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Research Paper

Detection of some virulence factors of staphylococcus aureus isolated from urinary tract patients in Kirkuk city, Iraq

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ABSTRACT

Background: Staphylococcus aureus is an important uropathogen that is a known cause of urinary tract infections (UTIs) and the virulence factors contribute to the pathogenicity and persistence of S. aureus.
Objective: To isolate and identify S. aureus from patients with UTI and to characterize some of the virulence factors present among the isolates.
Methods: The number of urine samples collected from patients with UTI was 200 of which 100 were male and 100 were female. The isolates were identified by biochemical tests and phenotypic and microscopic features and confirmed by API 20 Staph system. Confirmed S. aureus isolates were then tested for some of the virulence factors such as DNase production, biofilm formation, hemolysin production, production of broad spectrum beta-lactamase and adherence ability.
Results: 23 S. aureus isolates were confirmed from 200 urine specimens (11.0%) by female patients and 15 (7.5%) by male patients. Virulence factor analysis showed that 76.31% of the isolates produced DNase and all the isolates (100%) were biofilm producers. As far as hemolytic activity is concerned, 65.78% of the isolates showed complete (full) hemolysis. The rate of beta-lactamase production by the isolates was 55.26% (broad spectrum) and 81.57% of the isolates showed adherence ability.
Conclusion: S. aureus from UTI patients were highly virulent with 100% of the isolates producing biofilm and significant proportions of the isolates had DNase, hemolysin, beta-lactamase production, and adherence ability. The higher level of isolation in female patients and the broad spectrum of virulence factors and resistance determinants expressed indicates the pathogenicity of S. aureus in UTIs and the continued need to monitor virulence and resistance factors of uropathogenic isolates.

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1. INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium, spherical in shape, often arranged in the form of irregular clusters resembling a grape cluster, or maybe in the form of a single cell or the form of pairs, tetras, or short chains, and it is immobile, non-spore forming , non- capsulate except some species. [1] Staphylococcus aureus Catalase is positive, Oxidase – negative. Its cells are facultative anaerobic, grows easily on many media, the most important of which is the mannitol salt agar for its ability to withstand high salinity. Its nutrition is Chemoorganotrophs, it has fermentative metabolism, and it is active in the metabolism, exploiting carbohydrates and amino acids as a source of carbon and energy. Their colonies are circular, convex, with different white or golden-yellow colors. [2] They are a normal flora on the skin, nasal cavity and mucous membrane, [3] about (30%) of humans carry Staphylococcus aureus in their noses naturally and are more likely to contract it as an internal infection.

[4] Staphylococcus aureus is a bacterium that coexists with other bacterial species present in the human body but once it crosses the skin barrier it becomes an opportunistic pathogen, causing many purulent and systemic infections as well as acute and chronic infections. [3] Staphylococcus aureus is a major cause of nosocomial infection, as these bacteria cause diseases in hospitalized patients with impaired natural body defense mechanisms, with nosocomial infection. [5] Rising in recent years. Bacteria are considered pathogens when they can cause disease, and this susceptibility is called pathogenicity, while virulence is the severity or degree of pathogenicity and is represented by several elements produced by the pathogen and help cause disease. [6] Staphylococcus aureus possesses many virulence factors such as gluing, invasion, immune penetration, and many enzymes, toxins, and other virulence factors that increase their virulence and pathogenicity. [7], [8] To shed light on this topic and its health and scientific importance, I conducted this research.

1.1 Materials And Methods Specimen Collection

200 urine samples were collected from urinary tract patients for the age groups between 15 – 60 years and both sexes from patients visiting the following hospitals (Al-Nasr Hospital for Maternity, Gynecology, and Children, and Kirkuk General Hospital) after consulting the specialist doctor. The patients were referred to the laboratory and taking the information of each sample and writing it down in the special questionnaire form, where urine samples were collected and the middle diuretic drops were taken, and placed in sterile urine collection cups,and transferred directly to the laboratory.

1.2 Isolation and Diagnosis

After the samples were transferred to the laboratory, they were cultured on the pre-prepared mannitol salt agar, the dishes were placed in the incubator at a temperature of 37 °C and air conditions for 24 hours, and then the bacteria were diagnosed initially by observing the phenotypic characteristics of the colonies in terms of Shape, Size, Raised, Edge, Colour and their effect on the medium such as the fermentation of mannitol, and they were purified by re-culturing them on the nutrient agar and incubated at 37 °C for 24 h, after which microscopic examination was conducted to find out Bacterial isolation response to Gram stain , where part of a colony growing on a nutrient agar was taken by a loop and a bacterial smear was made on a clean glass slide and stained with gram stain and then Viewed with light microscope using an oil lens with a final magnification of 1000x in order to distinguish the shape and color of the cells. The diagnosis was confirmed using API 20 -staph system.

