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Study Of Some Virulence Factors And Virulence Genes Produced By Gram (+Ve) And Gram (-Ve) Cocci Isolated From Clinical Samples In Al-Anbar Province/Iraq

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***Abstract**: This study aimed to isolate the G (+ve) and G(-ve) cocci from different clinical samples and study some virulence factors and virulence genes produced by them .*

In this study, (177) samples were collected from different clinical sources, including (49) blood samples, (43) urine samples, (30) pus swabs, (40) cervical swabs and (15) sputum samples from patients attending Ramadi Teaching Hospital for Maternity and Children, Ramadi Teaching Hospital in Ramadi in Al-Anbar province/Iraq during the period from 19/12/2018 to 12/5/2019, and (203) isolates were identified as Gram (+ve) and Gram (-ve) bacteria, then (166) Gram (+ve) and Gram (-ve) cocci were chosen as study samples.

After diagnosis of the isolates, results of Gram staining showed that the highest number and percentage of Gram positive and Gram negative bacteria were from urine samples 48(28.9%) followed by blood samples 44(26.5%) then pus swab and cervical isolates 37(22.3%) and 23(13.9%) respectively, while the lowest number and percentage of isolates were from sputum 14(8.4%). Staphylococcus aureus showed the highest number and percentage of isolates 48(28.9%). For gram (-ve) cocci, only (10.4%) of Neisseria gonorrhea was isolated from urine samples and (14.2%) of Moraxella catarrhalis was isolated from sputum samples

Regarding virulence factor production, the hemolysin producer isolates were the highest 103(62.04%), and results of hemolysin producing bacteria was S.aureus, S.hominis, S.haemolyticus, S.chromogenes, S.xylosu and S.warneri showed (100%) beta hemolysis, while only one isolate of the genus Micrococcus was beta-hemolytic all isolates of Streptococcus pyogenes and Streptococcus agalactiae showed (100%) beta hemolysis (β), whereas the isolates of Strept. pneumoniae, Aerococcus urinae and Aerococcus viridans showed (100%) partial or alpha (α) type hemolysis, and (8) isolates of E.faecalis showed complete hemolysis (β-haemolysis).

All S. aureus isolates were (100%) gelatinase producers Kocuria kristinae was (100%) producer, all Strep. pyogenes, Strep. Agalactiae, Strep. Anginosus, Strep. Lc. lactis lactis and Strep.mitis isolates showed (100%) ability to produce this enzyme, The (24) isolates of the genus Enterococcus bacteria were (100%) able to produce the enzyme gelatinize, including (19) E.faecalis isolates and (5) E. faecium. As for the genus Gram (-ve) cocci, two isolates of Moraxella catarrhalis showed (100%) ability to produce the gelatinase enzyme.

The number and percentage of urease enzyme producers were 49(29.51%), where 38(79.16%) of S.aureus isolates were able to produce it. Also, 3(17.64%) of S.hominis isolates were able to produce it, while only one isolate of S.lentus (20%) was able to produce it. Moreover, both S.xylosus and S.warneri isolates were (100%) urease-producers.

From the total (166) isolates, 20(12.04%) isolates formed biofilms. E.faecalis bacteria was the most biofilm-producing isolate 9(47.36%), followed by S.aureus bacteria 3(6.25%) then

S. lentus isolates (40%), while Kocuria rosea bacteria produced biofilm as 1(100%), Strep.pneumoniae and Strep.pyogenes produced as 1(25%) for each isolate, and 1(50%) for Strep. anginosus isolate, whereas Strep.salivarius and Strep.mitis produced biofilm as 1(100%) isolate for each of them.

Also (100%) of the virulence genes icaC, srtC, and Emm were detected in S.aureus, E.faecalis and Strep.pyogenes isolates, which produced strong, moderate and weak biofilm types by using the PCR technology.

