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Molecular Identification of the enterotoxin B gene from *Staphylococcus aureus* and the enterobactin B gene from *Klebsiella pneumoniae*

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Abstract
One hundred and five of clinical samples were collected from Central Teaching Hospital of paediatric and Medical city / Educational laboratory, period from 17/10/2016 to 23/1/2017, *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates were diagnosed molecularly by using polymerase chain reaction, enterobactin B and enterotoxin B genes were amplified by using multiplex PCR. Multiplex PCR was performed for *K. pneumonia* and *S. aureus* isolates with two primers that target the *ent B* (enterobactin B) and *entB* (enterotoxin B) genes respectively. PCR was performed with the primer that targets the *ent B* (enterobactin B) gene, the result revealed that all eight samples of the *K. pneumoniae* isolates gave a band of 330 bp in size. On the other site the other primer that targets the *ent B* (enterotoxin B) gene, the result shows that only two isolate of *S.aureus* having a band of 411 bp in size. The reason leads to not all *S. aureus* species had enterotoxin B.

Keywords: Molecular Identification, enterobactin B, enterotoxin B

Introduction
Bloodstream infection are the most common cause of sepsis, so there are more than 45% of BSI are caused by single bacterial species, which can introduce in the blood, such opportunistic pathogens (e.g., *Staphylococcus aureus, Escherichia coli, Acinetobacter* spp, *Klebsiella pneumoniae*) (Wisplinghoff *et al*., 2004) [34]. *Staphylococcus aureus* have different virulence factors which give the bacteria the ability to invade the host, such as surface proteins that promote colonization and invasiveness (leukocidin, kinases, hyaluronidase) and surface factors that inhibit phagocytic engulfment (capsule, Protein A). *Klebsiella pneumoniae* have different virulence factors which gave the bacteria the ability to invade the host, such as capsular polysaccharide, lipopolysaccharide, serum resistance, siderophore production, fimbriae and other factors such as the production of urea and enterotoxin (Aher *et al*., 2012) [1]. The pathogenicity of *Staphylococcus aureus*, is related to production of wide variety of exoproteins, including alpha and beta haemolysins which contributes to its ability to cause diseases in humans (Dinges *et al*., 2000) [9]. Alpha-haemolysin or alpha toxin considered to be a main pathogenicity factor because of its haemolytic, dermonecrotic and neurotoxic effects. Additionally, beta-haemolysin contains sphingomyelinase that more active against sheep and bovine erythrocytes (Da-Silva *et al.*, 2005) [8]. Staphylococcal enterotoxin B (SEB) is one of the 20 exotoxin excreted by the *Staphylococcus aureus* bacterium; Staphylococcal enterotoxin B (SEB) is the toxin most commonly associated with classic food poisoning, It has also been demonstrated to cause a nonmenstrual toxic shock syndrome (TSS) (Hennekinne *et al*., 2012). PCR was used to amplify the chromosomal *entB* (enterobactin B) gene that found in all *Klebsiella pneumoniae* species, this gene is responsible for iron uptake system so it was used to identify the virulence of the bacteria that cause many diseases in bloodstream by using specific primers (Aljanaby and Alhasani, 2016) [3]. PCR was used to detect *ent B* gene encoding the enterotoxin B (Padmapriya *et al*., 2003) [27]. The *entB* (enterobactin B) chromosomal virulence gene responsible for an iron uptake system (codes for an iron uptake system), is a putative pathogenic gene (Schaible and Kaufmann, 2004) [31]. Therefore, this study was aimed to the isolation and identification of pathogenic bacteria in the blood and detection of some virulence factors such as haemolysin, capsule protease, gelatinase, siderophore, bacteriocin and molecular Identification of *entB* genes.

Materials and Methods
Collection of samples
One Hundred and five samples of blood were collected from patients suspected of having

blood stream infection and certain clinical symptoms. These samples were collected from Medical city Hospital / educational laboratories and Central Teaching Hospital of pediatric in Baghdad. Samples were collected from different age groups and genders from 17/10/2016 to 23/1/2017.

Blood samples
Blood is drawn from patients by using a syringe (5 ml). It is immediately transferred to a clean sterilized brain heart infusion broth tube, the blood is then allowed to clot for at least 10 to 15 minutes at room temperature, then kept in an incubator for 18 hours for further laboratory investigations (Tille *et al*., 2013) [32].

