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Characterizing the Virulence of Staphylococcus Aureus Isolates from Wounds: An Experimental Approach

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Abstract
In consequence of the feared superbug Methicillin-resistant Staphylococcus aureus (MRSA), people are gravely at risk for major health issues. The severity of an infection is influenced by the existence of virulence factors and medication resistance. The two most drug-resistant and virulent MRSA isolates that best exhibit the early characteristics of intestinal adhesion were chosen for this investigation in an effort to designate them for potential future using postbiotics and probiotics as antagonists to treat MRSA infections. The bacterial agent's level of pathogenicity towards the organism—i.e., its capacity to wreak disease is referred to as virulence. The virulence factors offer the virus certain qualities that allow it to fight the host's natural defences and so produce illness. The development of diverse enzymes, antibiotic resistance, and bacterial toxins are the most crucial of these elements since they all aid the pathogen's ability to adapt to varied situations. The results of the virulence factor assays revealed that each of the 50 MRSA isolates developed biofilm and hemolysin enzyme type beta and coloured blue in Gramme stain.

Keywords: Wound, Disease, Enzyme, Virulence, Isolates

Introduction

The ability to identify the gram-positive bacteria's virulence characteristics that have an impact on development of experimental endocarditis has advanced greatly in the last five years alone. These advances in the pathophysiology of heart valve infections have been made possible by potent molecular methods used in selective mutagenesis experiments (1). Staphylococcus aureus requires a variety of surface proteins (or "adhesins") attach to the substrates of extracellular matrix with eukaryotic cells in order to initiate an invasive infection. A range of sticky glycoproteins with strong eukaryotic cell attachment-promoting capabilities are found in biological substrates like a tissue wound or the damaged vessel wall. Many of these compounds have also been demonstrated to have a significant role in the viruses' first phase of adhesion. In real terms, thrombospondin, bone sialoprotein, glycosaminoglycans, elastin, and collagens, fibronectin (Fn), fibrinogen (Fg), vitronectin (Vn), make up the bulk about the host components that S. aureus engages to the study (2). Acute and persistent infections of the valvular and parietal endocardium as well as the valve itself might be signs of the life-threatening condition Infectious Endocarditis (IE). In many instances, the infection's origin and length are unclear, and patients show symptoms of an acute sickness. One of the most prevalent

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causing infectious agents is the Gram-positive bacteria Staphylococcus aureus (S. aureus). IE is often preceded by heart valve anomaly brought on by congenital or degenerative illnesses, which causes a mechanical modification of the endothelium (3). S. aureus is well known for acquiring a resistance phenotype to antimicrobial drugs in addition to its high pathogenicity for humans. Methicillin-resistant S. aureus (MRSA) isolates is primary source of most nosocomial and community-acquired staphylococcal infections, which are on the rise globally and pose substantial challenges for the management and prevention of infection in healthcare settings. A heterogeneous protein is expressed on a multi-layered biofilm that S. aureus strains also create and embed inside the slime layer. The important trait of the biofilms is their natural resistance to the host's immune system and antimicrobial drugs, which results in long-lasting and damaging infections (4). S. aureus, which has a reputation for acquiring genes that make it resistant to antibiotics may achieve this. S. aureus, particularly methicillin-resistant S. aureus, which often has a multidrug-resistance profile, has really become a substantial contributor to nosocomial infections. MRSA infections are hard to treat and a substantial source of morbidity and death, particularly in hospitalised patients and those with compromised immune systems. In developed nations, aureus is regarded as significant and prevalent bacteria that causes bloodstream infections and is the second most frequent cause of sepsis. Osteomyelitis, endocarditis, septic shock, and septic arthritis are some of the clinical signs and symptoms of MRSA bacteremia that is acquired in hospitals and the general public (5). Even while they often do not pose a danger to life, some S. aureus infections may nonetheless result in severe morbidity and suffering. Among them are fairly serious skin diseases including furuncles, abscesses, and wound infections (6). Regulators of Carbon Catabolite Repression (CCR) in pathogenic bacteria often affect the transcription of virulence genes, which are essential for utilising host-derived food sources (7).

Staphylococcal pathogenicity in such device-associated infections is largely determined by biofilm development, a crucial determinant in virulence (8). Significant human pathogen Staphylococcus aureus can result in mild skin infections, osteomyelitis, sepsis, and necrotizing pneumonia in infected individuals. (9).

