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Staphylococcus aureus isolated from various sites: Detection and diagnosis of some virulence factors production and antibiotic susceptibility

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Abstract

Background: Resistance of *Staphylococcus aureus* to commonly used antibiotics is linked to their ability to acquire and disseminate antimicrobial-resistant determinants in nature, and the marine environment may serve as a reservoir for antibiotic-resistant bacteria. Species of this genus can be distinguished by their capability to produce the coagulase enzyme that causes blood clotting, polypeptides that bind to activate prothrombin, in that way converting fibrinogen to fibrin and promoting the clotting of plasma or Blood Fifty skin swabs.

Methods: Samples were collected from patients who are burn and wound infections of Al-Yarmouk teaching hospital, during the period from November 2021 to March 2022, the researchers used different method to diagnosis of antibiotic resistance and alpha hemolysin assay by using a microtiter plate.

Results: The results were found by using cultural traits, microscopic analysis, and biochemical tests. Twelve *Staphylococcus aureus* isolates were discovered. A few antibiotics, including ciprofloxacin/CIP, cefotaxime/CTX, gentamycin (CN), tetracycline/TE, and chloramphenicol (C), were tested using the disc diffusion technique on *Staphylococcus aureus* isolates.

Conclusion: It was discovered that the twelve samples had alpha toxin production activity and their susceptibility to antibiotics was known.

Introduction

Staphylococci are facultative anaerobes that are non-motile and non-spore-forming, and they develop through aerobic respiration. They need a supply of organic nitrogen, such as arginine and valine [1]. The capacity of species in this genus to make polypeptides that bind to activate prothrombin, converting fibrinogen to fibrin, thereby promoting the clotting of plasma or blood, as well as the coagulase enzyme that causes blood clotting, distinguishes them from one another [2], As a result, *Staphylococcus epidermidis* is a coagulase-negative species, whereas *Staphylococcus aureus* belongs to the coagulase-positive staphylococcal species [3]. A serious threat to global health is the rise of drug-resistant bacterial infections. Many people believe that the widespread use of antibiotics has led to specific forces that have fueled the emergence of resistant strains. Less than a year after the second-generation beta-lactam antibiotics were introduced into clinical practice, Methicillin-resistant *Staphylococcus aureus* (MRSA) was first observed [4]. Due to the way that toxins work and the widespread gene expression of some virulence-determining factors, the present medical treatment methods are in difficulty [5]. Staphylococci species, such as *Staphylococcus aureus* [6], the bulk of infections can only be treated with methicillin and vancomycin because 90% of *Staphylococcus aureus* strains are penicillin resistant. However, with an increase in reports of methicillin-resistant *Staphylococcus aureus* (MRSA)and vancomycin-resistant enterococci (VRE), chemists are confronted with the challenging task of developing antibiotics with novel modes of action, and physicians with the task of treating infections that appear to have no hope of recovery [7]. because *Staphylococcus aureus* has coagulase production and beta-hemolytic activity, two traits that connect them to virulence in addition to alpha-toxin, which contributes to pathogenesis.

Methods

Isolation and identification of bacteria

From November 2021 to March 2022, fifty skin swabs were taken from patients at Al-Yarmouk Teaching Hospital who had burn and wound infections. Identification of these isolates was also done using the Gram stain and API staph [bioMerieux] as well as the shape, color, size, edges, and height of the colony on the surface of Brain Heart Infusion (HiMedia) agar and blood agar dishes [8]. The identification of these isolates also involved the use of several assays, such as the catalase test, the mannitol fermentation test, the coagulase test, the alpha-toxin test, and the hemolysis patterns on blood agar and Vitek 2 compact system [9].

Alpha hemolysin assay (Microtiter plate method)

Twelve isolates of Staphylococcus aureus were tested by the alpha hemolysin assay to determine toxin production in strain supernatants. In Tryptone Soya broth, bacteria were grown for 18 hours at 37 ºC. Each isolate's supernatant was moved to sterile test tubes after centrifugation (7000 rpm) for 15 minutes. 0.1 mL of 2% washed rabbit red blood cells and 0.1 mL of identical bacterial supernatants were added to the well of a U-shaped microtiter plate to test an isolate's capacity to produce alpha hemolysin. The first two wells of each row served as negative controls, containing 0.1 mL of ordinary saline and 0.1 mL of washed red blood cells. An hour was spent heating the microtiter plate to 37 ºC. Plates were maintained at 4 °C following incubation until the outcome (lysis of RBCs) was determined [10].

Vitek 2 compact system

The completely automated Vitek 2 compact microbiological system uses growth-based technology to identify microorganisms [11]. Colorimetric reagent cards are used in the system, and they are immediately incubated and interpreted. The reagent cards have 64 wells, each of which can hold a different test material. The substrates track a number of metabolic processes, including acidification, alkalinization, enzyme hydrolysis, and development in the presence of inhibitors.

Antibiotic sensitivity

The Kirby-Bauer disc diffusion technique was used to test the sensitivity of *Staphylococcus aureus* isolates [12]. During this research, the antibiotic discs ciprofloxacin/CIP, cefotaxime/CTX, gentamycin/CN, tetracycline/TE, and chloramphenicol/C were used.

Results

Morphological characterization

Twelve isolates (24%) were recognized as *Staphylococcus aureus* from the skin swab samples of all isolates. The outcomes showed that these strains were capable of fermenting mannitol and positive for the enzymes catalase, coagulase, and alpha-hemolysin (Hla). Characterizations based on morphology and biochemistry matched the information provided by [8,9]. In addition to the tests mentioned above, the API Staph system and the Vitek 2 system also perform biochemical identification, which verified the results of the earlier conventional identification shown in the table.