Identification of some virulence factors
After bacteria has been isolated by culturing, microscopic examination and finally identified morphologically and physiochemical characteristics, the most common bacteria has been isolated was *Staphylococcus aureus* and *Klebsiella pneumoniae*, some virulence factors will be detected for those two common bacterial isolates as follows:

Protease enzyme production test
This test was done by culturing bacterial isolates with surface streak with loop method on skim milk agar plates and then plates were incubated at 37 ºC for 24 hrs. The positive result indicated by the formation of the clear (halo) zone around bacterial growth (Elsner *et al*., 2000) [13].

Gelatinase enzyme production test
The gelatinase activity of the isolates was investigated by two different tests. In the first test, performed with tubes, a few cells from a pure culture was inoculated into 5 ml of medium consisting of trypticase soy agar and 3% gelatine, and then incubated at 37 ºC for 24 hours at 37 °C, gelatinase activity was revealed as liquefaction of the medium converting semi-solid agar tube to liquid. The second test, performed with plates. Bacterial cells were streaked as a single line across the center of a plate with trypticase soy agar supplemented with 3%gelatine and then incubated at 37 ºC for 24 hours at the optimal growth temperature. A clear zone around the growth of the bacteria indicated gelatinase activity (Goldman and Green, 2015) [16].

Siderophore production test
This test was performed by culturing bacterial isolates with surface streak with loop method on minimal 9 medium plates were previously attended. The results indicated by the growth of organism were present or not (Nassif and Sansonetti, 1986) [26].

Bacteriocin production test
The ability of bacterial isolates identified as *Staphylococcus aureus* and *Klebsiella pneumoniae* for bacteriocin production was screened by inhibition of the test microorganism (*E. coli*) growth on agar plates. The antagonistic effect against test microorganism was achieved by the cup assay method according to (Libertin *et al*., 1992) [23] and as follows:

1. Bacterial isolate that wants to detect were inoculated on brain heart infusion broth supplemented with 5% glycerol, then incubated at 37 ºC for 18 hours, with shaking (220 rpm).
2. After the end of incubation period, a drop with sterile loop was takenand inoculated on brain heart infusion agar plates (supplemented with 5% glycerol) by mate streaking then plates incubated at 37 ºC for 18 hours.
3. Holes or wells were done as disc agar in inoculated plates (step:2) by sterile cork pourer of 5 Mm in diameter.
4. The detected isolate was inoculated into brain heart infusion broth (37 ºC for 2 hours), then take 0.1ml from it by pipette and cultured on brain heart infusion agar and let to dry (37 ºC for 10 minutes).
5. The disc agar was transferred carefully into the surface of agar that inoculated with detected isolate and then incubated at 37 ºC for next day, positive result indicated by inhibition zone (can put 5 disc agar in the same plate of detected isolates).

DNA extraction
Kit: The following kit was used for isolation of DNA

Table 1: Used Kit

Kit	Company/origin
Presto™ Mini gDNA Bacteria Kit used for DNA extraction from bacteria	Geneaid/Taiwan

DNA markers : The following marker was used as ladder in DNA visualizing

Table 2: Used ladder

DNA Marker	Description	Company/ origin
100 bp Ladder	100-1500 base pairs (bp). The ladder consists of 11 double strand DNA fragment ladder with size of (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 bp). The 500bp present at triple the intensity of other fragments and serve as a reference. All other fragments appear with equal intensity on the gel.	Bioneer/ Korea

Electrophoresis solutions and buffers
10X Tris-Acetate-EDTA (TAE) buffer solution
This solution was prepared to be consisting of the followings:
Tris-OH 54 gm
Acetic acid 27.5 gm1
EDTA 20 ml
All components were dissolved in 1000 ml of distilled water, pH was adjusted to 8, and the solution was kept at room temperature until use.

Ethidium bromide solution (10mg/ ml)
It was prepared by dissolving 1 gm of ethidium bromide in 100 ml of distilled water, stirred on a magnetic stirrer until ethidium bromide was completely dissolved, then it was filtered using What man filter paper No. 1 and stored in dark bottle at 4 °C until use.