Keywords: Virulence factors, Virulence genes, Gram (+ve) cocci, Gram (-ve) cocci, Clinical samples, PCR

INTRODUCTION

The Gram-positive (G+ve) and negative (G-ve) cocci in their various spherical forms, whether they are single, paired or in chains or clusters, are classified according to the blue or pink in the staining method developed by Hans Christian Gram in 1884 [1]. These bacterial pathogens are important opportunistic pathogens for humans, being one of the widely spread contaminants in hospitals, and the acquisition of these bacteria to new characteristics enhances their pathogenicity [2]. The gram-positive cocci with their different types, whether staphylococcus, streptococcus or enterococcus, are able to penetrate the body's defenses, invade its tissues, and possess factors that increase its virulence and resistance to antibiotics, making it the cause of many types of infections and diseases in humans. The Gram positive cocci have highly variable growth and resistance forms, which is the project of epidemiologically significant pathogens [3].

As for the Gram-negative cocci, which are oval in shape and are arranged in pairs, including *Neisseria gonorrhoea*, *Neisseria meningitidis* and *Moraxella catarrhalis*, which are opportunistic pathogens and cause serious diseases such as gonorrhea, meningitis and pneumonia, each according to its type [4].

The lateral genetic transfer of microbial genome evolution played an essential role in shaping the genome content of bacteria as well as their metabolic capabilities [5,6].

Among the virulence factors is the biofilm which is a major clinical problem, mainly due to high levels of resistance to antibacterial immunotherapy and antimicrobial therapy and over-prescribing of antibiotics to treat infections is one of the causes of antibiotic resistance.

The biofilm is defined as a community of bacteria that live in an organized structure as cell assemblies or microcolonies, encapsulated by a matrix consisting of extracellular polymeric material separated by open water channels. These water channels act as a primitive circulatory system for introducing nutrients and removing the metabolic waste products. The biofilm allows bacteria to adhere to inert materials and to experience increased resistance to antibiotics [7].

Hemolysin has been described as a membrane toxin that damages the membrane of eukaryotic cells [8]. The alpha type toxin causes red blood lysis and destroys platelets, while the other type represents the beta type, which breaks down sphingomyelin and some other cells [9].

Among other products are urease and gelatinize enzymes, which are considered virulence factors that facilitate the spread of infection to neighboring tissues and thus play a role in bacterial pathology.

The polymerase chain reaction (PCR) is used to detect bacteria which cannot be detected by conventional culture methods, as it can detect the bacterial DNA. The positive element of microbial genome evolution played an essential role in shaping the bacterial genome content as well as their metabolic capabilities [5,6]. For example, the metabolic abilities of S. aureus are associated with the acquisition of both resistance and virulence characteristics [10,11].

MATERIALS AND METHODS

In this study, (177) samples were collected from different clinical sources, including (49) blood samples, (43) urine samples, (30) pus swabs, (40) cervical swabs and (15) sputum samples from patients attending Ramadi Teaching Hospital for Maternity and Children, Ramadi Teaching Hospital in Ramadi in Al-Anbar province/Iraq during the period from 19/12/2018 to 12/5/2019, and (203) isolates were isolated and identified as Gram positive and Gram negative bacteria, then (166) Gram positive and Gram negative cocci were chosen as study samples.

Samples were collected from cervical sites and infected pus using cotton swabs and culturing them directly on the culture medium, or by using transport medium swabs.
Urine and sputum samples were collected by sterile plastic containers, a drop of which was taken by a sterile loop and cultured directly on the culture media by the streaking method, while blood samples were obtained from the patient by sterile syringes under complete sterile conditions and placed in glass bottles containing Brain Heart and incubated for 5 to 7 days.

The collected samples were cultured directly on blood agar, MacConkey agar, chocolate agar and solid GC medium, by the streaking method, and incubated for (24) hours at (37) C. The growing bacteria were diagnosed on the basis of color, shape, size, edge and height of the growing colonies. The colonies were re-grown more than once to obtain pure single cultures.
To investigate the ability of the bacteria to produce hemolysin, they were incubated for 24 hours at 37°C on blood agar. The appearance of a transparent hemolysis area around the colonies indicates that the hemolysis is of the beta type (complete hemolysis), while if the hemolysis area is accompanied by a green zone around the colonies, the hemolysis is of alpha type, meaning partial hemolysis, whereas if no hemolysis appears around the colonies, it is an indication that the bacteria is not producing hemolysin.