The goal of this research was to identify the two MRSA isolates that were the most drug-resistant, virulent, and had the earliest symptoms of intestinal attachment in planning for the potential use of probiotics and postbiotics as antagonists in the treatment of MRSA infections (10). Clarifying the effect of fusidic acid at subinhibitory dosages [1/64, 1/32, and 1/16 MIC] on S. aureus biofilm development and toxin release was the aim of their research (11). The objective of this work is to describe the molecular interactions between two-component virulence factor (VF) systems with the lactamase gene production in bloodstream infection (BSI) and MRSA isolates. In this investigation, 640 samples from BSI were gathered and identified using phenotypic techniques (12). This research looked at the abundance of plasmids in S. aureus strains recovered from retail meats bought in Oklahoma. They also assessed relationships between rep families, particular antibiotic resistance along with virulence genes, and the source of the food supply (13). A development of effective weapons to combat Staphylococcus aureus despite great efforts has not been successful. The mistake may have been prevented by using more accurate experimental models (14). The prevalence of germs

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that are Multi-drug Resistant (MDR) and connected to wounds is rapidly rising. In order to determine the presence and features of MDR bacteria for potential use in antibacterial wound dressing designs, the current study evaluates a number of wounds in Alexandria hospitals in North Egypt (15). The findings of this research show that Pickering emulsions based on citral or borneol/citral may be an alternate antibiofilm strategy to control pathogenicity in chronic infection (16). The main objectives were to determine how often methicillin-susceptible S. aureus (MSSA) and MRSA clinical isolates developed biofilms and look into the relationship between resistance characteristics and biofilm production (17). This research aims to better understand how the virulence and pathogenicity of S. aureus are affected by interactions between the medications metformin (MET), metformin nano (MET-Nano), silver metformin nano structure (Ag-MET-Ns), and silver nanoparticles (AgNPs) (18). To examine the molecular epidemiology of and contrast the main methicillin-resistant Staphylococcus aureus biotypes in order to look for associations with patient traits in patients who received an implant for a closed bone fracture and experienced early Post-operative Wound Infections (POWI) in an Indian tertiary care hospital (19). There are 300 S. aureus strains collected from bacteremia and chronic wound patients were used in this investigation to analyse the pathogenicity profiles and rates of drug resistance to different antimicrobials (20). According to the findings, every S. aureus isolate had genes for well-known virulence factors such hemolysin and the soluble modulins for phenol and acetone (21).

The prevalence of these virulence traits was investigated in S. aureus strains collected from healthy individuals in Yangon, Myanmar, from hospital wound infections, from patient nasal swabs, or via chefs and patients' vomiting during a food poisoning event (22). In this study, they discuss how particular S. aureus virulence factors and clonal lineages affect wound colonisation and infection, with a focus on illnesses in diabetic feet (23). In patients who were referred to Minia University Hospital, S. aureus was isolated from a variety of wounds. This research investigated for virulence genes in S. aureus. Additionally, MRSA, MSSA, and vancomycin-resistant S. aureus (VRSA) isolates were investigated for the presence of these genes, along with the bacteria's sensitivity and resistance to other drug classes. (24). The experiments displayed fast bacterial growth, for the diabetic rat polymicrobial wound designs composed of S. aureus, Pseudomonas Aeruginosa, and Streptococcus pyogenes, large necrotic lesions that were kept open for as long as 40 days, and a sustained pro-inflammatory response, were observed. (25). In this study, The prevalence and treatment responsiveness of Staphylococcus aureus isolated from surgical-site infections (SSIs) and traumatic wounds will be quantified (26). The fast spread of multidrug-resistant, life-threatening bacterial infections necessitates the creation of fresh antibacterial drugs and alternative tactics (27).

Materials and Methods

The culture mediums used in this study have been sterilised by autoclave for a total of 15 minutes at 121 °C under 1 bar, as per the manufacturer's instructions. All glassware that requires drying and sterilisation was additionally sterilised for two hours at 180°C. A 0.22 mm diameter Millipore filter was used to sterilise solutions that had been harmed by high temperatures. (Figure 1) represents the identification of pathogenicity factors.