Agarose Gel (1%)
It was prepared by dissolving 1g of agarose in 50ml of freshly prepared TAE buffer 1X and

PCR Master mix: (Promega / U.S.A)
The PCR master mix was supplied to be containing the following components:

Table 3: PCR Master Mix components

Material	Concentration
PCR buffer (PH=8.5)	2X
MgCl2	3mM
dNTPs	400mM
Taq DNA polymerase	5 units

Primers (Promega / U.S.A)
Primers used in this study were provided in lyophilized form, and were dissolved in sterilized distilled water to give a final concentration of 10 picomole/µl; these primers have the following sequences, the following primers were used in this study to identify the target genes in *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Table 4: Used primers

No.	Target gene	Primer	Oligo sequence (5'-3')	Product size (bp)	Ref.
1	*ent B*	*entB*-F	ATTTCCTCAACTTCTGGGGC	371	(Fertas *et al*., 2013)
		entB-R	AGCATCGGTGGCGGTGGTCA		
2	*ent B*	*entB*-F	TCGCCTTATGAAACGGGATA	411	(Padmapriya *et al*., 2003) [27]
		entB-R	ACAAATCGTTAAAAACGGCG		

Extraction of DNA from the *Klebsiella* and *Staphylococcus* isolates
Genomic DNA was extracted according to the Geneaid company manufacturer's instructions.

Genomic DNA mini-kit bacterial protocol
- Bacterial culture was inoculated in 5 ml Brain heart infusion broth and incubated at 37 ºC for 24 hrs.
- A 1.0 ml was distributed in 1.5 ml Eppendorf tube, centrifuged at 14000-16000 xg for 1 min., and then the supernatant was discarded and thepellet was taken.
- A 200 µl of GT buffer was added, the cell pellet was resuspended by vortex or pipetting, and incubated at room temperature for 5 min.
- A 200 µl of GB buffer was added and mixed by shaking vigorously for 5 sec.
- The sample lysate was incubated at 60 Cº for 10 min. until is clear, during incubation the tube was inverted every 3 min.
- A 200 µl of absolute ethanol was added to the sample lysate and immediately mixed by vortex.
- A GD column was placed in a 2 ml collection tube then all mixture (including precipitate) was transferred to the GD column, centrifuged at 14000-16000 xg for 2 min. The 2 ml collection tube containing the flow through was discarded and the GD column was placed in a new 2 ml collection tube.
- A 200 µl of W1 buffer was added to the GD column, centrifuged at 14000-16000 xg for 30 sec. The flow through was discarded and the GD column was placed back in the 2 ml collection tube.
- A 600 µl of wash buffer was added to the GD column, centrifuged at 14000-16000 xg for 30 sec. The flow through was discarded and the GD column was placed back in the 2 ml collection tube, centrifuged again at 14000-16000 xg for 3 min. to dry column matrix.
- The dried GD column was transferred to clean 1.5 ml Eppendorf tube.
- A 100 µl of preheated elution buffer was added or TE to the center of the column matrix, let stand for 3-5 min., centrifuged at 14000-16000xg for 30 sec. to elute the purified DNA.

Agarose gel preparation: (Green and Sambrook, 2012) [17]
Agarose gel of 1% concentration was utilized to confirm the size of genomic DNA bands and to confirm the size of the PCR products. The agarose gel consists of 1g dissolved in 50 ml of 1X TAE buffer using a microwave. After the agarose solution cools down to 55-60 ºC, a 1 µl of 0.5 µg/ml final concentration ethidium bromide was added. Then, the solution was poured into the gel tank with the combs in place and let to cool for 45 min. The combs were removed carefully and the tank was placed in the electrophoresis system containing running buffer consisting of 1X TAE, the buffer is poured until it covers the gel for about 1-2 mm. Ten microliters of each PCR product and DNA ladder (100 bp) were loaded into the wells, the system cover was then placed and the system was turned on Electrophoresis was performed for 1hrs, with a 70 volt/35 mAmp current. The DNA bands were visualized with a UV transilluminator.

Preparation of primers solution
The lyophilized primer was dissolved using deionizer distal water DDH2O to obtain 100 pmol/µl in the master tube, then 10 pmol/µl was prepared as a working solution by taking 10 µl from the master tube and completed the volume to 100 µl by adding DDH2O.