The Asculin hydrolysis test is applied to detect the ability of bacteria for urease production, since the medium contains the red phenol indicator which turns the medium's color from yellow to pink if the bacteria has the ability to hydrolyze urea.

This test was conducted to investigate the ability of bacteria to produce the enzyme that causes gelatin lysis, where the medium tubes were inoculated with a pure culture by stabbing method, and then incubated at 37°C for (3-7) days after which the liquefaction of gelatin was investigated. The occurrence of liquefaction indicates gelatin enzyme activity [10].
Investigation of biofilm production by bacteria was performed by two methods: Congo Red Agar method (CRA) on blood agar and Micro- Titration Plates (MTP) on Trypticase Soya Broth (TSB).

The PCR technology was used to detect virulence genes icaC, srtC, and Emm in S.aureus, E.faecalis and Strep.pyogenes isolates.

Statistical analysis
Statistical Analysis System -SAS (2012) was used for data analysis to study the effect of different factors on the studied bacteria, and the significant differences between numbers and percentages were compared with the Chi-Square-2 test and the Kolmogorov-Smirnov Test.

RESULTS
The results in table (1) showed that the highest number and percentage of Gram (+ve) and Gram (-ve) bacteria 48(28.9%) were from urine samples followed by isolates from blood samples 44(26.5%), then came the isolates from pus and cervical sample 37(22.3%) and 23(13.9%) respectively, while the lowest number of isolates were from sputum, which was 14 (8.4%).

Table (1) showed that there were significant differences between the numbers of isolates in the different clinical sources (blood, urine, cervical, pus, and sputum) at the 1% probability level.

Table (1): No. and (%) of G(+ve) and G(-ve) cocci isolates from different clinical sources

Sample source	**No. of samples**	**No. of isolates**	**Percentage**
Blood samples	49	44	26.5%
Urine samples	43	48	28.9%
Cervical swab	40	23	13.9%
Pus swabs	30	37	22.3%
Sputum swabs	15	14	8.4%
Total	177	166	93.8%
Chi-square value	----	-----	8.026**

** P≤0.01

The No. and percentage of S. aureus was higher 48(28.9%) than other types of bacteria followed by E.faecalis 19(11.4%) followed by *Staph.hominis* 17(10.2%) followed by S. haemolyticus 13(7.8%) (13, while the lowest No. and percentage was S. epidermedis 10(6%), followed by E.avium 7(4.27%), followed by S. lentus, E.faecium and Neisseria gonorrhoeae, which recorded 5(3%) for each of them, followed by Micrococcus spp., S.pyogenes and S.pneumonia, reached 4 (2.4%) for each, followed by Staph.chormogenes, Kucoria kristinae, S. sanguinis, S.anginosus, S.agalactiae and Moraxilla catarrhalis 2(1.2%) (2) for each, while the remaining isolates recorded 1(0.60%) for each as shown in table (2).

Table (2): No. and (%) of G+ve and G-ve cocci types isolated from different clinical samples

Isolated bacteria	**No. and percentages of bacterial isolates according to clinical samples**					
	Total No. & %	**Blood**	**Urine**	**Cervical**	**Pus**	**Sputum**
Staphylococcus aureus	48(28.9%)	10(22.7%)	9(18.8%)	2(8.7%)	24(64.9%)	3(21.4)
Staphlyococcus hominis	17(10.2%)	13(29.5%)	---	---	3(8.1%)	1(7.1%)
Staphylococcus	13 (7.8%)	2(4.5%)	6(12.5%)	1(4.3%)	3(8.1%)	1(7.1%)