1.3 Detection of Hemolysin Production

Bacterial isolates were cultured on the Blood agar and the dishes were incubated at a temperature of 37°C for 24 hours, the presence of decomposition in the dishes as well as itsshape and type were observed.

1.4 Detection of Bioflim Formation

The bacterial Isolates were cultured on the medium of the red Congo Agar, by stabbing the medium with the tip of the loop with colonies of bacteria whose ability to form the biofilm isto be known. Positive results were inferred by the change in the color of the colonies to black [9].

1.5 Detection of DNase Production

A young colony was taken from the Agar Mannitol salt and cultured in a straight line on the medium of DNase agar, and incubated at a temperature of 37 °C for 24 hours. We then covered the dish with a HCI reagent at a concentration of 1 molar, the formation of a transparent halo around the transplanted colony is evidence of the ability of bacteria to produce the DNase [10].

1.6 Detection of the Production of Broad-Spectrum Beta-Lactamase Enzymes

The Disk Approximation method was used to detect bacteria secreting these enzymes according to. [11] Then, the Augmentin (Amoxicillin/Clavulanic acid) tablet was placed in the middle of the dish and the other tablets (Ceftazidime, Cefotaxime, Azitreonam) were arranged on the periphery and 3 cm from the center of the median disc. Inhibition zones between the central disk and one or more of the tablets used are observed to indicate a positive result and the production of these enzymes by the bacterial isolates used .

1.7 Detection of the Ability of Bacteria to Adhere to the Surface of Epithelial Cells

The ability of bacteria to adhere to the surface of epithelial cells was tested according to. [12] The results were recorded by calculating the adhesive rate based on the following law: adhering rate = total adherent bacteria / total epithelial cells.

2. RESULTS AND DISCUSSION

In this study, 200 urine samples were collected from patients with urinary tract infections for both sexes and for age groups between 15 – 60, for the period from 2022 August 1 to 2022 December 31. After the samples were transported directly to the laboratory, they were cultured on medium of mannitol salt agar, and incubated at 37°C for 18 h. Thirty – eight of the cultured media gave positive growth at a rate (19%). They were fermented mannitol, all isolates that fermented mannitol were diagnosed based on their phenotypic and microscopic characteristics and biochemical tests. Microscopic diagnostics showed that all of these isolates were gram-positive and had a spherical shape looks like a bunch of grapes. All were positive for coagulase and catalase, and all isolates were negative for oxidase. However, most of the isolates were positive for urease at a rate of 57.89%, and this is consistent with the results of. [13] Then these results were confirmed by the API 20 -staph system, and was consistent with. [14] The results of the current study showed that the majority of S.aureus wereisolated from within the city , 28 isolates, with a percentage of 73.86%, were isolated in the city and, this is due to the population momentum leading to increased contamination and bacterial infection.

The fact that antibiotics are used irregularly and in large quantities without health awareness. The percentage of their presence outside the governorate was estimated at 10 isolations by 26.31%, due to the nature of the area free of environmental pollutants, as well as the behaviors of the districts and aspects and how to live represented by agriculture and animal husbandry, dependence on natural sources in nutrition and treatment, and their lack of use of manufactured medicines .The figure below shows the distribution of isolates by mansions. In this study, the ability of 38 clinical Isolates to produce some virulence factors that give and increase the virulence and pathogenicity of bacteria was tested,

namely the production of hemolysin, the production of bioflim, the enzyme of DNase, the production of betalactamese, and the production of adherence factors. Hemolysin is one of the Important virulence factors possessed by many bacterial species, which has the ability to destroy the cell membrane of red blood cells. [15] The results of the findings of [16] by 66%.The results of the detection of biofilm production in S.aureus showed that all isolates arecapable of producing biofilms by 100%, and this percentage does not correspond to the findings of the researcher, 13 where the results of her study showed that 81.6% of isolates arecapable of producing biofilms, and the positive result was inferred by the transformation of the color of colonies producing biofilms to dark black.

