Gp-ID cards, which contained (64), biochemical tests. The results demonstrate that all (38) isolates were confirmed with ID message confidence level ranging excellent (Probability percentage from 94 to 99.7%). This technique is characterized by fast detection of bacteria as shown in Fig. 2.

Molecular Detection of *Moraxella catarrhalis*:

It was found that, 44/150 (23.3%) *M. catarrhalis* were detected under molecular level by using specific primers based on 16s rRNA gene, that is species –

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Fig. 6: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 37 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073768.1) with nucleotide sequence identity (100%).

specific gene, and less be affected with evolution. So, it that was used for distinguishing between bacterial throat infections, due to that molecular procedures depended mainly on neucleic acid of bacteria in specimens for detection that can minimize the conditions of traditional bacterial cultivation especially the unculturable one that led to lose it as low number of bacteria in the specimen, the result were shown in Fig. 3. The 16 s ribosomal RNA is component of the 30s small subunit of prokaryotic ribosome that bind to the Shine – Dalgar sequence. The genes coding for it are referred to as 16s rRNA gene and are used in reconstructing phylogenies (Woese and Fox, 1977), the gene sequences contain hyperactive variable regions that can provide species – specific signature sequences useful for identification of bacteria (Pereira *et al*., 2010). The 16s rRNA gene PCR targeting the variable region of the gene with species – level identification used for investigated the association between the presence of individual bacterial species and clinical diagnostic characteristics of *M. catarrhalis* by Bootsma *et al*., (2000). Petal, (2001) explained the reasons for using of 16s rRNA gene sequences to study

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Sbjct  320  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  379

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  380  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  439

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  499

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  549
```

Fig. 7: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 111 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (AM749012.1) with nucleotide sequence identity (100%).

bacterial species. these reasons included the presence in almost all bacteria, often existing as a multi gene family, or operons, the function of the 16s rRNA gene over time has not changed, suggesting that random sequence evolution take long time, finally the 16s rRNA gene (600 bp) is large enough for inforatics purposes. This agree with (Hoopman *et al*., 2008) when detected the *M. catarrhalis* and uses the 16s rRNA as potentially excellent markers and could be used as targets for clinical diagnosis by molecular approaches.

S. aureus Adhesive factor:

In our study the percentage of *fnbA* from (38) isolates of *S. aureus* only 13 (34%) have this gene. The results were shown in Figure (4). (Carla *et al*., 2005) founded that all isolates of *S. aureus* 191 (100%) have this gene.

```
Score            Expect   Identities        Gaps         Strand
979 bits(530)    0.0      530/530(100%)     0/530(0%)    Plus/Plus

Query  1    TAAAATATAAATTGAAATTCGAAGATGGACTaaaaaaaGGAGATTACTTTGATTTTACAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  20   TAAAATATAAATTGAAATTCGAAGATGGACTAAAAAAAGGAGATTACTTTGATTTTACAC  79

Query  61   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  139

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  199

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  259

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
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Sbjct  260  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  319

Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
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Sbjct  320  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  379

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  420
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Sbjct  380  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  439

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  499

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  549
```

Fig. 8: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 181(Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (AM749012.1) with nucleotide sequence identity (100%).

In another study founded, the prevalence of *fnbA* was high, the percentage of *fnbA* from (98) *S. aureus* was 58 (59%) have this gene (Netsvyetayeva *et al*., 2014). In other study founded the percentage of this gene among the *S. aureus* reach to (84%) (Shanmugaraj *et al*., 2016). A study by Mohsen *et al*., (2015) founded that the percentage of *fnbA* was reach to (82.5%) from isolates of *S. aureus* isolated from various pathological conditions and indwelling medical device related infections. The differences in percentage between current study and other studies may be due to difference in the site of infection and number of isolates in each study. In addition, the results explain the different percentages of this virulence factor due to differ between country to another, difference in seasons, weather, and immunity and may be the source of isolation. Microbial adherence to cells and matrix components is considered to promote colonization and infection, one of the most important stages

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Score           Expect   Identities      Gaps        Strand
979 bits(530)   0.0      530/530(100%)   0/530(0%)   Plus/Plus

Query  1    TAAAATATAAATTGAAATTCGAAGATGGACTaaaaaaaGGAGATTACTTTGATTTTACAC  60
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Sbjct  20   TAAAATATAAATTGAAATTCGAAGATGGACTAAAAAAAGGAGATTACTTTGATTTTACAC  79

Query  61   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  139

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  199

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  259

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  260  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  319

Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  320  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  379

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  380  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  439

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  499

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
            ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  549
```