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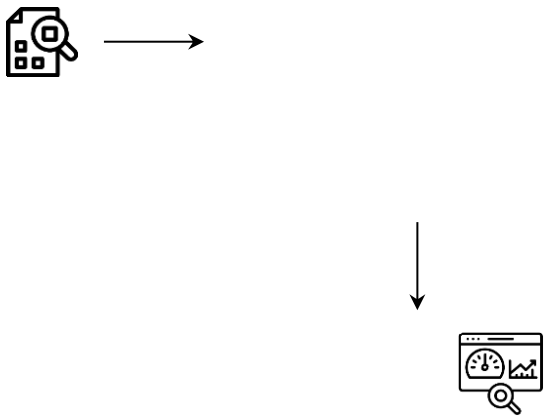

Figure (1) Collection of samples for Pathogenicity factors.

Samples gathering

A total of 70 various sources of wounds' Swab samples were used in the present investigation. By using sterile swabs with media, they were obtained from patients who visited Baaquba Teaching Hospital between June 2020 and the end of August 2020, and they were cultivated on blood agar and brain heart infusion agar.

Identification of pathogenicity factors

- Synthesis of Haemolycin
- Synthesis of Urease
- Synthesis of biofilms
- Monitoring the synthesis of Proteases
- Synthesis of Staphylokinase
- Protease synthesis

Phenotypic Confirmation of Methicillin Resistance

Considering the fact it just a few isolates were found in this study, bovine-associated isolates were more genetically diverse than their caprine or ovine counterparts. A more varied repertoire among the bovine isolates was also indicative of this. This study's isolates showed very little evidence of antibiotic resistance. These findings underline the need of preventing temperature misuse while minimizing any danger associated with production. They also imply that preserving the milk cold chain will do so.

MRSA isolates have virulence profling

Activity Haemolytic

MRSA isolates that have been cultivated over night were stained on blood agar base and then incubated for 24-48 hours at 37 °C with 5% calf blood. The cultivated plates were included and

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examined for signs of -hemolysis, such as a clear zone surrounding the streaking region, and alpha hydrolysis, such as partial hydrolysis with a greenish hue.

Inhibition of Coagulase

The human blood plasma and the rabbit Coagulase plasma are provided with three millilitres of the 2% staphylococcal test isolates independently, and the samples were then incubated at 37 °C for four hours. At intervals of 30 min, the incubated tubes were checked for coagulation. Coagulation was deemed positive if clots formed within 4 hours of development.

Capsule Manufacturing

Utilising a capsule staining approach and a capsule staining kit, the produced sample was examined under a bright field microscope to assess capsule formation.

Activity of the Nuclease

MRSA isolate live cultures were streaked over Deoxyribonuclease (DNase) test agar that had 0.2% Toluidine Blue added and then incubated at 38 °C for 24-48 hours. An effective reaction was identified on incubated plates by looking for clean patches surrounding the growth that had a reddish tint.

Results

Discovery of a few pathogenicity factors

The combination of Haemolysin, Urease, Biofilm, Protease, Lipase, Staphylokinase, and Bacteriocin was found in all MRSA isolates in this investigation along with some virulence factors were also examined.

Haemolycin synthesis: By growing the isolates on blood agar, production was discovered. A hemolytic zone surrounded the colonies for all Positive outcomes. This happens as a result of the exotoxin hemolysin, which is generated by S. aureus and which completely lyses red blood cells.

Urease synthesis: The urea agar slants underwent a manufacturing test. The capacity of the isolate to create urease, an enzyme that converts urea into ammonia and carbon dioxide and changes the color of the marker (phenol red) from yellow to pink to indicate a good outcome, was utilized to determine the isolate's ability to produce the enzyme. According to the results, 94% of the 50 isolates) generated urease.

Biofilm synthesis: Utilising Congo red agar, the meal was made. It was done in order to see whether the isolate could form biofilm. The findings demonstrated that 43 (84%) of the 50 isolates produced biofilm.

Protease synthesis: The capacity of the isolate to generate the protease enzyme was determined using this method. According to the findings, 32 (or 64%) of the isolates are protease producers.

Lipase: The isolate's capacity to generate the enzyme lipase was determined using this method. According to the findings, 15 people (or 30%) are lipase producers.

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Staphylokinase synthesis: It was used to determine if the isolate was capable of producing the enzyme Staphylokinase. 39 (78%) of the isolates produced staphylokinase, according to the findings.
Bacteriocin synthesis: It was used to determine if the isolate was capable of producing the Bacteriocin enzyme. According to the findings, 12 (or 24%) of the isolates generate bacteriocin.