PCR mixture and PCR program conditions
PCR reactions were performed in 20 µl volumes in PCR tubes under sterile conditions, all the volume of the reaction mixture was completed to 20 µl using sterile DDH2O. All amplification experiments included a negative control blank which contained all PCR material with the exception of target DNA. Mixture and program condition of primers were listed as follows:

Enterobactin diagnosis of *Klebsiella pneumoniae* by *entB* gene
Enterobactin was diagnosed with PCR by using the primer specific for *entB* gene. The master mix (lyophilized) contained optimum concentrations of reaction requirements ($Mgcl_2$ 1.5 mM, Taq polymerase 1 U, each dNTPs 200 µM). The reaction mixture was illustrated in table (5):

Table 5: PCR mixture

PCR reaction components	Volume (µl)
Master mix	12.5
Primer F (10 picomoles/µl)	1
Primer R (10 picomoles/µl)	1
DNA template 2.5	4
DDH2O	6.5
Total volume	25

The optimal condition for detection of *entB* gene was adjusted as in the table (6).

Table 6: Program of PCR thermocycling conditions

No.	Steps	Temp. (ºC)	Time	No. of cycle
1	Initial denaturation	94	5 min.	1 cycle
2	Denaturation	94	30 sec.	
3	Annealing	57	30 sec.	35 cycle
4	Extension	72	1 min.	
5	Final extension	72	10 min.	1 cycle

Enterotoxin diagnosis of *Staphylococcus aureus* by *entB* gene

The second set of PCR reaction was performed for diagnosing enterotoxin by using the primer specific for the *entB* gene.

Results and Discussion

Isolation of bacteria

Blood samples from a total of 105 clinical different blood samples were collected from Central Teaching Hospital of paediatric and Medical city / Educational laboratory, In Baghdad /Iraq. Table (7) samples were collected from different age groups and gender during the period from17/10/2016 to 23/1/2017. Seventy (66.6%) were clinical blood positive samples, while the rest (35) were negative blood samples (33.3 %). The relationship of BSI with the age of patients was investigated in this study and the patients were grouped into three categories according to their age as shown in table (7).

Table 7: Total number of samples used for the isolation of bacteria

Clinical sample	Positive (growth)	Negative (no growth)
(105)	70	35
Percentage	66.66%	33.33%

Identification of bacterial isolates

Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Seventeen isolates were obtained from one hundred and five samples.Results showed that *Klebsiella* spp. constitute 17.1% (12 isolates), and identified as *K. pneumoniae*, *Staphylococcus* spp. Constitute 15.7% (11 isolates), *Staphylococcus aureus* constitute 24.2% (17 isolates).

The other bacterial isolates were constituted *Escherichia coli* 14.2% (10 isolates), *Acinetobacter baumannii* 14.28% (10 isolates), *Proteus* spp. 2.82% (2 isolates), *pseudomonas aeruginosa* 7.14% (5 isolates), and *Enterobacter* spp. 4.28% (3 isolates). Figure (1) illustrates the percentages of each bacterial species found in the collected samples.

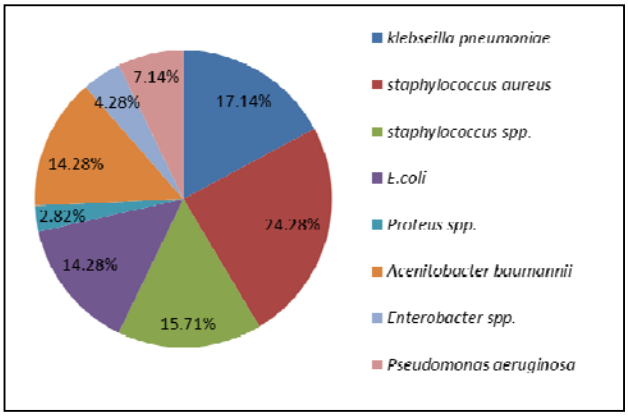

Fig 1: Bacterial isolates obtained from blood samples.

Bacterial isolates were identified according to their cultural, microscopical and biochemical characteristics that were in agreement with Holt *et al.*, (1994) [20], Atlas *et al*., (1995) [5] and Collee *et al*., (1996) [7].

