Isolation and Molecular Diagnosis of Bacterial Species Isolated from Burns and Wounds Using PCR Technique in Salah Al-Din Governorate

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Abstract:

Biochemical tests results showed unique pattern of reactions for each tested bacterium. In this study, P. aeruginosa isolates were positive for catalase, citrate and oxidase +ve, and negative for the indole, MR, VP and urease tests. aureus isolates gave positive results in most tests whereas (Indole –ve). K. pneumoniae tested positive for the (Catalase, VP and Citrate) tests and negative for (Indole, Oxidase and MR) tests. At the molecular level, a number of genes related to virulence and resistance factors, like mecA, sea, ermA and hlb, were amplified and sequenced, and their presence in some strains was also demonstrated, further suggesting potential pathogenicity of these bacteria. Discriptions: Isolation and identification of aerobic bacterial species causing burn and wound infections in burn patients at different hospitals in Salah Al-Din Governorate was the aim of this study. Characterization of their phenotypic and biochemical features and their susceptibility to a number of standard antibiotics was performed. Some genetic virulence factors were also tested by Polymerase Chain Reaction (PCR). One hundred and fifty five clinical samples from wound and burn infections were received, and the most frequent isolates were S. aureus. K. pneumoniae, P. aeruginosa and E. coli. DNA migration analysis validated the purity of genomic DNA required for precise PCR analysis, thus enhancing reliability of the results.

Keywords: Staphylococcus aureus, Klebsiella pneumonia, Psedomonas aeruginosa،.PCR, Burns .

عزل وتشخيص جزيئي لأنواع بكتيرية معزولة من الحروق والجروح باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR) في محافظة صلاح الدين

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الملخص:

أظهرت نتائج الاختبارات الكيميائية الحيوية نمطًا فريدًا من التفاعلات لكل بكتيريا مُختبرة. في هذه الدراسة، كانت عزلات الزائفة الزنجارية إيجابية لاختبارات الكاتالاز، والسترات، والأوكسيديز الموجبة، وسلبية لاختبارات الإندول، وMR، وVP، واليورياز. أعطت عزلات المكورات العنقودية الذهبية نتائج إيجابية في معظم الاختبارات، بينما أعطت نتائج سلبية لاختبار الإندول. أظهرت نتائج اختبارات (كاتالاز، في بي، وسيترات) لبكتيريا K. pneumoniae نتائج إيجابية، وسلبية لاختبارات (إندول، أوكسيديز، و .(MRعلى المستوى الجزيئي، تم تضخيم وتسلسل عدد من الجينات المرتبطة بعوامل الضراوة والمقاومة، مثل mecA و seaو hlbوermA، كما تم إثبات وجودها في بعض السلالات، مما يشير إلى احتمالية تسبب هذه البكتيريا في الأمراض. الوصف: كان الهدف من هذه الدراسة هو عزل وتحديد أنواع البكتيريا الهوائية المسببة لالتهابات الحروق والجروح لدى مرضى الحروق في مستشفيات مختلفة بمحافظة صلاح الدين. تم تحديد خصائصها الظاهرية والكيميائية الحيوية وحساسيتها لعدد من المضادات الحيوية القياسية. كما تم اختبار بعض عوامل الضراوة الجينية بواسطة تفاعل البوليميراز المتسلسل .(PCR) تم استلام مائة وخمسة وخمسين عينة سريرية من التهابات الجروح والحروق، وكانت أكثر العزلات شيوعًا هي المكورات العنقودية الذهبية. أثبت تحليل هجرة الحمض النووي لبكتيريا K. pneumoniae و P. aeruginosaو E. coliنقاء الحمض النووي الجينومي اللازم لإجراء تحليل تفاعل البوليميراز المتسلسل (PCR) بدقة، مما عزز موثوقية النتائج.

الكلمات المفتاحية: المكورات العنقودية الذهبية

1.Introduction:

The skin represents the biggest and is the largest organ of the human body BD ~ 2m². It also acts as a defence to the outside world, serves to balance fluid and body temperature. And it also functions a central part in the sense of touch [1]. The skin is richly innervated with numerous sensory receptors that are distributed throughout the epidermis and dermis as well as the subcutaneous tissues. They are sensors for the variety of internal and external insurgencies that allow us to see, hear, and touch the world [2]. The skin fulfils numerous sophisticated functions such as creating for underlying tissues an appropriate living environment that isolates them from the external world but allows contact to it by exchanging and receiving stimuliAdditionally, it plays an important role in water balance (skin barrier and sweat glands) and the lymphatic response. Furthermore, it is a vital sensory organ through free nerve endings, and it also participates in maintaining equilibrium, removing materials, selectively absorbing them, and storing them [3].