haemolyticus						
Staphylococcus epidermidis	10(6%)	4(9.1%)	1(2.1%)	3(13%)	1(2.7%)	1(7.1%)
Staphylococcus lentus	5(3%)	---	2(4.2%)	3(13%)	---	1(7.1%)
Staphylococcus chromogenes	2 (1.2%)	1(2.3%)	1(2.1%)	---	---	---
Staphylococcus saprophyticus	1(0.6%)	---	1(2.1%)	---	---	---
Staphylococcus xylosis	1(0.6%)	---	---	1(4.3%)	---	---
Staphylococcus lugdunensis	1(0.6%)	---	1(2.1%)	---	---	---
Staphylococcus warneri	1(0.6%)	---	---	1(4.3%)	---	---
Staphylococcus capitis	1(0.6%)	---	---	1(4.3%)	---	---
Micrococcus ssp.	4(2.4%)	---	---	3(13%)	1(2.7%)	---
Kocuria kristinae	2(1.2%)	---	2(4.2%)	---	---	---
Kocuria rosea	1(0.6%)	---	1(2.1%)	---	---	---
Streptococcus pneumonia	4(2.4%)	2(4.5%)	---	---	---	---
Streptococcus pyogenus	4(2.4%)	1(2.3%)	1(2.1%)	---	2(5.4%)	2(14.3%)
Streptococcus agalactia	2 (1.2%)	---	---	1(4.3%)	---	---
Streptococcus sanguinis	2 (1.2%)	1(2.3%)	1(2.1%)	---	---	1(7.1%)
Streptococcus anginosis	2 (1.2%)	---	---	---	1(2.7%)	---
Aerococcus urina	1(0.6%)	---	1(2.1%)	1(4.3%)	---	---
Aerococcus viridans	1(0.6%)	---	---	1(4.3%)	---	---
Streptococcus alactolyticus	1(0.6%)	1(2.3%)	---	---	---	---
Lactococcus lactislactis	1(0.6%)	1(2.3%)	---	---	---	---
Streptococcus salivarius	1(0.6%)	---	---	---	1(2.7%)	---
Streptococcus mitis	1(0.6%)	---	---	---	---	1(7.1%)
Enterococcus faecalis	19(11.4%)	1(2.3%)	---	5(21.7%)	---	1(7.1%)
Enterococcus avium	7(4.2%)	5(11.4%)	12(25%)	---	1(2.7%)	---
Enterococcus faecium	5(3%)	1(2.3%)	1(2.1%)	---	---	---
Enterococcus durans	1(0.6%)	1(2.3%)	4(8.3%)	---	---	---

Neisseria gonorrhoeae	5(3%)	---	5(10.4%)	---	---	---
Moraxella catarrhalis	2 (1.2%)	---	---	---	---	2(14.3%)
Total	166	44	48	23	37	14

Results of ability of G(+ve) and Gram (-ve) cocci for virulence factor production showed variations in their ability to produce hemolysin, gelatinases, urease and biofilms. The highest No. and percentage 103(62.04%) were among the hemolysin producers, followed by the gelatinase producers 86(51.80%), followed by urease producers 49(29.51%), then 20(12.04%) biofilm producing isolates as shown in table (3).

The results showed a difference in the No. and percentages of hemolysin-producing isolates Where all S.aureus isolates were beta-hemolytic at 100%, The isolates of the genus Kocuria were all 100% non-hemolytic. Regarding the genus Streptococcus, all Strep.pyogenes and Strep.agalactiae isolates were beta-hemolytic at 100%, while Strep.pneumoniae, urinae Aerococcus and viridans Aerococcus were partially hemolytic i.e. α-type and 100% of the rest of the isolates Strep.sanguinis and Strep. Anginosus, Strep.alactolyticus, and Lc. lactis lactis, Strep.salivarius, and Strep.mitis were 100% non- hemolytic. From the genus Enterococcus,(8) isolates were completely hemolytic and 24 isolates were non y-haemolysis, as shown in table (3).