Identification of some virulence factors

Haemolysis production

The result shows complete hemolysis to the blood type β-haemolysis, figure (2) this result agrees with Dinges *et al*. (2000) [9] that shows *Staphylococcus aureus* cause hymolysis to blood in three types α, β, and γ. The results shows 17 (100%) isolate of *Staphylococcus aureus* are positive result for production of heamolysin enzyme. This result agree with Wiseman, (1975) [33], which shows that all isolates of *Staphylococcus aureus* cause heamolysis to blood, also agrees with Zhang *et at.,* (2016) [36], and disagrees with Ariyanti *et al.,* (2015), which shows that 27,27% of β-hemolysis of *Staphylococcus aureus*

Figure (3) shows *Klebsiella pneumoniae* on blood agar medium, bacterial isolates were large, mucoid, white to grey and Non-heamolytic colonies. The result shows that non-heamolytic to blood (γ-hemolytic), all 12 isolates are negative for heamolysis. The result agrees with Kang *et al*., (2003), Lee *et al.,* (2005) [22], which shows of non- haemolytic activity of *klebsiella pneumonia.*

Fig 2: *K. pneumonia* on blood agar (A), *S. aureus* on blood agar (B)

Capsule production

The *Klebsiella* isolates were found to be Gram negative, non-motile, small straight rods and arranged singly or in pairs under the compound light microscope (figure 3 A) as described by Garrity (2005) [15]. All isolates showed a distinct capsule as clear zone surrounding bacteria when they examined under oil immersion after capsule staining as shown in figure (3 B).

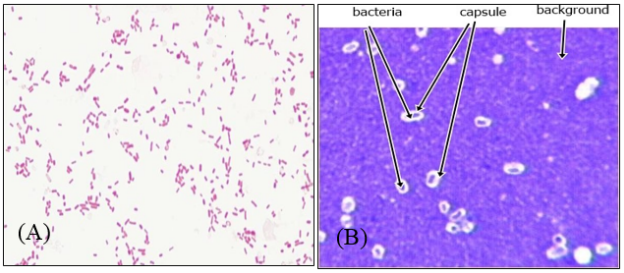

Fig 3: *K. pneumoniae* gram stains (A), *K. pneumoniae* capsule stain (B).

Protease production

Figure (4) shows surface streak with loop method, all *Klebsiella pneumoniae* isolates were found to produce protease enzyme. The formation of clear zone (12 mm or

more) in the medium surrounding the well indicated positive protease activity as described by Mazzucotelli *et al*., (2013) [25]. All *Staphylococcus aureus* isolates has been found to produce protease enzyme. Positive result indicated by formation of halo around streaking as described by Dapeau *et al*., (1972) [11].

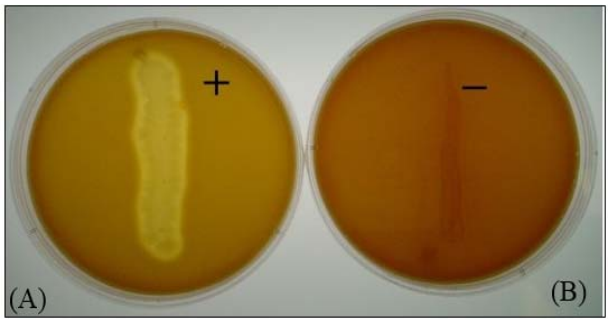

Fig 4: Surface streak with loop, positive result (A), negative result (B)

Gelatinase production

Staphylococcus aureus isolates when cultured on trypticase soy agar plates supplemented with gelatine shows to be small, white, circular, slightly convex, entire, smooth, glistening colonies. Compared with *Klebsiella pneumoniae* which shows to be big, white, muccoid, singly in pairs or short chains. This shows in figure (5, 6).

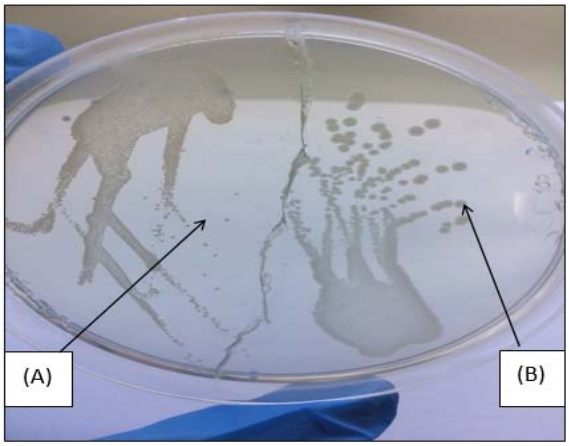

Fig (5): *S. aureus* (A), *K. pneumoniae* (B).On trypticase soy agar with gelatine

This result agrees with Chakraborty *et al*., (2011) [6]. While all *klebsiella pneumoniae* isolates are negative for gelatinases production, this result agrees with Dootittle *et al*., (2008) [10].