Burns and wounds are quickly colonized by microorganisms, including fungi and Gram-positive bacteria, especially Staphylococcus aureus, due to the contaminated environment the patient encounters. Within hours to days, burns and wounds are colonized by Gram-negative bacteria [4]. Early treatment of burns is critical to prevent colonization by microorganisms. The first step in burn treatment is cleaning the burn area and removing necrotic tissue. Local dressings and antibiotics are applied, and if the patient does not respond, intravenous antibiotics are administered. However, rapid development of resistance to multiple antibiotics can

result in skin graft failure [5]. Patients with burns and wounds quickly acquire a variety of pathogens from their skin and surrounding environment, and when they seek medical care, they are likely to acquire pathogens that form biofilms [6]. The World Health Organization has ranked burn and wound injuries as the seventh leading cause of traumatic injuries, with a total mortality rate of 5%. Around 2.65 million deaths occur annually worldwide due to burn injuries, with these cases being more prevalent in developing and underdeveloped countries. In these cases, the mortality rate can rise to 100%, especially when burns cover more than 40% of the body surface. Approximately 80% of burns occur in homes, and burn injuries are more common among children and adolescents [7]. Molecular diagnosis is one of the latest laboratory techniques that uses modern methods to detect all types of living organisms. Currently, molecular methods, along with morphological techniques, are used to accurately identify bacterial species. With advancements in molecular techniques, specific amplification and RFLP mapping of certain genomic regions have been possible. The laboratory molecular detection method for identifying microorganisms relies on the Polymerase Chain Reaction (PCR), which is highly sensitive and rapid for detecting microorganisms, including bacteria [8]. Given the danger of these bacterial species and the severe damage they cause, which can result in death if not accurately and early detected, the idea of this study was developed. The study focuses on isolating the aerobic bacterial species responsible for burn and wound infections and diagnosing them based on their phenotypic and biochemical characteristics, along with a molecular study of important resistance genes in the isolated species using PCR.

2.Materials and Methods

1.2 Sample Collection:

A total of 155 swabs were collected from patients with burn and wound infections under the supervision of the attending physician at Tikrit Hospital, Dujail Surgical Hospital, and Balad Surgical Hospital, from October 1, 2024, to January 16, 2025. Relevant information, including medical history, gender, age, and type of procedure, was gathered. The swabs were taken before stitch removal, with a focus on areas with pus present in the wounds. The swabs were immediately transported to the laboratory for cultivation on two types of agar plates: Blood agar and MacConkey agar. The plates were incubated at 37°C for 24 hours. Catalase, Coagulase, IMVIC tests, and Gram staining were performed. The bacterial isolates were preserved in long-term storage in test tubes containing 4 ml of Brain Heart Infusion Broth, followed by the addition of 1 ml glycerol, and stored at -20°C.

2.2 Bacterial Genetic Content Study:

1.2.2 Genomic DNA Extraction:

Nineteen bacterial isolates were selected based on their production of virulence factors and resistance to several antibiotics. Fourteen isolates were selected based on virulence factors, and fifteen isolates were selected for their antibiotic resistance. The genomic DNA was extracted using a commercial kit (Geneaid) according to the manufacturer's instructions [9][10]. The following steps were followed:

1. Add 1.5 ml of bacterial culture (S. aureus) to a 1.5 ml Eppendorf tube. Centrifuge at 14,000 rpm for 5 minutes to obtain a pellet and discard the supernatant.
2. Add 25 µl of Lysozyme enzyme (0.8 mg/200 ml) to the tube and vortex gently. Incubate at 37°C for 20 minutes.
3. Add 200 µl of Lysis Buffer, vortex, and incubate at 70°C.
4. Add 20 µl of Proteinase K, vortex, and incubate at 60°C for 15 minutes with intermittent mixing every 3-5 minutes.
5. Add 200 µl of absolute ethanol, vortex for 30 seconds, and transfer the mixture to a GD column. Centrifuge at 10,000 rpm for 2 minutes, discard the supernatant, and wash the column with 500 µl Wash Buffer. Repeat the centrifuge step.
6. Elute the DNA by adding 100 µl Elution Buffer, incubate for 3 minutes, and centrifuge at 13,000 rpm for 3 minutes. Store the extracted DNA at -20°C until use.

2.2.2.DNA Purity Check:

The concentration and purity of the extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer. One microliter of rehydration solution was used for calibration, and the DNA sample's concentration and purity were determined by reading the absorbance at 260 nm. The DNA concentration was calculated, and purity was assessed based on the A260/A280 ratio

3.2 PCR Amplification Reactions

1.3.2 Preparation of Gene Primers:

The primers were prepared in the form of lyophilized powder by the manufacturer (Scientific Researcher Co. Ltd.) at various concentrations in picomoles. Each primer was dissolved in an appropriate, sterile, acid-free distilled water to obtain a stock solution with a concentration of 100 picomoles/µl. The primers were thoroughly mixed before use to ensure uniform distribution in the PCR reaction

solution. The stock primers were stored at -20°C. For use in the PCR reaction mixture, the primers were diluted to a concentration of 10 picomoles/µl by adding 90 µl of sterile distilled water to 10 µl of the stock primer.

In this study, six types of primers were used. These primers target genes for Staphylococcus aureus diagnosis, including virulence genes (nuc1, lip1, blaZ), and resistance genes (mecA, parC, mph), which are associated with resistance to some antibiotics.