The biofilm is one of the most widespread virulent factors, as it works to provide full protection of the bacterial cell from theimmune defence of the host and phagocytes and from antibiotics, which makes the strains produced by it more virulent and more pathogenic. [17] The production of DNase is one of the characteristics of S.aureus, as the results showed that out of 38 isolates 29 isolates have the ability to produce this enzyme by 76.31%, and this percentage is close to what he found, [18] where the results of his study showed that 75% of isolates are capable of producing the enzyme, and it is evidenced by the formation of a transparent halo around the colonies of bacteria grown in a straight line shape by adding concentrated HCI acid. As for the production of beta-lactamese enzymes, the results showed that only 55.26% produce this enzyme, and this is not consistent with the findings of the researcher [19] as the percentage of isolates producing these enzymes reached 19.4%. This kills sensitive isolates and allows the spread and reproduction of resistant isolates. [20] The results of the detection of adhesion factors showed that 31 isolates of S.aureus are able to adhere by 81.57%, this percentage differed from the results of, [21] where the results of his study showed that 62.74% of Staphylococcus aureus is able to adhere . The ability of bacteria to adhere depends on the presence of fimbria-specific receptors found in cell membranes. [22] The walls of the reproductive system are covered with a layer of mucus. This layer forms an effective barrier against bacterial invasion as this layer constantly changes due to various factors, including hormones leading to peeling and erosion of the outer layer of the surfaces of epithelial cells, which helps in the arrival of pathogens such as bacteria to the wall of the urethra and gluing and infection events. [23] As shown in the figures below.

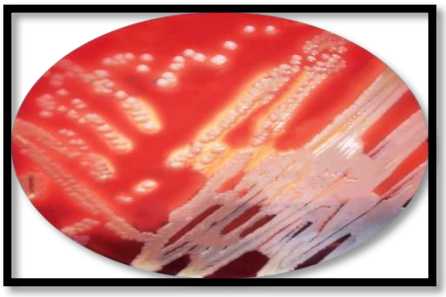


Figure 1. Shows Hemolysin Production

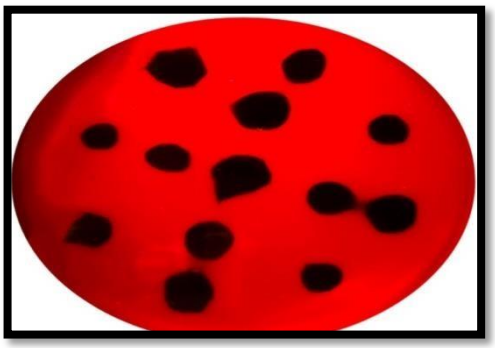


Figure 2. Shows Production of Biofilms



Figure 3. Shows DNase Production

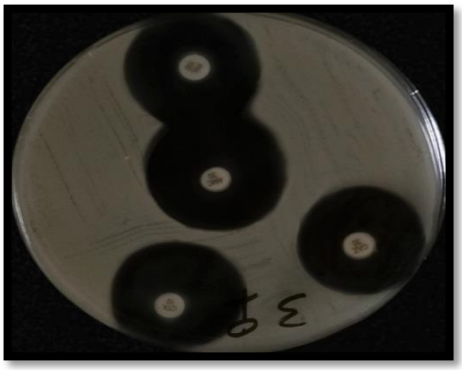


Figure 4. Shows the Production of the Beta-Lactamase

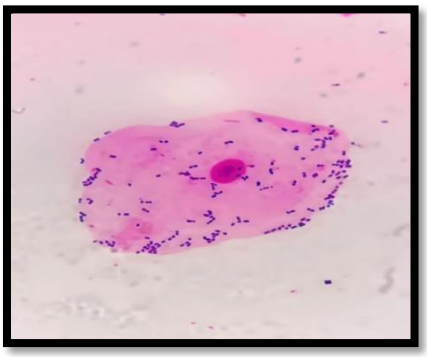


Figure 5. Shows Susceptibility S.Aureus to Adhere on Epithelial Cells

3. CONCLUSION

The highest incidence of S.aureus was found in females than in males, also the prevalence of UTI in the city center was higher than in the districts. The results of this study showed that most of isolates of S.aureus had the ability to produce many virulence factors such as hemolycin, biofilm, DNase, beta-lactamase, adhesion factors.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Fatima Mahmood Tariq	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓		✓
Najdat Bahjat Mahdi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Akeel Hussain Ali Al- Assie	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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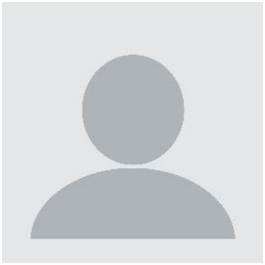
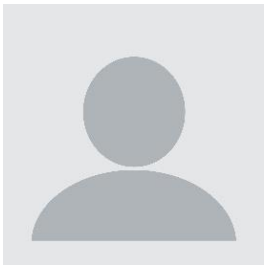
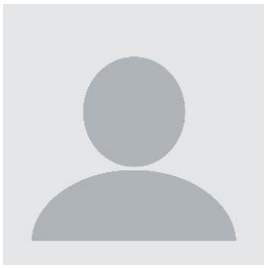
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