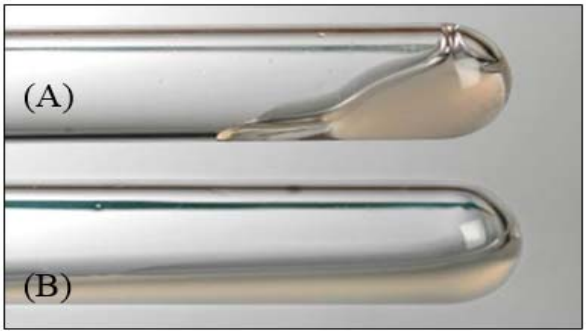

Fig 6: Gelatine liquefaction, positive result (A), negative result (B).

Siderophore production

In this study (17) isolate of *Staphylococcus aureus* and (12) isolate of *klebsiella pneumonia* were collected, siderophore production assay is used to detect the ability of both S. aureus and *K. pneumoniae* isolates to produce this virulence factor. (Table 8).

Table 8: Number of siderophore production isolates

Total Isolation No.	Bacterial isolate	Siderophore producer isolate No.
17 isolate	*S. aureus*	13 (76.4%)
12 isolate	*K. pneumoniae*	9 (75%)

Siderophore production has been shown more frequent in *Klebsiella* isolates than in *Staphylococcus*. Figure (7, 8) shows *Staphylococcus aureus* can produce siderophore appear pink on minimal 9 agar plates, this result agrees with Reniere *et al*., (2010) [30]. This test can differentiate S. *aureus* from other species such as S. *epidermis* with is negative for siderophore production, this agrees with Lindsay *et al*., (1995). This result agrees with Aljanaby and Alhasani (2016) [3] and Holden *et al*., (2016) [19]. While Podschun *et al*., (1992) [28] demonstrated that all *klebsiella* species could produce siderophore.

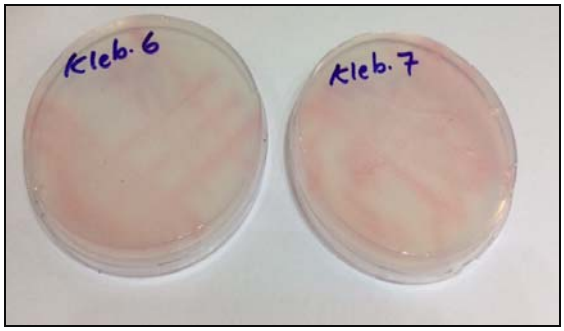

Fig 7: *Klebsiella pneumoniae* on minimal agar plate

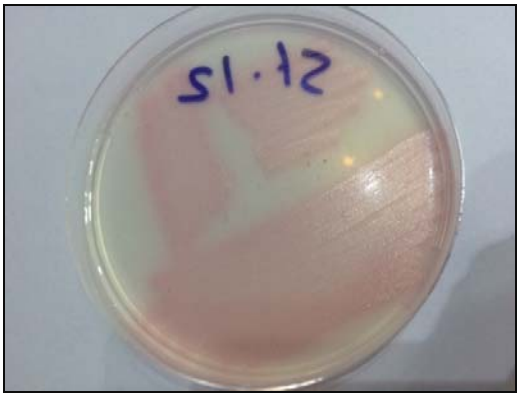

Fig 8: *Staphylococcus aureus* on minimal 9 agar plate

Bacteriocin production

Cup assay or agar-well diffusion method has been used to detect of 17 *Staphylococcus aureus*, 12 *Klebsiella pneumoniae* for bacteriocin production by using brain heart infusion agar plate supplemented with 5% glecerol as growing of producing isolates, the result shows that 5 isolates (K1, K9, K10, K11, K12) of *klebsiella pneumoniae* have the ability to produce bacteriocin that had its effect on some isolates of bacteria (*E. coli* and *Staphylococcus aureus*) as showed in table (9).