2.3.2Polymerase Chain Reaction (PCR) Method:

The PCR reaction mixture was prepared by mixing the DNA sample with the appropriate primer for each gene, combined with the Premix components, in a 0.2 ml Eppendorf tube, which was pre-prepared by the manufacturer (Promega, USA). The total reaction volume was set to 20 µl with distilled water, as shown in Table 5-3.

The mixture was vortexed using a Microfuge for 3-5 seconds to ensure proper mixing of the reaction components.

The reaction tubes were then transferred to a thermal cycler to undergo the amplification process, using the program designed for each reaction, as outlined in Table 2-3. After amplification, the samples were loaded into pre-prepared 2% agarose gel wells, with 55 µl of DNA Ladder (prepared by Biolaps) added to one of the wells. The samples were subjected to electrophoresis for 45 minutes, after which the gel was visualized using a UV-Transilluminator.

Table 1: PCR Conditions for Genes Used in the Study

Gene	Main Steps	Number of Cycles	Temperature (°C)	Time
ErmA	**Step 1: Initial Denaturation**	1	95°C	5:00
	Step 2: DNA Denaturation	35	95°C	0:55
	Primer Annealing		53°C	0:55
	Primer Extension		72°C	0:50
	Step 3: Final Extension	1	72°C	5:00
	Step 4: Hold		4°C	-
sea	(Same as above)	(Same as above)	(Same as above)	(Same as above)

hlb	(Same as above)	(Same as above)	(Same as above)	(Same as above)
MecA	(Same as above)	(Same as above)	(Same as above)	(Same as above)

4.2 Detection of PCR Products Using Gel Electrophoresis:

After the PCR amplification phase is completed, the reaction products are detected by examining the specific bands of the products based on their molecular sizes. This is done by performing gel electrophoresis of the samples on 1% agarose gel, which is prepared according to the size of the DNA fragment corresponding to the gene being studied.

The PCR products are loaded into the gel wells, with 5 µl of the product loaded in each well. A DNA Ladder (bp100) is then added to a dedicated well on one side of the gel. After filling all required wells, an electric current is applied for a period of 1.5 - 2 hours. Once electrophoresis is complete, the gel is examined under ultraviolet (UV) light and photographed using a UV-Transilluminator.

5.2 Monoplex PCR Mixture:

In this process, a Green Master Mix *GoTaq from Promega is used to prepare the reaction mixture for conducting the monoplex PCR. The manufacturer's instructions are followed during the preparation of the reaction mixture. The components are collected in PCR reaction tubes and mixed in a sterile environment on an ice pack. The tubes are then vortexed for a few seconds (approximately 5 seconds), as shown in Table 2. Afterward, each PCR reaction tube is transferred to the programmed thermal cycler, as outlined in Table 3.

PCR Reaction Mixture PCR	Volume (uL)
Master Mix (Taq)	**10ul**
Primer forward(10uL)	**0.5**
Primer reverse(10uL)	**0.5**
DNA template	**2ul**
Nuclease-Free Water	**7ul**
Total Volume	**20ul**

5.2 Agarose Gel Electrophoresis:

This method was used to separate DNA molecules of different sizes, and the electrophoresis was carried out according to the method described by [11] as follows:

1. **Preparation of Agarose Gel:**

A 2% agarose gel was prepared by dissolving 1 gram of agarose powder in 50 mL of S.B (X1) buffer.

2. **Gel Preparation:**

The solution was placed in a flask and heated using a heat source until it dissolved, with constant stirring until it boiled. It was then allowed to cool to a temperature of 50-60°C. Once cooled, 1 µl of Red Safe dye was added.

3. **Gel Casting:**

The agarose gel was poured into the tray of the electrophoresis apparatus, and the comb was placed to form wells at the edges of the gel. The gel should be poured slowly to prevent the formation of air bubbles. If bubbles were formed, they were removed using a pipette. The gel was left to solidify for 20 minutes.

4. **Electrophoresis Setup:**

The tray was placed in the electrophoresis tank, which was then filled with an appropriate amount of TBE (X1) buffer. The comb was gently lifted.

5. **Sample Preparation:**

The samples were prepared by mixing 3 µl of loading buffer with 5 µl of DNA sample. The mixture was carefully loaded into the wells.

6. **Electrophoresis Process:**

The electrophoresis apparatus was turned on to apply a voltage of 5 volts per centimeter. The process was carried out in two stages:

Stage 1: 35 volts for 15 minutes (to ensure the samples stay within the wells).

