The prepared gel was poured to cool and solidify at the room temperature waiting 30 min. and still in its plastic tray after that, the combs and seal were removed gently from the casting tray, the combs made wells that used for loading DNA samples.

The samples containing DNA mixed with 1/10 of loading buffer then, pipetted into the wells. Lid was placed on the apparatus and the power supply, then, the current was applied. Generally, the gel was run at 5 v/cm for about 30 min to 1.5 h to separate the DNA fragments for detect of presence of DNA in samples and for PCR products respectively. DNA bands were visualized by U.V. trans illuminator at 365 nm wave length documentation system, then photographed by digital or phone camera.

Detection of specific genes by polymerase chain reaction:

The detection of *S. aureus* specific species gene, in addition to the genes that encoding the resistance for oxacillin and cefoxitin antibiotics were done by amplification of specific sequences within the target gene by using the polymerase chain reaction technique. The experiment was performed by using the mixture of a specific sets of primers designated for each targets genes that were mixed with the DNA samples (as a template) and master mix reagent which contain (Taq polymerase, PCR buffer, MgCl2 and dNTPs), the final constituents was the nuclease free water, the reaction mixtures were mixed for each targets genes then, transfer to thermal cycler machine to start the reaction depending on the steps of specific program [19,20].

Detected of *S. aureus* isolates from clinical samples by conventional methods

Out of 150 clinical specimens that were included in this study, a total of 61 *Staphylococcus aureus* were isolated and identified. Twenty (20) of these bacterial isolates were from ear pus samples will percent of (62.5 %) followed by eighteen (18) were from sputum in percent (60 %) then, eleven (11) were from urine with (26.82 %), six (6) were from nasal with (28.57 %), four (4) were from throat and two (2) were from wound samples, with percent of (21.05 %) and (28.57 %) respectively as shown in **Table 5**.

Table 5. Prevalence of *Staphylococcus aureus* that isolated from clinical specimens of study group.

Type of clinical specimens	No. of specimens	*S. aureus*	
		No.	(%)
Wound	7	2	28.57
Sputum	30	18	60.0
throat	19	4	21.05
Nasal	21	6	28.57
Ear Pus	32	20	62.5
Urine	41	11	26.82
Total	150	61	40.66

Identification of MRSA isolates by using the modified Kirby-Bauer method according to (CLSI, 2021)

Out of 150 samples, 61 (40.66 %) were found to be infected with *S. aureus* isolates and the results of (61) isolates of *S. aureus* against cefoxitin (30 µg) and oxacillin (1 µg) antibiotic disks by disk diffusion assay showed that, all bacterial isolates were resistant to both antibiotic disks in rate (100 %) and were considered as (MRSA), **(Table 6 & Figure 1)**.

Table 6. Antibiotic susceptibility test towards 61 *S. aureus* isolates for determine MRSA strains by using disc diffusion assay.

No	Antibiotics	Con.	No. of bacterial isolates	
			R (%)	S (%)
1	Oxacillin (OX)	(1 mg)	61 (100)	0
2	Cefoxitin (CX)	(30 mg)	61 (100)	0

Figure 1. MRSA isolates detected by resistant to cefoxitin and oxacillin by disk diffusion method.

Frequency of coagulase negative (CONS) Staphylococcus spp. from clinical specimens:

Out of 150 specimens, the coagulase negative (CONS) Staphylococcus spp. were isolated from patients in 56 (37.33 %) and they consisted of Staphylococcus epidermidis which was appeared as the high frequently isolated bacterial species 41 (73.21%) followed by Staphylococcus saprophyticus 15 (26.78 %) as appeared in **Table 7**.

Table 7. Staphylococcal spp. that isolated from clinical specimens.