Fig. 9: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 195 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073768.1) with nucleotide sequence identity (100%).

in the occurrence of infection caused by *S. aureus* is able to adhere of the bacterium to the cells and the extracellular matrix. Among adhesions, two fibronectin binding proteins, (*FnbA* and *FnbB*) have been proved significantly to contribute to tissue colonization ,family of staphylococcal surface adhesions, called MSCRAMMs (for "microbial surface components recognizing adhesive matrix molecules") are known to mediate adherence of the bacteria to host extracellular matrix components, such as collagen, fibrinogen, and fibronectin (FN). *S. aureus* can express up to 20 different potential MSCRAMMs among these, two fibronectin binding proteins, (*FnbA* and *FnbB*) have been well characterized, FN-binding activity is mediated by two closely related FN-binding proteins (FnBPs), encoded by two adjacent genes, *fnbA* and *fnbB* (Vazquez *et al*., 2011).

M. catarrhalis Adhesive factor:

The PCR detection for *McaP* gene among DNA from direct detection of throat swabs find that from (44) specimen there were 44 (100%) have the *McaP* gene (*M. catarrhalis* adherence protein). The results were shown in Fig. 5. This result was in agreement with Suzanne, (2008), which founded from 195 sample there were 194 (99.5%) have this gene. Several recently described genes have also been associated with biofilm formation, including *McaP* (Lipski *et al*., 2007) and *pil* genes for type IV pili (TFP) (Luke *et al*., 2004). Luke *et al*., (2007) showed that, TFP play an important role in nasopharyngeal colonization of *M. catarrhalis* and that biofilm formation is enhanced by TFP expression; and that TFP genes are ubiquitous within *M. catarrhalis*. *M. catarrhalis* populations may be subdivided into two distinct genetic lineages, phenotypically characterized by (1) their ability to resist the destructive effect of human serum (*i.e.* complement resistant versus complement sensitive), and (2) differences in their ability to adhere to

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Sbjct  18   TAAAATATAAATTGAAATTTGAAGATGGACTAAAAAAAGGAGATTACTTTGATTTTACAT  77

Query  61   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  120
Sbjct  78   TATCAAATAATGTAAATACTTATGGGGTTTCAACAGCGAGAAAGTTACCAGAGATTAAAA  137

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
Sbjct  138  ATGGCTCTGTCGTAATGGCTACTGGTCAACTTCTTGGAGATGGAAAAATTAGATACACGT  197

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
Sbjct  198  TTACAGATTATATTGATTATAAAGTGAATGTAATAGCAAATTTAAACTTGAATTTATTTA  257

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
Sbjct  258  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGAAACACTTACTTCAAAATTAAATGGGA  317

Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
Sbjct  318  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGATGGAGTTGGAAAGTATTATACAA  377

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  420
Sbjct  378  ACCTGAATGGGTCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  437

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
Sbjct  438  ACGTTAAACCGATAAACGGAAATAAATCTGAAAGTGTATCTATTACTGGTAGTTTGACAC  497

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
Sbjct  498  AAGGTAGTAATGTAAGTGGTGATTCACCCATTGTTAAAGTGTATGAGTAT  547
```

Fig. 10: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 37 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073762.1) with nucleotide sequence identity (94%).

human epithelial cells (Wirth *et al*., 2007). Several virulence-associated genes have been identified in *M. catarrhalis*, including the outer-membrane proteins *UspA1*, *UspA2*, *Hag*, *OMPCD*, *CopB* and lipooligosaccharide *LOS* (Akgul *et al*., 2005). The *M. catarrhalis* Auto transporter *McaP* is a conserved surface protein that mediates adherence to human epithelial cells, which also displays esterase and phospholipase B activities (Serena *et al*., 2007). Phospholipases are known to be involved in a diverse range of cellular functions, inducing host inflammatory responses, changing membrane composition, and altering