For Gram-negative bacteria, Neisseria gonorrhoeae and Moraxella catarrhalis isolates were shown to be 100% non-hemolysin producers. It can be concluded that there was a significant difference between the bacterial isolates in their ability to produce the enzyme hemolysin below the level of probability $P \leq 0.01$.

The results showed that all S. aureus isolates produced 100% gelatinase enzyme and all Strep.pyogenes, Strep.agalactiae and Strep isolates were able to produce it. Strept. anginosus and Lc. lactis lactis and Strep.mitis produced 100% of this enzyme, while the rest of the species were unable to produce it, and Entreococcus.faecalis and (5) E.faecium isolates were 100% gelatinase producers, and E.faecium produced 20% of the gelatinize enzyme.

As for the negative bacterial genus, two isolates of Moraxella catarrhalis were (100%) able to produce the gelatinase enzyme, while Neisseria gonorrhoeae isolates were unable to produce it. there were significant differences between isolates in their production of gelatinase enzyme at $p<0.01$.

The results showed (38) isolates of S.aureus produced urease enzyme at a rate of (79.16%) and (3) S.hominis isolates produced urease with a percentage of (17.64%) and both isolates of S. xylosus and S.warneri were 100% producers, and the results showed inability of the genus kocuria, genus streptococcus and the G (-ve) cocci produce this enzyme. There was significant differences between the isolates in their production of urease at $P \leq 0.01$.

Table (3) showed through the use of the two methods (the Central Concorde method (CRA)) And the method of micro-titration dishes (MPT) that the most biofilm producing bacteria was E.faecalis 9(47.36%), followed by S.aureus bacteria 3(6.25%), while one Kocuria rosea bacteria produced biofilm at (100%), and one isolate of Strep.pneumoniae produced it at (25%), and one isolate of Strep.pyogenes at (25%), and one isolate of Strep bacteria.

Anginosus at (50%), while one isolate of each of Strep.salivarius and Strep.mitis produced it at 100%.

Table (3): No. and (%) of ability of G (+ve) and G (-ve) bacterial isolates to produce virulence factors

Bacterial isolates	**No.**	**No. and (%) of hemolysin-producing isolates**	**No. and (%) of gelatinase-producing isolates**	**No. and (%) of urease-producing isolates**	**No. and (%) of biofilm-producing isolates**
Staphylococcus aureus	48	48β(100%)	48(100%)	38(79.16%)	3(6.25%)
Staphlyococcus hominis	17	17β(100%)	0(0%)	3(17.64%)	0(0%)
Staphylococcus haemolyticus	13	13β(100%)	0(0%)	0(0%)	0(0%)
Staphylococcus epidermidis	10	0(100%)	0(0%)	5(50%)	0(0%)
Staphylococcus lentus	5	0(100%)	0(0%)	1(20%)	2(40%)
Staphylococcus chromogenes	2	2β(100%)	0(0%)	0(0%)	0(0%)
Staphylococcus saprophyticus	1	0(100%)	0(0%)	0(0%)	0(0%)
Staphylococcus xylosis	1	1β(100%)	0(0%)	1(100%)	0(0%)
Staphylococcus lugdunensis	1	0(100%)	0(0%)	0(0%)	0(0%)
Staphylococcus warneri	1	1β(100%)	0(0%)	1(100%)	0(0%)
Staphylococcus capitis	1	0(100%)	0(0%)	0(0%)	0(0%)
Micrococcus ssp.	4	1β(25%)	0(0%)	0(0%)	0(0%)
Kocuria kristinae	2	0(100%)	2(100%)	0(0%)	0(0%)
Kocuria rosea	1	0(100%)	0(0%)	0(0%)	1(100%)
Streptococcus pneumonia	4	4α (100%)	0(0%)	0(0%)	1(25%)
Streptococcus pyogenus	4	4β(100%)	4(100%)	0(0%)	1(25%)
Streptococcus agalactia	2	2β(100%)	2(100%)	0(0%)	0(0%)
Streptococcus sanguinis	2	0(0%)	0(0%)	0(0%)	0(0%)
Streptococcus anginosis	2	0(0%)	2(100%)	0(0%)	1(50%)
Aerococcus urina	1	1α (100%)	0(0%)	0(0%)	0(0%)