Coagulase negative *Staphylococcus spp*.	**No. and percentage (%) of isolates (56)**
Staphylococcus epidermis	41 (73.21)
Staphylococcus saprophyticus	15 (26.78)
Total	56 (37.33)

Molecular part:

Detection of 16S rRNA and mec A genes from *S. aureus* isolates in different clinical specimens:

The purified fragments DNA of 61 *S. aureus* isolates from different sites of infection in human were conducted for PCR assay to detect the presence of 16srRNA, and mec A genes as shown in **Table 8**. The results of PCR amplification of 16srRNA gene from *S. aureus* isolates revealed that 52/61(85.24 %) isolates had a clear band of 228 bp which corresponds to identification of *S. aureus* strains. While, PCR amplification of 42/52(80.76 %) isolates were generated a clear band of 310 bp which corresponding to detect mec A gene in methicillin resistant strains of *S. aureus* isolates, as appeared in **Figures 2-4**.

Table 8. Frequency and percentages of 16srRNA & mec A genes detection in *S. aureus* isolated from clinical specimens.

The genes	**No. of isolates from total samples**	**No. of positive isolates**	**%**
16S rRNA	61/150	52/61	85.24
mec A	52/61	42/52	80.76

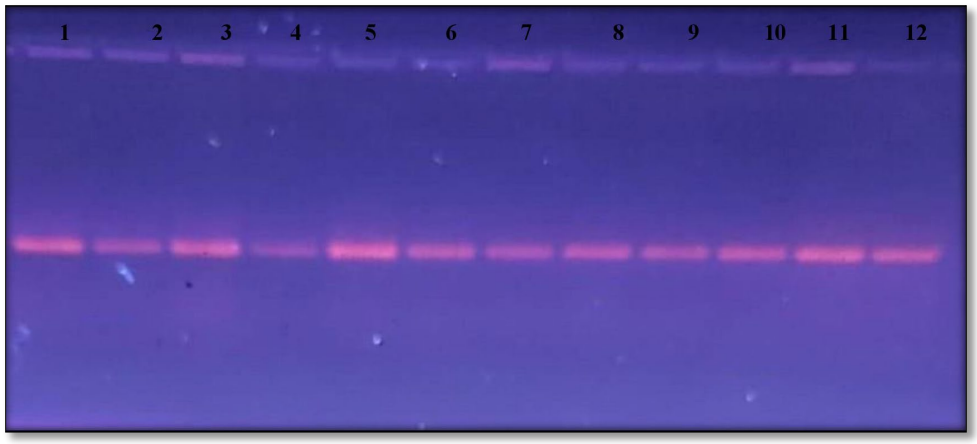

Figure 2. Agarose gel electrophoresis for detection purified fragments DNA of *Staphylococcus aureus* isolates. Line 1: numbers of *S. aureus* isolates that loaded in agarose gel.