```
Score           Expect   Identities       Gaps          Strand
802 bits(434)   0.0      498/530(94%)     0/530(0%)     Plus/Plus

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Sbjct  18   TAAAATATAAATTGAAATTTGAAGATGGACTAAAAAAAGGAGATTACTTTGATTTTACAT  77

Query  61   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  120
Sbjct  78   TATCAAATAATGTAAATACTTATGGGGTTTCAACAGCGAGAAAGTTACCAGAGATTAAAA  137

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
Sbjct  138  ATGGCTCTGTCGTAATGGCTACTGGTCAACTTCTTGGAGATGGAAAAATTAGATACACGT  197

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
Sbjct  198  TTACAGATTATATTGATTATAAAGTGAATGTAATAGCAAATTTAAACTTGAATTTATTTA  257

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
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Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
Sbjct  318  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGATGGAGTTGGAAAGTATTATACAA  377

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  420
Sbjct  378  ACCTGAATGGGTCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  437

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
Sbjct  438  ACGTTAAACCGATAAACGGAAATAAATCTGAAAGTGTATCTATTACTGGTAGTTTGACAC  497

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
Sbjct  498  AAGGTAGTAATGTAAGTGGTGATTCACCCATTGTTAAAGTGTATGAGTAT  547
```

Fig. 11: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 111 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073762.1) with nucleotide sequence identity (94%).

signaling cascades (Tan and Riesbeck, 2007).

Sequencing and Alignment of *S. aureus and M. catarrhalis adhesion genes:*

The results of DNA sequencing should be at the beginning of test to confirm the nucleotide sequences and closed relationship with others world strains. *S. aureus and M. catarrhalis* isolates were examined by sequencing technology to diagnosis of isolates and recorded it by adherence genes. All isolates were successful in processing of sequencing that was performed and based on NCBI-Blast Alignment identification and un weighted Pair Group method with Arithmetic Mean (UPGMA tree) (MEGA 6.0 version) (Tamura *et al.*, 2013). The sequence variability within particular genes can be used in molecular imprinting plans to determine the relatedness of bacteria. Genetic material of microorganisms are predisposes mutableness due to

```
Score           Expect   Identities       Gaps        Strand
802 bits(434)   0.0      498/530(94%)     0/530(0%)   Plus/Plus

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Sbjct  78   TATCAAATAATGTAAATACTTATGGGGTTTCAACAGCGAGAAAGTTACCAGAGATTAAAA  137

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
            |||| || || || |||||||||||||||||||||| ||  || ||||||||||||||||
Sbjct  138  ATGGCTCTGTCGTAATGGCTACTGGTCAACTTCTTGGAGATGGAAAAATTAGATACACGT  197

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
            |||||||||| |||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  198  TTACAGATTATATTGATTATAAAGTGAATGTAATAGCAAATTTAAACTTGAATTTATTTA  257

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
            ||||||||||||||||||||||||||||||||| |||||||||||||||||||||||   
Sbjct  258  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGAAACACTTACTTCAAAATTAAATGGGA  317

Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
            ||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  318  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGATGGAGTTGGAAAGTATTATACAA  377

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTCGCTT  420
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  378  ACCTGAATGGGTCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  437

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
            | |||||||||||||| |||||||||||||||||||||||||||||||| || || ||||
Sbjct  438  ACGTTAAACCGATAAACGGAAATAAATCTGAAAGTGTATCTATTACTGGTAGTTTGACAC  497

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
            |||| ||||||||||||||| | ||||| |||||||||||||||||||||
Sbjct  498  AAGGTAGTAATGTAAGTGGTGATTCACCCATTGTTAAAGTGTATGAGTAT  547
```

Fig. 12: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 181(Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073762.1) with nucleotide sequence identity (94%).

mutation or recombination. Roetzer *et al*., (2013) suggested that Whole Genome Sequencing WGS was superior to conventional genotyping for pathogen tracing and investigating micro-epidemics. WGS provides a