Table 9: bacteriocin production of *Klebsiella pneumoniae*

Isolates	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12
E. coli	+	-	-	-	-	-	-	-	+++	+++	+	+++
S. aureus	+	-	-	-	-	-	-	-	+	-	-	+++

No production; + weak production; +++ very good production

The results also shows that 4 isolates (S2, S6, S12, S13) of *Staphylococcus aureus* have the ability to produce bacteriocin that had its effect on some isolates of bacteria (*E.coli* and *Klebsiella pneumoniae*) as showed in table (10).

Table 10: bacteriocin production of *Staphylococcus aureus*

Isolates	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17
E. coli	-	-	-	-	-	+++	-	-	-	-	-	+	+	-	-	-	-
K. pneumoniae	-	+++	-	-	-	-	-	-	-	-	-	+++	-	-	-	-	-

No production; + weak production; +++ very good production

Recent studies had classified Gram + and Gram – bacteria according to producing bacteriocin and declared that S. epidermis also produce bacteriocin (Yang *et al*., 2014) [35]. Other studies determined bacteriocin of *K. pneumoniae* (Klebocin) by cup assay method (Al-Charrakh *et al*., 2011) [2]. The positive result shows clear inhibition zone on brain heart infusion agar plate as shown in figure (9).

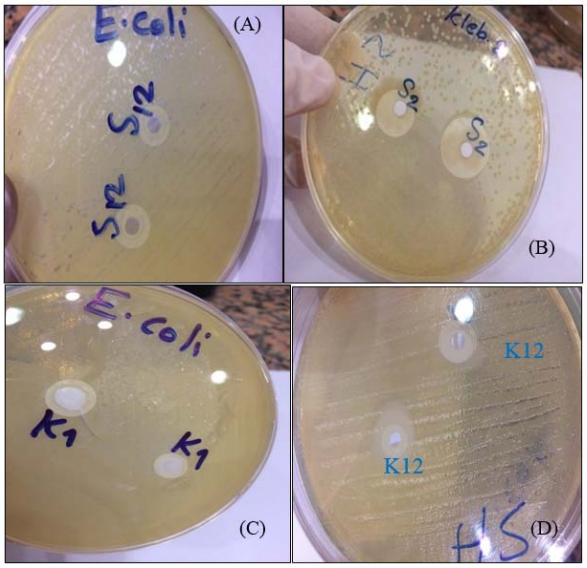

Fig 9: Inhibitory effect of bacteriocin produced by isolated *Staph. aureus* against (A) *E. coli*, (B) *Klebsiella pneumoniae* and *Klebsiella pneumoniae* against (C) *E. coli*, (D) *Staph. aureus* on brain heart infusion agar medium after incubation at 37 °C for 16 hours under anaerobic conditions.

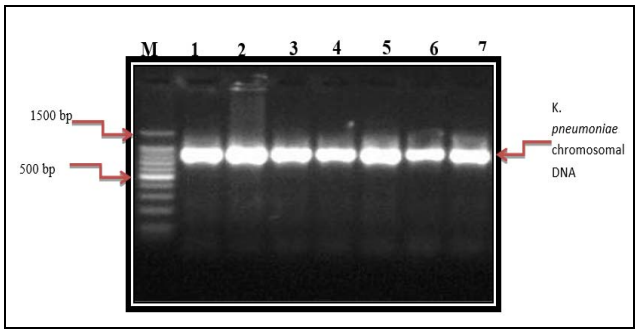

Fig 10: Gel electrophoresis for genomic DNA of *K. pneumoniae* isolates. Lane M is a (1 Kb) ladder, Line: 1 –SH5, 2 – SH9, 3 – SH6, 4 – SH12, 5 – SH8, 6 – SH11, 7 – SH10.

The results were detected for the most active (7) isolates of both *K. pneumoniae* and *S. aureus* and also observed when the DNA samples analysed by gel electrophoresis, in which DNA bands were detected indicating purified DNA samples as shown in figure (11, 12).