Aerococcus viridans	1	1α (100%)	0(0%)	0(0%)	0(0%)
Streptococcus alactolyticus	1	0(0%)	0(0%)	0(0%)	0(0%)
Lactococcus lactislactis	1	0(0%)	1(100)	0(0%)	0(0%)
Streptococcus salivarius	1	0(0%)	0(0%)	0(0%)	1(100%)
Streptococcus mitis	1	0(0%)	1(100%)	0(0%)	1(100%)
Enterococcus faecalis	19	8β(42.10%)	19(100%)	0(0%)	9(47.36%)
Enterococcus avium	7	0(0%)	0(0%)	0(0%)	0(0%)
Enterococcus faecium	5	0(0%)	5(100%)	0(0%)	0(0%)
Enterococcus durans	1	0(0%)	0(0%)	0(0%)	0(0%)
Neisseria gonorrhoeae	5	0(0%)	0(0%)	0(0%)	0(0%)
Moraxella catarrhalis	2	0(0%)	2(100)	0(0%)	0(0%)
Total	166	103	86(51.80%)	49(29.51%)	20(12.04%)

The results in table (4) and figures (1,2 and 3) showed that the virulence gene srt-C was detected in 14(100%) of *Enterococcus faecalis*, and ica-C gene was detected in 5(100%) of *Staphylococcus aureu*s, while the gene emm was detected in 4(100%) of *Streptococcus pyogene*s isolates by using the PCR technology.

Table (4): No. and percentage of bacterial isolates whose virulence genes were detected by PCR

Genes	**Bacterial isolates**	**No. of isolates**	**(%) of isolates**
srt-C	*Enterococcus faecalis*	14	100%
ica-C	*Staphylococcus aureus*	5	100%
emm	*Streptococcus pyogenes*	4	100%

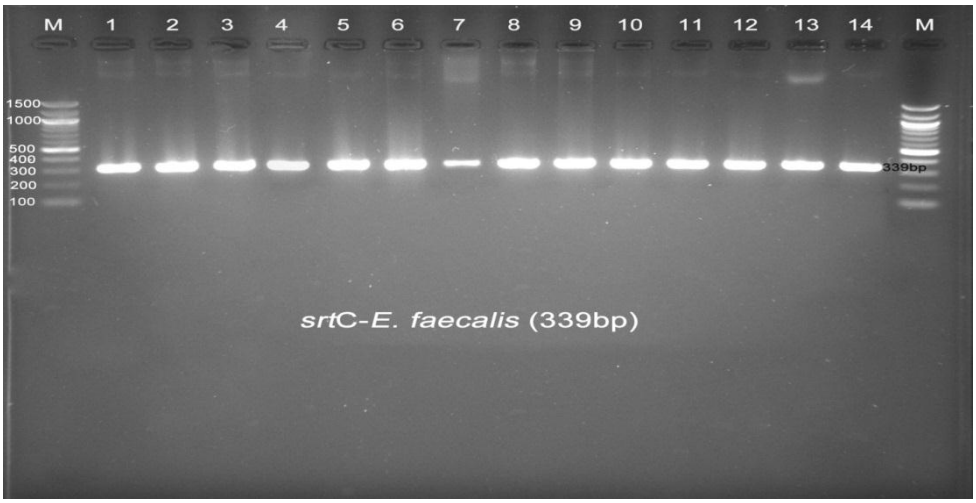

Figure (1): Eectrophoresis of (2%) agarose gel used in PCR for detection of srtC gene in *E.faecalis* using 75mV/50 mA/ hour. as M: pb Marker ladder 100-2000 and isolates (1, 2, 3, 4, 5, 6, 7, 8, 9, 75, 10, 11, 12, 13 and 14)