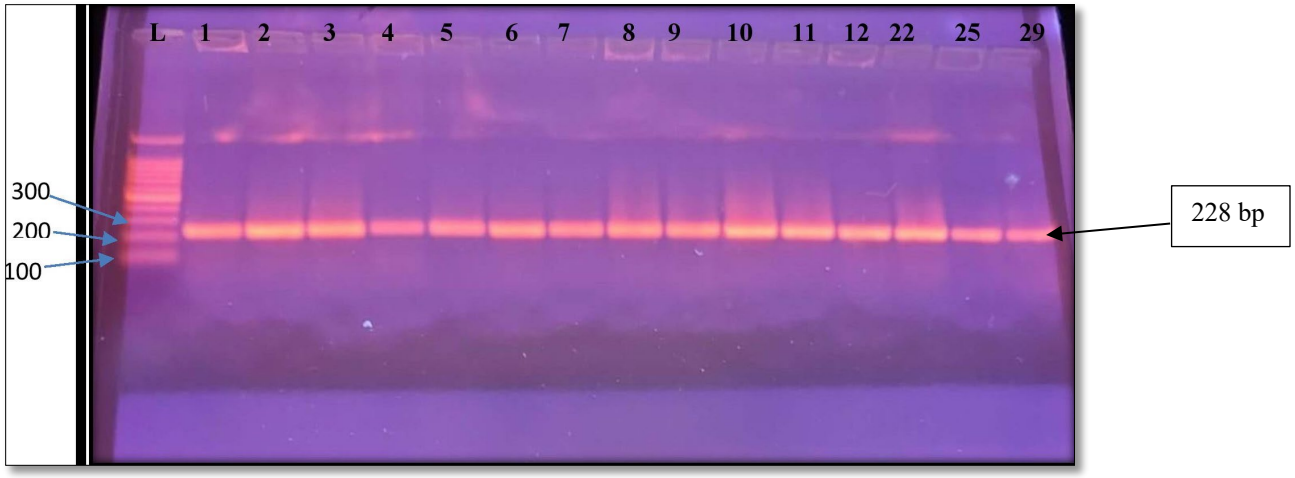

Figure 3. Agarose gel electrophoresis of PCR-amplified for 16S rRNA gene of Staphylococcus aureus isolates. Line L: DNA marker (ladder) 1000 bp. Lines (1-12, 22, 25, 29) numbers of S.aureus isolates that loaded in agarose gel for PCR to amplify the 16S rRNA gene of Staphylococcus aureus with confirm detection of 16S rRNA gene 228 bp in these isolates.

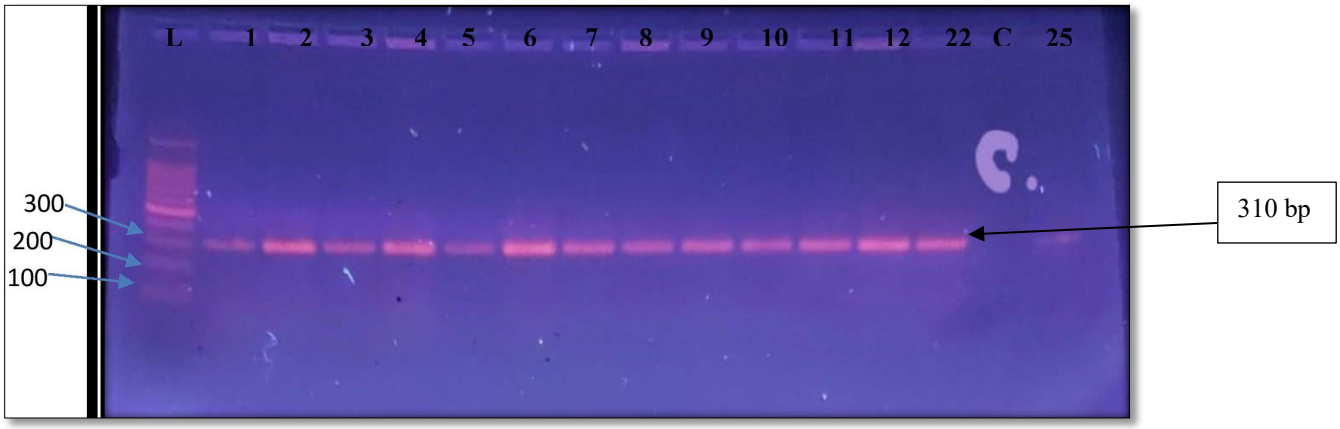

Figure 4. Agarose gel electrophoresis of PCR-amplified for mecA gene of Staphylococcus aureus isolates. Line L: DNA marker (ladder) 1000 bp. Lines (1-12, 22, 25) numbers of S.aureus isolates that loaded in agarose gel for PCR to amplify the mecA gene of Staphylococcus aureus with confirm detection of mecA gene 310 bp in these isolates. C: Negative control.