```
Score           Expect   Identities       Gaps          Strand
808 bits(437)   0.0      499/530(94%)     0/530(0%)     Plus/Plus

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Sbjct  18   TAAAATATAAATTGAAATTTGAAGATGGACTAAAAAAAGGAGATTACTTTGATTTTACAT  77

Query  61   TATCAAATAATGTAAATACTTATGGAGTTTCAACAGCTAGAAAAGTACCAGAGATTAAAA  120
Sbjct  78   TATCAAATAATGTAAATACTTATGGGGTTTCAACAGCGAGAAAGTTACCAGAGATTAAAA  137

Query  121  ATGGATCAGTTGTTATGGCTACTGGTCAACTTCTTGAAGGGGGTAAAATTAGATACACGT  180
Sbjct  138  ATGGCTCTGTCGTAATGGCTACTGGTCAACTTCTTGGAGATGGAAAAATTAGATACACGT  197

Query  181  TTACAGATTACATTGATTATAAAGTGAATGTAACAGCAAATTTAAACTTGAATTTATTTA  240
Sbjct  198  TTACAGATTATATTGATTATAAAGTGAATGTAATAGCAAATTTAAACTTGAATTTATTTA  257

Query  241  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGTAACACTTACTTCAAAATTAAATGAAC  300
Sbjct  258  TAGACCCTAGAATCGTTAAAAATAATGGTGAAGAAACACTTACTTCAAAATTAAATGGGA  317

Query  301  AAAATACTGaaaaaaaGATTGAAGTTGAGTATAAAGACGGAGTTGGAAAGTATTATACAA  360
Sbjct  318  AAAATACTGAAAAAAAGATTGAAGTTGAGTATAAAGATGGAGTTGGAAAGTATTATACAA  377

Query  361  ACCTGAATGGATCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  420
Sbjct  378  ACCTGAATGGGTCAATTGAAACATTTAATAAAGCGGATAATAAATTCACACATGTAGCTT  437

Query  421  ATGTTAAACCGATAAATGGAAATAAATCTGAAAGTGTATCTATTACTGGCAGCTTAACAC  480
Sbjct  438  ACGTTAAACCGATAAACGGAAATAAATCTGAAAGTGTATCTATTACTGGTAGTTTGACAC  497