Stage 2: 50 volts for 60 minutes (to allow separation of the DNA fragment)

7. **Gel Visualization:**

After electrophoresis, the gel was examined under ultraviolet (UV) light using a UV-Transilluminator to visualize the DNA bands and PCR product. This method enables the separation and visualization of the PCR-amplified DNA fragments

based on their size, providing crucial information for analyzing the results of the PCR reaction

3.Results and Discussion

1.3Collection and Isolation of Clinical Samples:

The current study involved the collection of 115 clinical samples from burn and wound infections of patients admitted to Tikrit Teaching Hospital, Dujail Surgical Hospital, and Balad Surgical Hospital between October 1, 2024, and January 16, 2025. A total of 57 wound samples and 58 burn samples were collected from both genders. After cultivating the samples on suitable growth media, the bacterial isolates were diagnosed morphologically and microscopically, in addition to performing biochemical tests. A total of 95 bacterial isolates were obtained, representing 82% of the samples. The laboratory results identified three main bacterial species: *Staphylococcus aureus* with 30 isolates, *Pseudomonas aeruginosa* with 33 isolates, and *Klebsiella pneumoniae* with 32 isolates, as well as other species such as *Escherichia coli*. These findings are consistent with the results of a similar study by [12]. Table 4 presents the distribution and percentage of each bacterial species.

Table 4: Distribution of Bacterial Isolates According to Species and Sample Source

Bacterial Species	Number of Isolates	Percentage
Staphylococcus aureus	30	31.6%
Pseudomonas aeruginosa	33	34.7%
Klebsiella pneumoniae	32	33.7%
Escherichia coli	4	2.1%
Total	95	100%

2.3 Diagnosis:

The bacterial isolates were initially diagnosed based on their morphological characteristics, particularly after being cultured on various growth media. The results of the culture revealed that some isolates grew on certain media and not others. For example, some isolates grew on *Mannitol Salt Agar* and formed yellow, round, small colonies, turning the medium from pink to yellow due to the

fermentation of mannitol sugar. This is a distinguishing characteristic of *Staphylococcus aureus* [15], as shown in Figure 1.

The results indicate a significant prevalence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, which are commonly associated with wound and burn infections. These findings highlight the need for timely and accurate diagnosis, as these pathogens can contribute to the severity of infections if not properly managed.

Figure 1: Morphological Appearance of S. aureus Colonies on Mannitol Salt Agar.

The isolates that grew on MacConkey Agar showed bright pink colonies due to lactose fermentation, and the colonies were mucous in appearance. This characteristic is attributed to certain enteric bacteria, including Klebsiella pneumoniae, which possesses a capsule [14]. This is shown in Figure 2. These morphological characteristics help in the preliminary identification of bacterial species and are crucial for differentiating between the various pathogens present in wound and burn infections

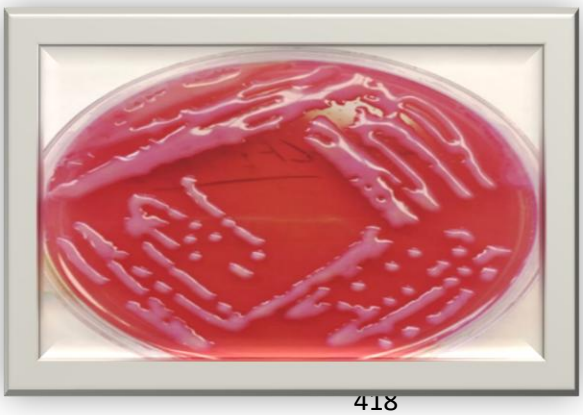


Figure 2: Klebsiella pneumoniae on MacConkey Agar.

The isolates of Pseudomonas aeruginosa appeared as colorless, pale colonies on MacConkey Agar due to their inability to ferment lactose. Additionally, they were characterized by a distinctive odor similar to fermented grapes. On solid nutrient agar, the colonies appeared green due to the secretion of the pigment pyoverdine [17]. This is shown in Figure 3.

These distinctive features of Pseudomonas aeruginosa can be used to help in its identification, as the odor and pigmentation are key indicators for distinguishing it from other bacterial species in clinical samples.

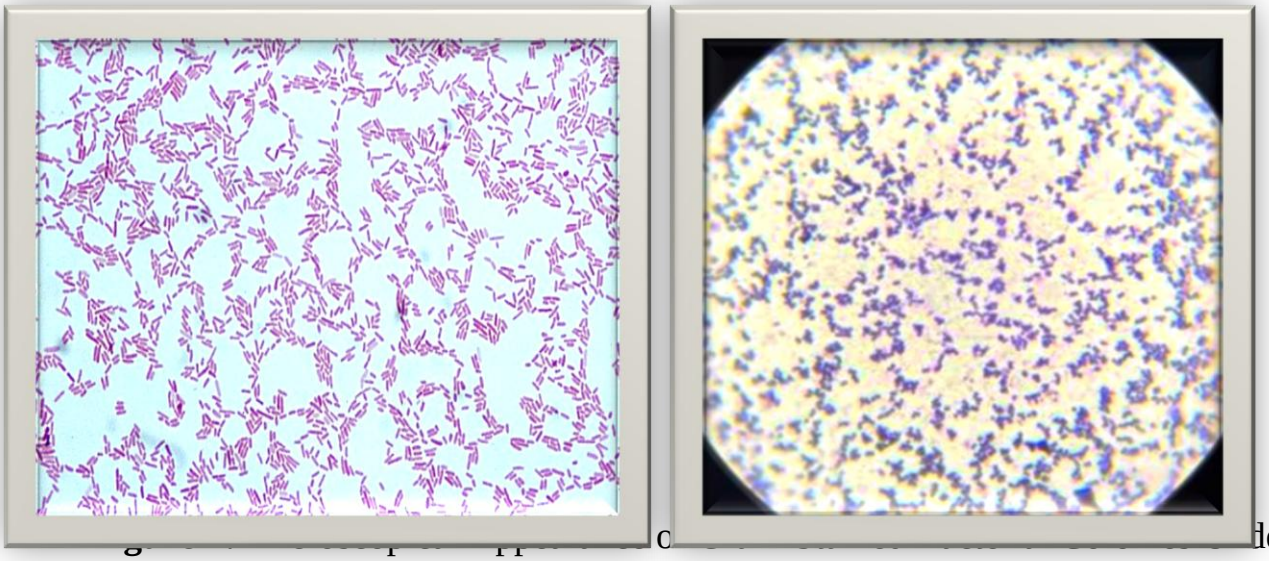
Figure 3: Morphological Diagnosis of P. aeruginosa on Solid Nutrient Agar.