Detection of *S. aureus* isolates by molecular and conventional methods:

PCR assay was used to detect the presence of 16srRNA specific for *S. aureus* and mec A gene in bacterial isolates through the amplification of 16srRNA gene and mec A gene from *S. aureus* isolates as shown in **Table 9**. The results were revealed that 52/61 (85.24 %) isolates had a clear band of approximately 228 bp which corresponds to specific identification of 16srRNA gene in *S. aureus* isolates, and 42/52 (80.76 %) isolates were generated a clear band of approximately 310 bp which corresponding to detect mec A gene in methicillin resistant strains of *S. aureus* isolates. While, in conventional methods which that be used for detection of *S. aureus* and methicillin resistant strains from different clinical specimens were revealed, out of 150 tested samples, 61 (40.66 %) were found to be infected with *S. aureus* isolates and all isolates of S. *aureus* were (100 %) resistant to oxacillin. Cefoxitin, ampicillin, methicillin and amoxicillin respectively that previous mentioned with details in **Tables 5 & 6**.

Table 9. Percentages of *S. aureus* isolates that detection by molecular and conventional methods from clinical specimens.

Method to detect of *S. aureus*	No. of specimens & isolates	No. of positive	%
Conventional methods	150	61	40.66
16S rRNA	61	52	85.24
mec A	52	42	80.76

In this study the identification of the *S. aureus* and other *Staphylococcus spp*. from clinical samples were performed depending on morphological and biochemical assay including gram stain, catalase test, coagulase test, changes on mannitol salt agar and blood agar media in addition to susceptibility against Novobiocin disc. This results that were obtained from the traditional methods for isolation and identification of *S. aureus* isolates from clinical samples agreed with the results of Oladipo [21]. Also, from 150 clinical specimens that were implicated in current study, a total of 56 other Staphylococcal spp. (CONS) that were identified and represented the rate of (73.21%) in 41 isolates of *Staphylococcus epidermis*, followed by *S. saprophyticus* with rate of (26.78%) in 15 isolates. The results of current study were in congruence with the results of Mohammed [22] and Deyno [23] while the study of AL-Mosawi [24], show the low rates in prevalence of (CONS) which isolated from UTI patients in compare with rates of (COPS), these results were somewhat in accordance with our results.

The identification of the methicillin resistant *S. aureus* (MRSA) from clinical specimens were performed depending on morphological and biochemical assay based positive cultures of *S. aureus* from clinical samples. Out of 150 a total of 61 *Staphylococcus aureus* were isolated and identified. All isolates of *S. aureus* were examined to detect the methicillin resistance strains, that were perform by disc diffusion assay to Oxacillin and Cefoxitin antibiotics with the modified Kirby-Bauer method on Muller Hinton agar (MHA), the cultured plates of bacteria were examined after incubation period at 37°C for 24 h then by the following of Clinical and Laboratory Standard Institute were determined the zone diameter stop points of inhibition for the isolates of *S. aureus* to the closest millimeter (mm). shown that, the rates of resistance were highest and record (100%) for Oxacillin and Cefoxitin. In present study, the percentage of antibiotic resistance towards Oxacillin was (100%), that is accordance with the study of Ifra [25] who found that, the *S. aureus* was recorded in highly resistant towards Oxacillin in rate 100%. Also, the study of and Onemu [26] in university of Benin Hospital, Benin City which report the prevalence of MRSA in rate 79%, this finding was, to some extent, in agreement with the results of current study concerning with the isolation rate of MRSA. And in study of Lin [27] which was detected, the infectious cases by *S. aureus* (MRSA) particularly nosocomial infection in a Medical Center, that corresponding to 53 (66.3%) which represent the rate of MRSA prevalence, those finding were to some accordance with on the other hand, both studies were recorded a low percentages of MRSA isolates than detected in current study. Also, in study of detected the cefoxitin resistance 19 (73.07 %) of *S. aureus* isolates from patients with CSOM infections this result was constant with our results and out of 82 clinical specimens (31.70 %) percent of *S. aureus* isolates which detect low rat in compare with current study. In addition, other study shows the percentage rate of identification *S. aureus* isolates from patients with caries were, out of 50 clinical samples 26 (52 %) this results of were constant with present study.