Query  481  AAGGCAGTAATGTAAGTGGTAAATCACCAATTGTTAAAGTGTATGAGTAT  530
Sbjct  498  AAGGTAGTAATGTAAGTGGTGATTCACCCATTGTTAAAGTGTATGAGTAT  547
```

Fig. 13: Multiple sequence alignment analysis of *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence for local of *S. aureus* No. 195 (Query) with NCBI-Blast of *S. aureus fnbA* gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast *S. aureus fnbA* gene (LC073762.1) with nucleotide sequence identity (94%).

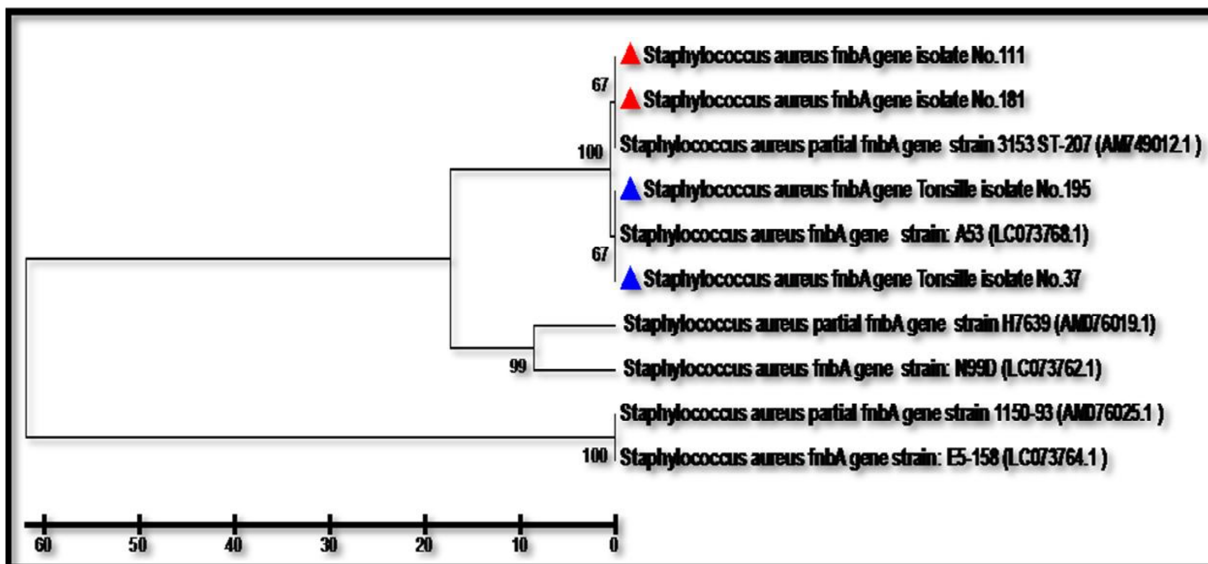

Fig. 14: Phylogenetic tree analysis based on the *S. aureus fnbA* gene for fibronectin-binding protein. A partial sequence that used for genetic analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *S. aureus* isolates (No.37 and No.195) were show closed related to NCBI-Blast *S. aureus fnbA* gene (LC073768.1) and the local *S. aureus* isolates (No.111 and No.181) were show closed related to NCBI-Blast *S. aureus fnbA* gene (AM749012.1)whereas other NCBI-Blast *S. aureus fnbA* gene were show different out of tree.

Table 3: The NCBI-BLAST Homology Sequence identity (%) between local *S. aureus fnbA* gene isolates and NCBI-BLAST submitted *S. aureus fnbA* gene Isolates.

Isolate No.	Isolate source	NCBI-BLAST Homology Sequence identity (%) of *S. aureus fnbA* gene			
		LC073768.1	AM749012.1	LC073762.1	AM076019.1
37	Tensile	100%	99%	94%	94%
111	Oropharynx	99%	*100%*	94%	94%
181	Oropharynx	99%	100%	94%	94%
195	Tensile	100%	99%	94%	94%

measure of genome evolution over time in its natural host context. Adherence genes were amplified using specific PCR primers as it was mentioned in Table 2 for *S. aureus and M. catarrhalis* isolates which gave a specific PCR products (200 and 120 bp respectively as shown in Fig. 4 and 5. The results of sequencing of specific gene to detect adherence associated bacteria *S. aureus* isolates, revealed that the analysis results of amino acids for adherence gene for isolates (No. 37, No. 111, No.181 and No.195) was identified in percentage range of 530/530 (100%), by comparing its sequence with that in the database in gene bank by blast program with gaps in percentage range 0/530 (0%) as shown in Fig. 6, 7 , 8, 9 and 499/530 (94%) with gaps percentage 6/530 (6%) as shown in Fig. 10, 11, 12 and 13. The results of current study was shown there is more than one mutation in one isolate. This displays that, the type and location of mutation that there were found could lead to a differences in the effect of these mutations, and some of these mutations leading to change in the genetic code. then change in the amino acids at the translation. However, these mutations in our isolates missense unaltered the function of the gene. ORF program was a perfect tool, which used for reading each nucleotide sequence to 4-6 segments of the genetic codes with its translation to amino acids with six open reading frames three in direction 5-3 and other three reading in the direction 3-5, one of them is proper Methionine (start codon) and the ends by stop codon. Its provide high information about amino acids translation for each sequence, also provide information used in the submission data of this study to Gene Bank databases information for recording and publishing isolate of this study. *fnbA* gene were appear to be conserved the identity of four isolates were (100%) as shown in Fig. 6, 7, 8 and 9, whereas isolates with were lass conserved which gene (94%) identity where compared with the standard isolates as shown in Fig. 10, 11, 12 and 13. This was explained because was a conserved region of the active gene in which there annealing primer. In a study, there was no differences were found among local and standard isolates which associated with clinical samples. The results of *fnbA* gene sequence analysis was showed that there was some variation the identity was (94%) when compared with the standard isolates,

all this mutations were unfunctional because they don't change the genetic code or stop the portion translation but gene variation. While in Fig. 14, the local *S. aureus* isolates (No. 37 and No. 195) were show closed related to NCBI-Blast S. aureus fnbA gene (LC073768.1) and the local *S. aureus* isolates (No.111 and No. 181) were show closed related to NCBI-Blast *S. aureus fnbA* gene (AM749012.1) whereas other NCBI-Blast *S. aureus fnbA* gene were show different out of tree. The compared between local and global isolates by using adherence gene revealed variety in relationship as shown in Table 3. The results of sequencing of specific gene to detect adherence associated bacteria *M. catarrhalis* isolates revealed that the analysis results of amino acids for adherence gene for isolates (No.10) was identified in percentage of 475/475(100%) by comparing its sequence with that in the database in gene bank by Blast program with gaps in percentage 0/475 (0%). While second isolate (No. 49) was identified in percentage of 478/479 (99%) by comparing its sequence with that in the database in gene