A toxin from *Staphylococcus aureus* called alpha-haemolysin can lyse various cell types and damage membranes. Because alpha- hemolysin disrupts ion transport across host cell membranes, it has several negative effects on host cells that eventually cause

apoptotic cell death and oedema [13]. This is why alpha- hemolysin is believed to be essential in infection. Alpha-hemolysin has been shown to play a part in the virulence *of Staphylococcus aureus* in a variety of infection types, including mastitis and pneumonia. The immunomodulatory characteristics of alpha-haemolysin are also noteworthy, particularly its capacity to promote the release of pro-inflammatory cytokines [14]. As a result, photodynamic therapy that activates alpha hemolysin may help to both get rid of infectious organisms and defend against damaging inflammatory processes. Several antibiotics, including ciprofloxacin/CIP, cefotaxime/CTX, gentamycin/CN, tetracycline/TE, and chloramphenicol/C), were tested using the disc diffusion technique on *Staphylococcus aureus* isolates.

Test	Activity
Blood hemolysis	100% (12/12 isolates) [+]
Catalase	100% (12/12 isolates) [+]
Coagulase	100% (12/12 isolates) [+]
Mannitol fermentation	100% (12/12 isolates) [+]
Alpha-toxin	100% (12/12 isolates) [+]

Table 1: Biochemical Tests *S. aureus.*

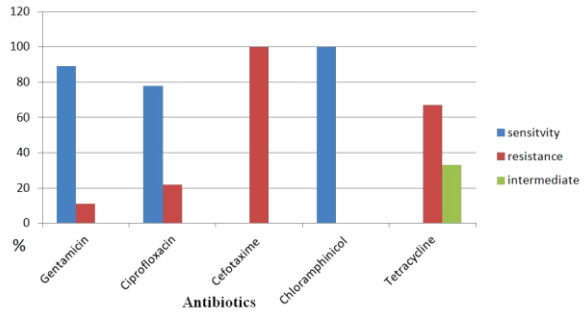

Figure 1: The percentage of susceptibility of isolates toward the selected antibiotic discs.

Discussion

Previous studies have shown that humans can act as contributors of antibiotic-resistant organisms in diverse environments, including aquatic settings, through horizontal gene transfer mechanisms such as transposons, plasmids, and integrons [15,16]. In contrast, the present study focused on *Staphylococcus aureus* isolated from skin swabs of burn and wound patients in a hospital setting.

The prevalence of *Staphylococcus aureus* and MRSA is increasing, leading to a higher frequency of hospital-acquired and community-acquired infections globally. This presents a significant public health issue [17]. In addition, the presence of diverse antibiotics of industrial origin in aquatic environments has the potential to modify microbial communities [18]. *Staphylococcus aureus* is regarded as a very proficient and adaptable human pathogen owing to its ability to acquire mechanisms of antibiotic resistance and pathogenic determinants, resulting in its development in both healthcare-associated and community contexts [19]. The nosocomial colonization of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) has the potential to remain unnoticed, with manifestations of the infection becoming apparent many months after patient exposure to the pathogen. Infected individuals might potentially operate as reservoirs for further transmission, particularly due to the prevalence of these strains carrying SCCmec types that encode resistance to methicillin and other beta-lactams [20].

To the best of our knowledge, this is the first study which has used a mixture of phenotypic approaches simultaneously to determine the occurrence and antibiotic resistance profiles of *Staphylococcus aureus* strains from beach water and sand in the study area. However, the frequency (12.2%) of isolation was lower in our study than observed in other studies [21].This study only analyzed a single isolate for every sample, which could account for the lower detection frequency.

The findings of Bimanand's research in 2018 [22], which examined the sensitivity of *Staphylococcus aureus* isolates to Gentamicin and Tetracycline, disagreed with those of the present study illustrated in figure 1. Their findings showed that 76% of the isolates were sensitive to Tetracycline and that 69% of the isolates were susceptible to Gentamicin. The findings were consistent with another research on S. aureus isolates conducted in India [23], which found that 96% of the isolates were susceptible to chloramphenicol, 76% of the isolates were susceptible to gentamicin, and 8% of the isolates were susceptible to tetracycline. According to the [24] research, about 97.2% of *Staphylococcus aureus* isolates were susceptible to Chloramphenicol ,while 61.2% of them were susceptible to Gentamicin. Chloramphenicol showed the highest sensitivity percentage, followed by Gentamicin.

The primary sources and producers of coagulase, catalase, and mannitol fermentation with alpha-hemolysin are skin infections brought on by burns and wounds contaminated with *staphylococcus aureus*. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) was not performed in this study, as no cefoxitin/oxacillin disc or mecA PCR testing was included. *Staphylococcus aureus* isolates were sensitive to ciprofloxacin, gentamicin, and chloramphenicol. A limitation of this study is that the alpha hemolysin assay was qualitative only, based on visual assessment of RBC lysis. Quantitative hemolytic titers or standardized scoring were not determined, which may limit reproducibility and comparison with other studies.

Author Contributions

Morooj Ali Fahad: data collection, writing the introduction, objectives, methods, results, and discussion. Nabaa Ali Jasim: data analysis, abstract, keywords, references, and some additions to the discussion, writing the conclusions and making all revisions requested by the editors, Shahad Nazar Mustafa: data collection, and linguistic review. Fatima Malallah Mohammed: reading and reviewing the full article with change or add some sentences.

Competing Interests

The authors declare that they have no conflicts of interest.

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