In study of study was recorded the MRSA prevalence in rate (48.8%) which is comparable to that obtained by Olowe [28] who is reported the prevalence of MRSA in rate (47.8%) in Oshogbo, Ladoke Akintola University of Technology College of Health Sciences, South western Nigeria. Furthermore, in study of, the results of detected MRSA isolates from clinical specimens was recorded in rate of prevalence (24.75%) which is comparable to the results that

reported by the study of Hujier [29] in Gaza, Palestine. While, in present study the rate of resistance in MRSA isolates are higher and were recorded (100%) for Oxacillin, Cefoxitin, Ampicillin, Amoxillin and Methicillin, these results were consistent with the results that reported in study of Aboud [30]. Our finding was disagreement with the results of that related with rates of sensitivity for Methicillin (51.2%) and Oxacillin (46.5%) and consistence with its results which related to other antibiotics used in this study.

The results of antibiotic susceptibility test and detection of mecA gene in study of Naorem [31] showed, 33 (94.28 %) strains were MRSA while 2 (5.72 %) strains were MSSA, this study agrees with present study and such a validation process was reported by other researchers [32-35]. The results of current study that related with PCR amplification of 16srRNA gene from *S. aureus* isolates revealed that 52 isolates in (85.24 %) percentage had a clear bands of approximately 228 bp which corresponds to identification of *S. aureus* strains, While, PCR amplification of 42 isolates in (80.76 %) percentage were generated a clear bands of approximately 310 bp which corresponding to detect mec A gene in methicillin resistant strains of *S. aureus* isolates, The present study is constant with the study of Al-Ashmawy [36] in which most of recovered *S. aureus* isolates were genetically verified as MRSA strains by molecular detection of the mecA gene. Further, in study of the percentage (90.7 %) of *S. aureus* isolates which identified by conventional methods were confirmed by amplification of the 16S rRNA gene, the current study agreed of those finding. The results of current study were somewhat in agreement with the results of Malihe [37] also, the high prevalence rates (57 %-70 %) of MRSA isolates in Alzoubi [38] study which documented among Jordanian hospitalized adults were coinciding with results of present study. All *S. aureus* isolates contained the resistance genes for (mecA) and penicillin were reveled in study of de which is consistent with results of this study. In study of Azimian [39] the results of PCR for mecA showed that 110 strains of *S. aureus* with percent (47 %) had mecA gene lower than we detected in present study but the study of Kot [40] which detect the methicillin resistant *S. aureus* (MRSA) in higher percentages about (80 %) by PCR amplification of mecA gene this result is agreement with current study. Gadban [41] study showed the result of amplifying 16S rRNA was given positive for identify the *S. aureus* and MRSA strains in all isolates, the 18 (100 %) were pvl positive, results of present study were consistent with those finding. While, in conventional methods that used for detection of *S. aureus* and methicillin resistant strains from different clinical specimens in present study were revealed, out of 150 samples, 61 (40.66 %) were found to be infected with *S. aureus* and all isolates of S. *aureus* were (100 %) resistant to oxacillin, Cefoxitin, ampicillin, methicillin and amoxillin respectively. Kader [42] study reported that, (88.24 %) of *S. aureus* isolates were resistant to Oxacillin and methicillin discs, and the current results were in constant with those results. Also, in study of showed the highest percentage of MRSA strains were isolated from respiratory tract samples (49%) followed by urine cultures (43%) and nasal swabs (35%), the results of present study were somewhat in consistent with these results. In addition, the current study is along with the studies of whose found that, the coagulase positive *S. aureus* isolates were recorded a highly resistant against oxacillin antibiotic (100 %). Furthermore, study detected out of 150 swab samples of diabetic foot ulcer only 21 isolates which include 18 (85.7 %) was identified as *S. aureus*, the other 3 (14.3 %) isolate was identified as Staphylococcus spp. and the results of vitek *2 showed all 18 (100 %) *S. aureus* isolates were resistance to oxacillin and Cefoxitin discs, the results of current study were somewhat in agreement with those finding.