```
Score          Expect   Identities       Gaps        Strand
878 bits(475)  0.0      475/475(100%)    0/475(0%)   Plus/Plus

Query  1    TGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGAAGATGT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  404  TGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGAAGATGT  463

Query  61   GGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATCAGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  464  GGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATCAGCACA  523

Query  121  AACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGCATTGTA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  524  AACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGCATTGTA  583

Query  181  TACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCAGTTGCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  584  TACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCAGTTGCA  643

Query  241  AGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCAGCTTGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  644  AGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCAGCTTGG  703

Query  301  GCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCACGCCAGA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  704  GCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCACGCCAGA  763

Query  361  ATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTACAACCA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  764  ATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTACAACCA  823

Query  421  AACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTAATACC  475
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  824  AACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTAATACC  878
```

Fig. 15: Multiple sequence alignment analysis of *M. catarrhalis* (*mcaP*) gene partial sequence for local of *M. catarrhalis* (No. 10) (Query) with NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (AY291294.1) with nucleotide sequence identity (100%).

```
Score            Expect   Identities       Gaps          Strand
880 bits(476)    0.0      478/479(99%)     0/479(0%)     Plus/Plus

Query  1    ATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGAAG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  400  ATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGAAG  459

Query  61   ATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATCAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  460  ATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATCAG  519

Query  121  CACAAACTCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGCAT  180
            ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  520  CACAAACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGCAT  579

Query  181  TGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCAGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  580  TGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCAGT  639

Query  241  TGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCAGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  640  TGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCAGC  699

Query  301  TTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCACGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  700  TTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCACGC  759

Query  361  CAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTACA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  760  CAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTACA  819

Query  421  ACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTAATACC  479
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  820  ACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTAATACC  878
```

Fig. 16: Multiple sequence alignment analysis of *M. catarrhalis* (*mcaP*) gene partial sequence for local of *M. catarrhalis* (No. 49) (Query) with NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (AY291294.1) with nucleotide sequence identity (100%).

bank by blast program with gaps in percentage 0/479 (0%). The third isolate (No. 112) was identified in percentage of 477/477 (100%) by comparing its sequence with that in the database in gene bank by Blast program with gaps in percentage 0/477 (0%) and the last isolate (No. 117) was identified in percentage of 473/473 (100%) by comparing its sequence with that in the database in gene bank by blast program with gaps in percentage 0/473 (0%) as shown in Fig. 15, 16, 17 and 18. While in Fig. 19. The local *M. catarrhalis* isolates (No. 10, No.

Score	Expect	Identities	Gaps	Strand
881 bits(477)	0.0	477/477(100%)	0/477(0%)	Plus/Plu

```
Query  1    CCAATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  397  CCAATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAG  456

Query  61   AAGATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  AAGATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCAT  516

Query  121  CAGCACAAACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517  CAGCACAAACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAG  576

Query  181  CATTGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  577  CATTGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCC  636