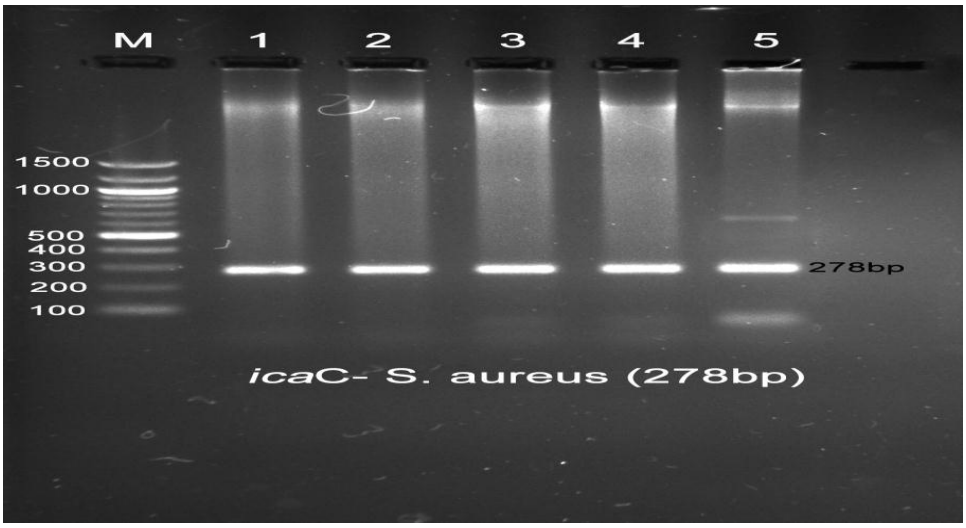

Figure (2): Eectrophoresis of (2%) agarose gel used in PCR for detection of icaC gene in *S. aureus* using 75mV/50 mA/ hour. as M: pb Marker ladder 100-2000 and isolates (1, 2, 3, 4, 5)

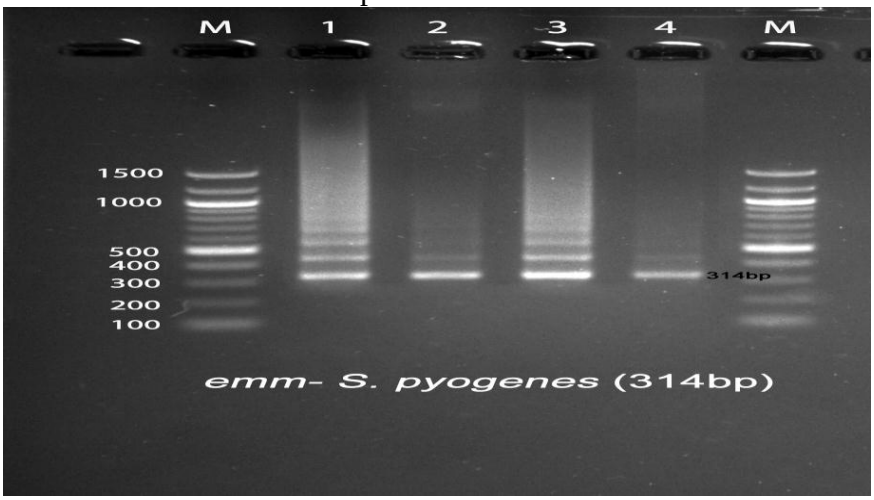

Figure (3): Eectrophoresis of (2%) agarose gel used in PCR for detection of emm gene in *St. pyogenes* using 75mV/50 mA/ hour. as M: pb Marker ladder 100-2000 and isolates (1, 2, 3, 4)

DISCUSSION

Results in this study showed that genus staphylococcus was the first causative agent of nosocomial infections which agreed with [12], who found that the genus Enterococcus was the second after the genus Staphylococci as a causative agent of hospital infections in the United States. It was also close to the studies that confirmed that the strains of the two types E.faecalis and E.faecium constitute up to 90% of the infections caused by Enterococcus bacteria, while only 10% are caused by the rest of the species [13], while it differed from the study of [14], who indicated that the isolate percentage of E.faecium was higher than that of E.faecalis, and this increase may be due to the difference in the number and type of samples. On the other hand, the result was close to the study of [15,16] who showed that E.faecalis are the most isolated, followed by E.faecium and then of other species.