3.3 Microscopical Identification:

Bacterial cells were microscopically diagnosed by performing Gram staining. A fresh pure colony from each bacterial species isolated during the study was taken individually. A smear was made from each colony on a glass slide, which was then stained using the Gram staining technique, following the sequential steps. After the slide dried, it was examined under the oil immersion lens of a microscope.

Some bacterial colonies appeared as small clusters with a purple color, indicating that they were Gram-positive. In contrast, other colonies appeared as rod-shaped cells or short chains with a pink color, indicating they were Gram-negative. These findings are shown in Figure 4.

This method is crucial for the initial identification and differentiation between Gram-positive and Gram-negative bacteria, which provides key information for further species identification and treatment decisions.



Light Microscope at x100 Magnification.

4.3 Biochemical Identification for Isolated Bacterial Strains:

The results of the biochemical tests performed on the bacterial isolates during this study showed the following:

1. ***P. aeruginosa*:**

The colonies in MacConkey Agar were large with a fermentation odor that was like grapes that were fermented and were pale yellow color [17]. The isolates were also positive on the Oxidase test as the reagent turned purple. The purple color change was an implication that they had Cytochrome Oxidase as a hydrogen acceptor. The isolates also tested positive on the Catalase test, as evidenced by the bubble. They were also positive on the Citrate test since the medium turned greenish-blue and citric acid was being produced [18]. The isolates tested negative on the Indole test since no red ring was formed, Methyl Red test since there was no color change, and on Voges-Proskauer. The Urease test was also negative since no pink color was formed [19]. These results were in line with the study by [20].

2. ***S. aureus*:**

Biochemical assay Catalase test All Staphylococcus aureus isolates were positive for Catalase, (bubbles was formed when reagent was added denoting degradation of hydrogen peroxide into water and oxygen). On the other hand the isolates were Methyl Red (red ring formation), Voges-Proskauer, Simmon's Citrate (bluish-green color) and Urease (pink color formation) positive [21]. These findings were consistent with [22].

3. ***K. pneumoniae*:**

The isolates were confirmed as K. pneumoniae by Catalase (bubbling), Voges-Proskauer and Citrate (growth on citrate as the sole carbon source changed the medium into bluish-green) [23]. The isolates were Indole negative (failed to produce a red ring), Oxidase and Methyl Red negative (did not turn red) [23]. These results are consistent with [24].

The results of these biochemical tests were further supported by using the *Vitek2 Compact* system.

Table 3: Biochemical Tests for Bacterial Isolates

Biochemical Test	P. aeruginosa	S. aureus	K. pneumoniae
Growth on MacConkey Agar	Pale yellow colonies, fermented grape odor	Yellow colonies	Mucous, pink colonies
Oxidase	+	-	-
Catalase	+	+	+
Citrate (Simmon's Citrate)	+	+	+
Indole	-	-	-
Methyl Red	-	+	-
Voges-Proskauer (VP)	-	+	+
Urease	-	+	+

These biochemical results help confirm the identity of the bacterial isolates and provide insight into their physiological properties, which are crucial for understanding their pathogenic potential and treatment response.

5.3 Molecular Study Results

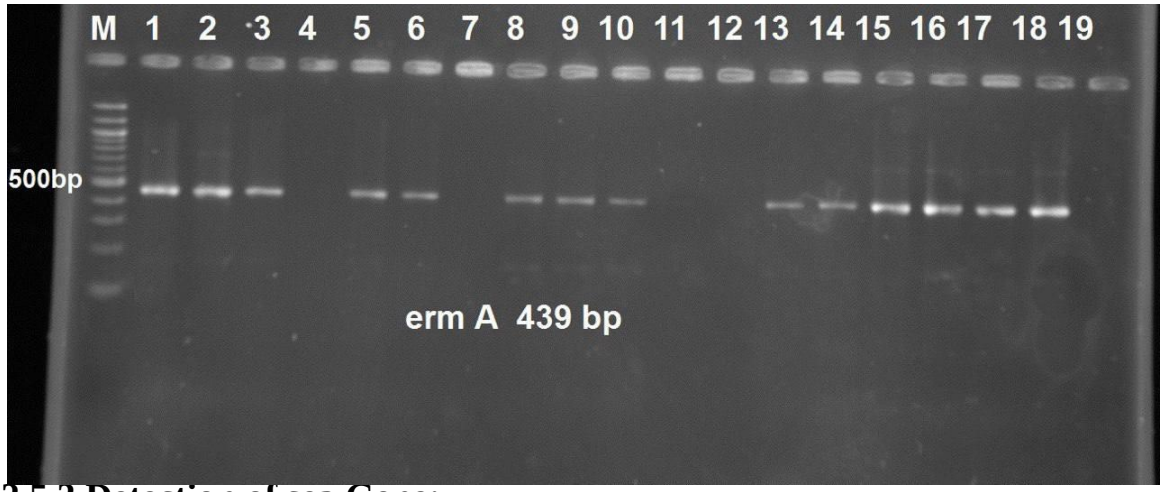
1.5.3 Detection of the ermA Gene:

Presence of the antibiotic resistance gene ermA (439 bp) was tested by PCR (Figure 5). They tested 15 bacterial samples and found that 53.3% (8/15) were positive for said gene. Positive samples were; 1, 2, 3, 6, 7, 8, 9 and 10. You're right! 7 samples (46.7%) indicated negative (4, 5, 11, 12, 13, 14, 15).

These data indicate that the prevalence of the ermA gene is moderate in the tested bacterial isolates. The ermA gene indicates possible resistance to antibiotics of the macrolide family including erythromycin. The higher positive rate (53.3%) may suggest the selection pressure of either drug treatment or environmental conditions that might facilitate the existence and dissemination of this resistance gene [25].

In another work of [26], out of 215 Staphylococcus aureus isolates, 82 (40.9%) were methicillin-resistant Staphylococcus aureus (MRSA). The rates of phenotypic resistance of iMLSB, cMLSB and MS, were 4.18%,26.9% and 5.1%, respectively. Of the nine iMLSB phenotypic resistance isolates, four isolates contained the ermC gene, two isolates harbored the ermB gene, and one isolate harbored the ermA gene. No erm genes were detected in two isolates.

Also in an investigation by [23] that included 172 bacterial isolates ermA gene was found less prevalent both in methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant Staphylococcus epidermidis (MRSE), methicillin-sensitive Staphylococcus aureus (MSSA), as well in methicillin-sensitive Staphylococcus epidermidis (MSSE); lower than 14%. These findings reveal the prevalence of the ermA gene in clinical bacterial isolates and types of resistance to macrolides, which are important for antimicrobial treatment.



2.5.3 Detection of sea Gene:

PCR was performed for the detection of sea gene (Staphylococcal enterotoxin A) of the size 342 bp, in 15 suspected Staphylococcus aureus samples. Of these DNA, it was confirmed that 8 (53.3%) of them were positive for sea gene by a 342 bp clear band in the gel. The other 7 (46.7%) were negative, as presented in Fig. 6.

These findings revealed that over half of the samples analyzed were positive for the sea gene of Staphylococcus aureus, which is responsible for encoding enterotoxin A. The fact that this gene is present means these strains have the capacity to produce enterotoxin, which is related to food poisoning and is an indication of the bacteria's virulence. The moderately high percent positive samples 53.3%) recorded reveals that the gene of this bacterium is widespread in the tested isolates, and it poses a potential threat especially when these strains were isolated from food and from clinical sources such as burns and wounds. The remaining 46.7% of the samples what are negative could belong to strains that harbored no sea gene or another enterotoxin gene, such as seb or sec, needed to be analyzed in order to determine the other enterotoxin genes found in these strains.

Five enterotoxin genes (SEA to SEE) were detected by a study from Assiut, Egypt in S. aureus isolates from various clinical samples. The findings revealed that in 75 clinical samples, 61.3% were positive for S. aureus. The sea gene was found by PCR in 32.6% of the isolates, being the most frequent gene among those studied [24]. In another research carried out in Tehran the frequency of sea gene among clinical isolates of S. aureus was investigated and 46.9% of 128 clinical isolates harbored the sea gene (17). The study indicated a significant association between the presence of the sea gene with type of infection and antibiotic resistance [26].

These results reveal the general occurrence of the sea gene in clinical strains and the need for detection of enterotoxin production in Staphylococcus aureus isolates, particularly in the clinical and food areas.