Query  241  AGTTGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  637  AGTTGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGC  696

Query  301  AGCTTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  697  AGCTTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCA  756

Query  361  CGCCAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  757  CGCCAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCT  816

Query  421  ACAACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTA  477
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  817  ACAACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTGCTA  873
```

Fig. 17: Multiple sequence alignment analysis of *M. catarrhalis* (*mcaP*) gene partial sequence for local of *M. catarrhalis* (No. 112) (Query) with NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (AY291294.1) with nucleotide sequence identity (100%).

112 and No. 177) were show closed related to all NCBI-Blast *M. catarrhalis* in Phylogenetic tree whereas The local *M. catarrhalis* isolates (No. 49) were show different out of tree. The compared between local and global isolates by using adherence gene revealed variety in relationship as shown in Table 4.

Recording of Iraqi *S. aureus* and M. catarrhalis in gene bank-NCBI.

Four isolates of *S. aureus* and four isolate for *Moraxella catarrhalis* were isolated from throat infection in AL-Hilla city and each isolate have a symbol

```
Score           Expect   Identities       Gaps        Strand
874 bits(473)   0.0      473/473(100%)    0/473(0%)   Plus/Plus

Query  1    CAATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  399  CAATGATGGAACAACCTTAACTGGTACAAACTATGCCGTTGGTGGTGCAAGAACTAAAGA  458

Query  61   AGATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  459  AGATGTGGTCAAAAATGCACCTGTTCCTTTTTTCACCATTCCTTTATTTACCATCCCATC  518

Query  121  AGCACAAACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  519  AGCACAAACCCAAATCAATCGCTACCTGACCTTAAATAATCATCAAGCCGACCCCAAAGC  578

Query  181  ATTGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  579  ATTGTATACTGTTTGGACAGGTGCCAATGATTTGTTTGAGGCAGCCAAAGCACCAACCCA  638

Query  241  GTTGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  639  GTTGCAAGCAGCCGAAATCATTACCACCGCTGCCAATGACCAAGCCAATTTGGTTGGGCA  698

Query  301  GCTTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  699  GCTTGGGCAAGCAGGTGCAAAACACATTTTAGTACCCAGTCTTCCTGATGTTGGCGTCAC  758

Query  361  GCCAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  759  GCCAGAATACGCCCAAGATCCGACCAAATCTGCCACAGCATCATTGTCTGCTCATATCTA  818