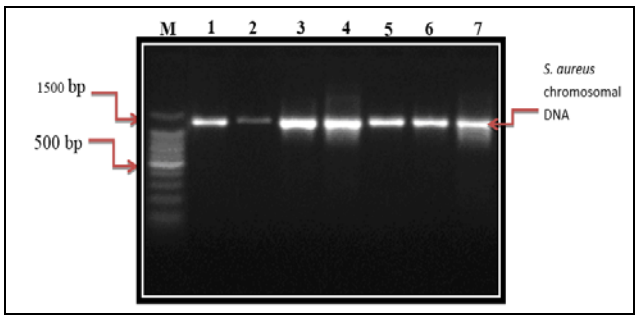

Fig 11: Gel electrophoresis for genomic DNA of S. aureus isolates. Lane M is a (1 Kb) ladder, Line: 1 –SH514, 2 – SH13, 3 – SH8, 4 – SH12, 5 – SH17, 6 – SH7, 7 – SH10.

Amplification of *ent B* gene of *Klebsiella pneumoniae*

Enterobactin-B is a virulence factor was studied, which is receptor that translocate ferric enterobactin across the outer-membrane, as well as its iron uptake system gene (*ent B*) required for the metabolism of iron to sustained growth in the host (El-Fertas-Aissani *et al*., 2013) [12].

In present study the most active eightisolate of *Klebsiella pneumoniae* were subjected to molecular identification through PCR amplification of *ent B* gene using *ent B*-F and *ent B*-R primers which represents specific primers for the PCR amplification of K. pneumonia enterobactin (*ent B*) gene. Results in figure (4-18), showed that the amplified fragments were about 330 bp in size, this result agrees with Aljanaby and Alhasani (2016) [3] whom concluded the same size and same primer. All (8) isolates gave positive results (330 bp bands), and identified as *K. pneumoniae*.

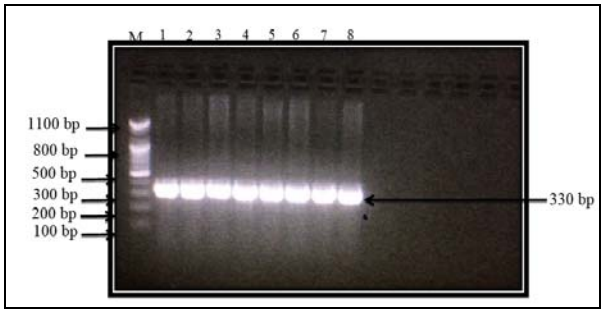

Fig 12: Gel electrophoresis for amplification of entB gene of *K. pneumoniae*. Lane M is a (100 bp) ladder, Line: 1 – SH5, 2 – SH9, 3 –SH6, 4 – SH12, 5 – SH8, 6 – SH11, 7 – SH10, 8 – SH7.

Amplification of *ent B* gene of *Staphylococcus aureus*

Enterotoxin-B is a virulence factor was studied, which is one of the toxins responsible for staphylococcal food poisoning in humans and has been produced by some countries as a biological weapon (Fries and Varshney, 2013) [14]. The most active eightisolate of *Staphylococcus aureus*were introduce to molecular identification by PCR amplification of *entB* gene using *entB*-F and *ent B*-R primers which represents specific primers for the PCR amplification of S. *aureus* enterotoxin (*entB*) gene. Results demonstrated that the amplified fragments were about 411 bp in size as shown in figure (13), this result agees with Ramesh *et al*., (2002) [29] who used the same primer and obtained the same size. Two isolates gave positive results (411 bp bands), while six are negative results. That illustrate not all S. aureus can produce enterotoxin B (Dinges *et al*., 2000) [9]. The positive result identified as S. *aureus*. Resuts of PCR amplification proved that all isolates were S. *aureus*, and confirmed the previous results.

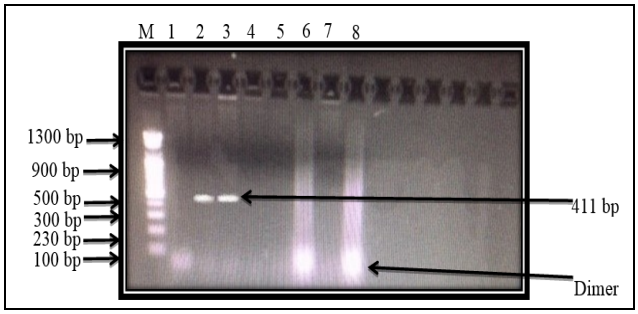

Fig 13: Gel electrophoresis for amplification of entB gene of *S. aureus*. Lane M is a (100 bp) ladder, Line: 1 – dimer, 2 – SH13, 3 – SH8, 4 –negative, 5 –negative, 6 – dimer, 7 –negative, 8 – dimer.

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