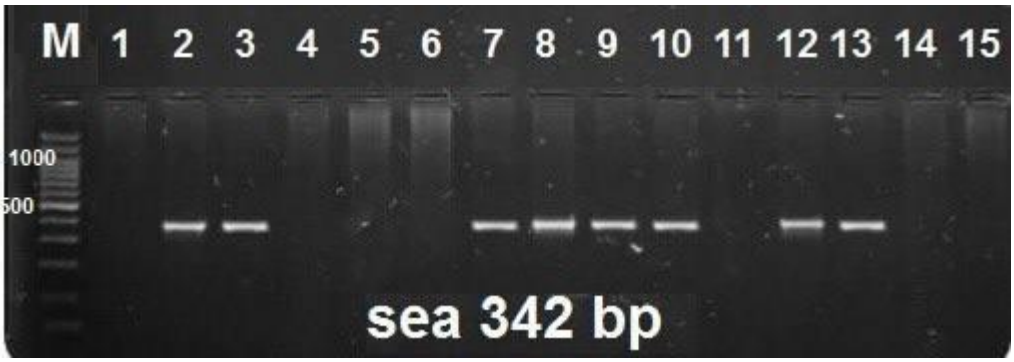


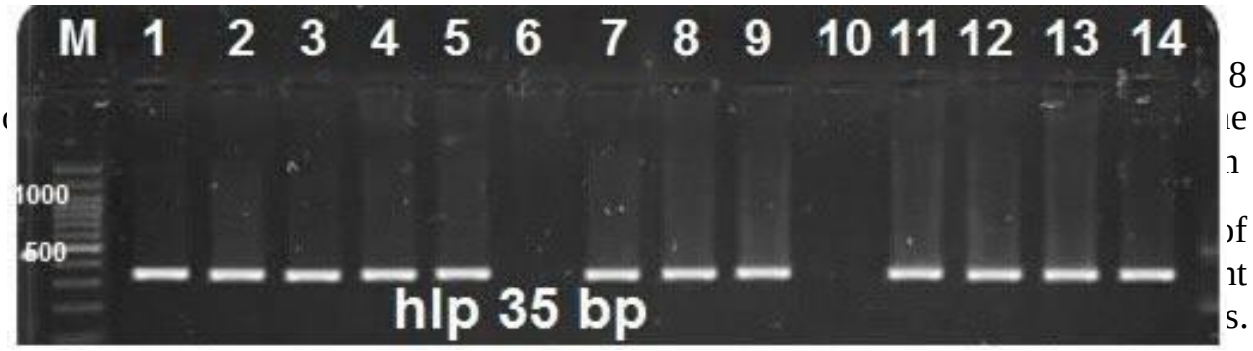
Figure 6: Detection of sea Gene at 342 bp.

3.5.3 Detection of hlp Gene:

The gene hlp was observed to be in 8 out of 14 S. aureus isolates (Figure 7), at a rate of 57.1%. The other 6 samples (42.9%) were negative for this gene.

This result indicates that hlp is harbored by a large number of the S. aureus isolates studied. It is optional that the hlp gene is coding for a genetic regulator or a histone-like protein participating in the maintenance of DNA structure or in the regulation of gene expression. The 57.1% prevalence could represent the relevance of this gene for some strains, and the absence of the gene in those samples may be indicative of genetic or functional diversity among them. Therefore, additional research is necessary to investigate the relationship between the existence of this gene and virulence genes, antibiotic resistance, or other differences.

To our best knowledge however, no such published study has been found on PCR detection of hI p (Histone-like protein) gene of Staphylococcus aureus isolates. Nevertheless, various other virulence and resistance genes have been reported for this bacterium. S aureus prevalence and its antibiotic resistance (2014 through 2017) An example is a study carried out in Algeria between 2014 and 2017 to evaluate the prevalence of S aureus isolates and their antibiotic resistance. The study identified the genes mecA and nuc by PCR, while the hlp was not incorporated [28].



.Figure 7: Detection of hlp Gene at 35 bp

4.5.3 Detection of mecA Gene:

The presence of the mecA gene of methicillin resistance among S. aureus isolates was investigated by PCR. This gene is the major determinant for the identification of MRSA, and the specific genetic product is mecA, which is 475 bp in size.

There were 15 isolates which have been tested for PCR and 8 (53.3%) of them were positive for mecA at 475 bp which represent methicillin resistant according to the PCR. The other 7 samples (46.7%) did not present this band and were considered negative for the mecA gene, and hence for MSSA. The results are presented in Fig 8.

These data indicate that more than half of the tested bacterial isolates were positive for the mecA gene and that MRSA were highly prevalent in these specimens. This represents a key element for the resistance to β-lactams and to methicillin by these isolates.

This high rate of mecA may be due to the uncontrolled use of antimicrobials in hospital or health care service, or the community and also to the inter-patients, health care work resistance or through unsterilized equipment. These findings emphasize the significance of molecular tests for rapid and accurate detection of MRSA compared with the available traditional methods which have implications for the control of infection and treatment [29]

A recent study which also utilized PCR for detection of mecA gene of S. aureus isolates from different clinical specimens also observed similar prevalence rates.For instance, a study in Pakistan found a mecA prevalence of 58% in isolates from surgical wounds, which is close to the findings of our study. In contrast, a study in Gaza reported a higher prevalence of 65%, indicating greater spread of methicillin resistance in that region. A study in Nepal revealed an even higher prevalence of 94%, reflecting significant health challenges in antibiotic resistance there.The variability in the prevalence of mecA across these studies can be attributed to several factors, including the sources of samples (e.g., blood, wounds, urine, community vs. hospital samples), the level of infection control measures in healthcare settings, and the indiscriminate or excessive use of antibiotics, especially in developing countries. Geographic and environmental differences may also influence the dynamics of bacterial resistance spread [32].