Many laboratories still favor using of Oxacillin in detection of MRSA strains, that because of the Oxacillin antibiotic preserve its activity through the storage best than the Methicillin antibiotic also, more likely to detect hetero resistant strains [43]. In hospital and according to the doctors, there are many critical cases that were infected with *S. aureus* that are treated with glycopeptides (as Vancomycin or Teicoplanin) which may lead to create of a new strain of resistant bacteria to these mention antibiotics. In a particular concern of MRSA strains that are starting to develop the resistance to Vancomycin antibiotic, which is at present the most effective of antibiotic inverse MRSA. In current study many factors may have participated in the above levels that mentioned of resistance towards the tested of antibacterial drugs which selected for study the susceptibility levels to *S. aureus* isolates including, the antibiotics that are misused by the health occupational, un skilled practitioners and a lay person. The incidence of MRSA in present study could be imputed to many agents, despite of the Methicillin not being routinely used against *S. aureus* infections. Additional to antibiotic stress, the horizontal gene transfer is considered as a contributing agent in the incidence of antibiotic resistance in clinical isolates. Then, it has been proposed that a high prevalence in resistance to a particular antibiotic dose not constantly reflected in antibiotic consumption [44,45]. Another contributed agent by the using of antimicrobials in food of animals. Antibiotics are usually added to feed for enhance the growth in animals, in particular the dairy sheep, cattle and poultry [46,47]. Recurrent traveling is a supplementary factor for transmitting the resistance in bacterial strains between countries, the misusing of antimicrobial is another participate factor [48]. In Iraq, there is a current practice that antibiotics can be purchased in absence of formula which leads to misusing of antibiotic drugs by a public thus, participating to the emanation and diffusion of antimicrobial resistance. Other causal factors involve a condition that relating to the public health as poor hospital hygienic accounting for the spreading of resistant bacteria and

inadequate surveillance [49]. In present study, the prevalence of MRSA isolates were high as in studies of these studies in Iraq country thus, compulsory institute guidelines for detection of MRSA and controlling in the hospital by the daily oversight of a clinical laboratories for MRSA isolates, approach of monthly predictable cultures that monitoring of inpatients believed to be at high risk in conquest of MRSA infection also, inspection of hospital personnel, regular of hand washing and disinfecting by hospital personnel with policy regulation of antibiotics in usage and prescription, acquisition of a new and additional information routinely from antimicrobial susceptibility testing to bacterial isolates and monitor the testing of these bacterial isolates with surveillance the antibiotics resistance. All of which that mention above, are decisive for a good clinical practice and for logical polices against the resistance of antibiotics [50].

CONCLUSION

The results of current study that related with PCR amplification of 16srRNA gene and mec A gene in *S. aureus* isolates which previously detected by conventional methods showed, most *S. aureus* isolates had a clear band of 228 bp and 310 bp that identical to these genes in this bacterium with corresponds to identification of *S. aureus* (MRSA) strains. So, the PCR analysis effective in confirm detection the specific 16S rRNA gene of *S. aureus* isolates and mecA gene in methicillin resistant strains of these bacterial isolates.

Also, in current study, the higher resistance of MRSA isolates was illustrious to Oxacillin, Cefoxitin and in many other studies in Iraq country detected that so, some rules must follow to reduce the spreading of MRSA strains include, inspection to MRSA isolates among the reservoir and distributer of MRSA strains in hospitals (healthcare workers and patients). Also, the nomination of antimicrobial agent should be based on the in vitro susceptibility test with a hospital based antibiotic policies that must be robustly followed and firm surveillance of drug resistance to all bacterial pathogens are required in both hospital patients and the national level.

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