Some studies confirm that the genus Enterococcus is widespread in several environments, in the human body and in different animals, and this wide spread is one of the reasons for the emergence of multiple resistance to antibiotics and thus the cause of many pathological conditions [17].

Another study indicates that these two species (E.faecalis and E.faecum) are the most commonly isolated from health and environmental sources, and the reason may be due to the virulence factors that this species possesses [18]. The main reason for the prevalence of these two species in the hospital environment may be due to their natural resistance to many commonly used antibiotics and their ability to acquire resistance to all available antibiotics, either by mutation or acquisition of new genetic material [19].

The results showed a difference in the percentages of hemolysin-producing isolates, where all S.aureus isolates were (100%) β-hemolytic and this is a good indication of the virulence of these bacteria because hemolysis is closely related to bacterial pathogenicity, and emphasized by [20] who stated that this factor plays a very important role in the bacterial pathogenesis of S. aureus. It has been observed that there is a close correlation between the ability of bacteria to produce hemolysin and their ability to coagulate plasma.

S.hominis, S. haemolyticus, S.chromogenes, S. xylosus, and S.warneri showed (100%) complete hemolysis, while the rest of the isolates of S.epidermidis, S.lentus, S. saprophyticus, S.lugdunensis and S.capitis were (100%) non hemolytic. It is observed that all S. epidermidis and S. saprophyticus were unable to cause hemolysis, while S. haemolyticus isolates were positive for this phenomenon.

All the S. aureus isolates produced 100% gelatinase enzyme and this is identical to the study of [21], who found the same results. E.faecalis isolates and (5) of E.faecium isolates were (100%) gelatinase producers and these results were in agreement with the study of [16,17,22] who confirmed that E.faecalis isolates are the most capable of producing this enzyme, followed by E.faecium isolates. The results of this study were in agreement with a study by [23], who indicated the inability of any isolate of E.faecalis isolates to produce gelatinase enzyme.

A great variation in the production of the biofilm between bacterial isolates was found as shown in table (3). The variation in the ability of bacterial isolates to produce the biofilm is due to the environmental conditions surrounding the bacteria in terms of food content, temperature and pH, as well as endocarditis, wound and burn inflammation, and otitis media, thus the biofilm contribute to an increase in disease [24].

The results of PCR for detection of virulence genes showed the possession of 14(100%) of the E.faecalis the (srtC) gene which is responsible for encoding the enzyme (SrtC). This enzyme is widely present in Gram positive bacteria and its function is to bind the basic units forming the cilia, which are important in the process of bacterial attachment to the surface of host cells, as well as in the formation of the biofilm [25]. The icaS gene can be considered necessary to initiate biofilm production, and in the study [26], the icaC gene was detected in a large number of isolates and was also expressed by the icaABCD operator. The production of intercellular adhesion molecules, by icaABCD and other genes, plays an important role in staphylococcus, and biofilm production may be the primary cause of increased antibiotic resistance in these bacteria. In their study, [27], they detected 12 pure and active genes that encode for biofilm synthesis including icaABCD and other genes.

This study revealed the presence of 100% virulence genes icaC, srtC, and emm in S.aureus, E.faecalis, and Strep.pyogenes bacterial isolates, respectively, of which there were isolates with high, moderate and weak biofilm formation.

In a previous study, it was demonstrated that MTP, CRA, and / or PCR techniques are important to identify serious virulence factors, especially the ability to form biofilms in many bacterial types including Staphylococcus bacteria [28].

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