Query  421  CAACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTG  473
            |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  819  CAACCAAACTTTATATCAAAGTTTAAACAACCAAACCACCAATGTCATTGCTG  871
```

Fig. 18: Multiple sequence alignment analysis of *M. catarrhalis* (*mcaP*) gene partial sequence for local of *M. catarrhalis* (No. 117) (Query) with NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (Sbjct) by using (NCBI-BLAST online). The multiple alignment analysis were shown no SNP compared to standard NCBI-Blast of *M. catarrhalis* (*mcaP*) gene (EF075933.1) with nucleotide sequence identity (100%).

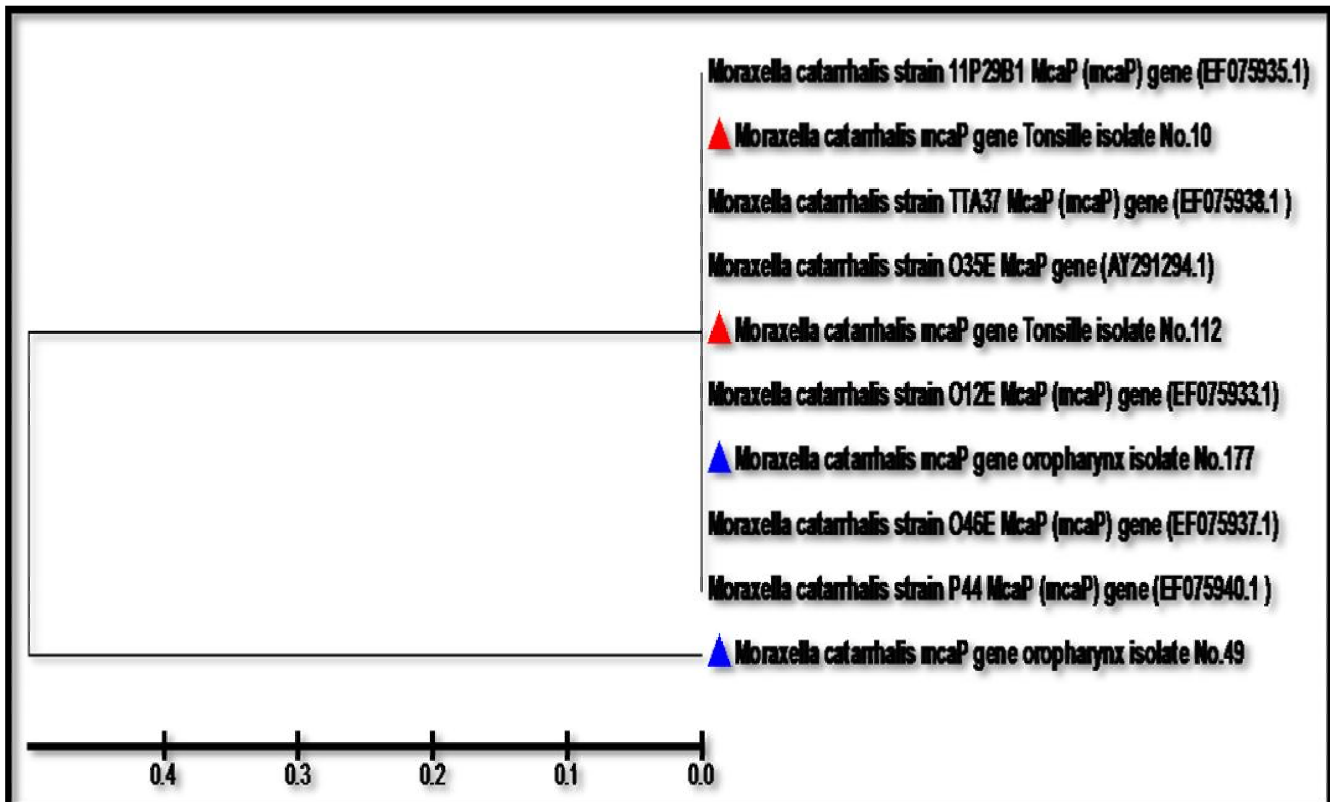

Fig. 19: Phylogenetic tree analysis based on the *M. catarrhalis* (*mcaP*) gene partial sequence that used for genetic analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *M. catarrhalis* isolates (No.10, No.112, & No.177) were show closed related to all NCBI-Blast *M. catarrhalis* in Phylogenetic tree whereas The local *M. catarrhalis* isolates (No.49) were show different out of tree.

Table 4: The NCBI-BLAST Homology Sequence identity (%) between local *M. catarrhalis* (*mcaP*) gene isolates and NCBI-BLAST submitted *M. catarrhalis* (*mcaP*) gene Isolates.

Isolate No.	Isolate source	NCBI-BLAST Homology Sequence identity (%) of *M. catarrhalis (mcaP) gene*					
		AY291294.1	EF075933.1	EF075935.1	EF075937.1	EF075938.1	EF075940.1
10	Tensile	100%	99%	99%	99%	99%	99%
49	Oropharynx	99%	99%	99%	99%	99%	99%
112	Tensile	100%	100%	100%	100%	100%	100%
177	Oropharynx	100%	100%	100%	100%	100%	100%

code for each gene in Gen-Bank. The *fnbA* gene for isolate (37) for *S. aureus* have the code bank MH379989. Isolate (111) have the code MH379990. Isolate (181) have the code MH379991, and isolate (195) have the code MH379992. The *mcap* gene for isolate (10) for *M. catarrhalis* have the code bank Mh379993. Isolate (49) have the code MH379994. Isolate (112) have the code MH379995 and isolate (177) have the code MH379996. All these sequences are accepted in gene bank for the first time in gene bank, take place in the gene bank database under the above submission number, and realized in 24 / 2018.

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