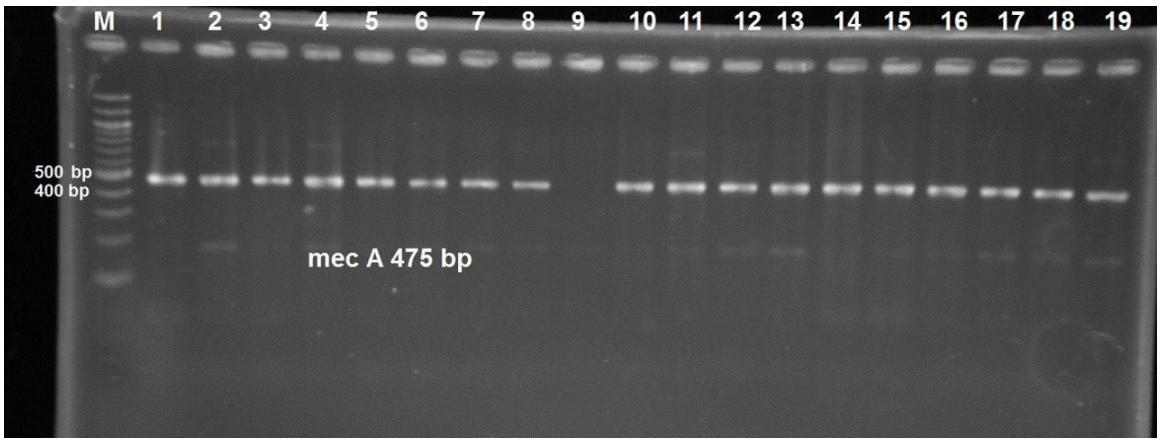


Figure 8: Detection of mecA Gene at 475 bp.

5.5.3 DNA Genomic Extraction Results:

Obtaining pure, high-quality genomic DNA is a fundamental step in molecular studies, especially when using Polymerase Chain Reaction (PCR) to detect target genes such as the mecA gene responsible for methicillin resistance. Genomic DNA of 15 isolates used in this study was isolated. The quality and quantity of the extraction were accessed using gel electrophoresis. The agarose gel electrophoresis result revealed that there were bright, complete bands in all groups, indicating a successful extraction of DNA.

All samples provided clear and single band in DNA quality which appeared to be highly pure without degradation and fragmentation. Also, no smearing or shadowing was seen, which might be suggestive of DNA damage or nuclease contamination, respectively. Equal intensity of the bands (of about the same strength) in the different samples demonstrated equal amounts of DNA extracted.

The reliability of the molecular data that will be drawn from these material is confirmed by the successful genomic DNA extraction obtained in all samples. Inovirus DNA preparation Good quality DNA is a prerequisite to the success of molecular detection techniques, including classical PCR, real-time qPCR and DNA sequencing [34]. Many reports have emphasized that the quality of isolated DNA may influence the PCRs. For example, [35] filtered for clear and clean genomic DNA bands in their results to determine successful amplification of resistance genes such as mecA and blaZ.

Additionally, [36] noted that the presence of smearing in the gel negatively impacts the genetic analysis results, further highlighting the importance of visual assessment of DNA extraction, as demonstrated in this study .The current results confirm that all samples contain high-quality genomic DNA, which is fully suitable for use in subsequent molecular analyses, thereby enhancing the accuracy and reliability of the results when detecting target genes such as mecA. This is shown in Figure 9.

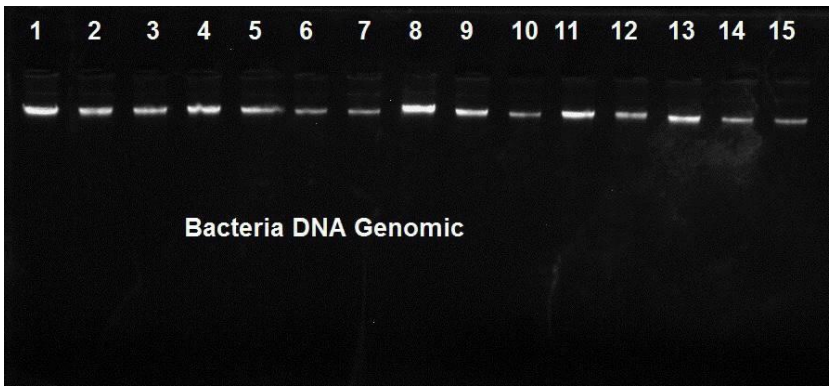


Figure 9: Detection of Genomic DNA.

4. Conclusions:

The study demonstrated a widespread presence of antibiotic-resistant bacterial species in wound and burn samples, particularly S. aureus and P. aeruginosa. PCR testing confirmed the presence of critical resistance genes such as mecA, ermA, and sea, highlighting the potential danger of these isolates in clinical environments. The results emphasize the importance of relying on molecular diagnostic techniques to accurately identify resistance genes, in comparison to traditional diagnostic methods, ensuring more precise and timely detection of antibiotic-resistant bacteria in clinical settings.

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