Antibiotic Resistance Epidemic

It was discovered that MRSA isolates 12/241 and 12/206 shared a 77.7% resistance to 14 of the 18 tested antibiotics, whereas MRSA 5/255 was found to be resistant to 12 antibiotics (66.6%) that represent in (Figure 2) & (Table 1). MRSA 25/224, on the other hand, showed the least resistant phenotype was MRSA 9/265 (38.8%), which was followed by it (22.2%).

Table (1): MRSA isolates percentage of resistant, sensitive and intermediate

MRSA isolates	Percentage resistant/sensitive/intermediate		
	Intermediate	Sensitive	Resistant
12/241	90	80	79
12/241	95	80	79
25/214	95	50	45
5/255	90	65	65
9/265	90	40	35
25/224	0	20	25
25923	0	0	0

The studied medications were only marginally resistant to MRSA 25/214 (50%) and 5/255 (66.6%). Several routinely used medicines includes cefoxitin (5/6) , oxacillin (5/6) along with gentamicin (4/6) , amoxicillin (6/6) and methicillin (6/6), and ciprofoxacin (4/6), were able to withstand most of the MRSA isolates being examined. However, none of the MRSA isolates were tetracycline (6/6) and vancomycin (6/6) resistant.

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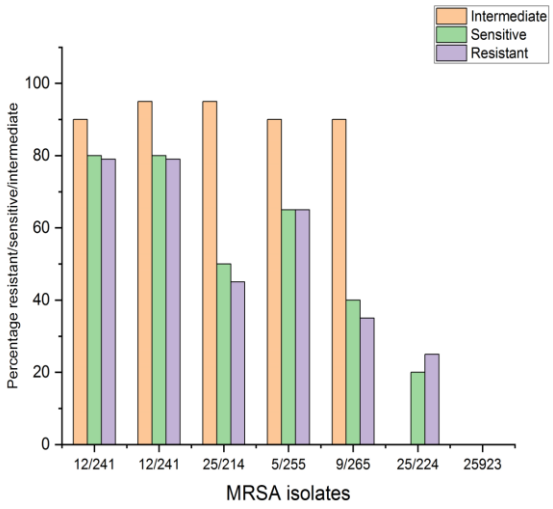

Figure (2): MRSA isolates based on percentage of resistant, sensitive and intermediate levels.

Cellular Characteristics of MRSA Isolates

The % hydrophobicity of MRSA isolates ranged from 24.271.3 to 72.314.4 (MRSA 5/255). S. aureus had a 47.600.9% adhesion to diethyl ether, indicating a considerable cell surface hydrophobicity. However, some of the isolates included in the study demonstrated moderate to high hydrophobicity, with diethyl ether concentrations ranging from 52.782.4 to 64.112.6%.

Automatic aggregation

The proportion of MRSA isolates that exhibited the self-aggregative phenotype ranged from 37.901.8 (MRSA 9/265) to 51.533.1 (MRSA 5/255). The moderate aggregative potential of MRSA 12/206 and MRSA 12/241 was 49.61 2.7 and 47.89 2.6%, respectively as shown in (Figure 3) & (Table 2). In a same manner, it was discovered that the estimated auto aggregation was 40.970.4%.

Table (2): The moderate aggregative potential of MRSA 12/206 and MRSA 12/241 was 49.61 2.7 and 47.89 2.6%

Auto aggregation (%)	S. aurous isolates
25923	41
12/242	48
12/208	49
25/214	42
5/255	50
9/265	38
25/224	43

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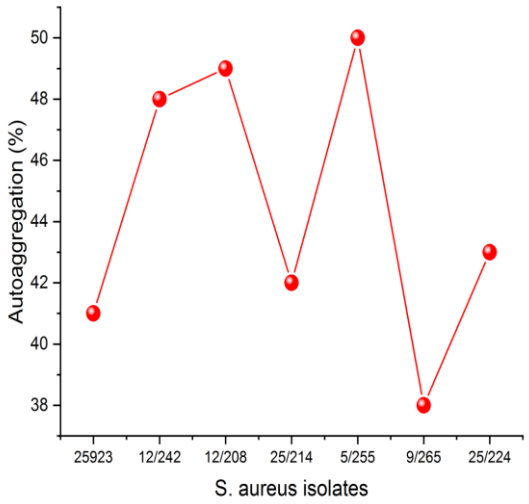

Figure (3): Proportion of MRSA isolates that exhibited the self-aggregative phenotype

Analysis of MRSA Isolate Adhesion Capability

The capacity to form Biofilms

By counting the quantity of crystal violet attached to the formed biofilm, researchers were able to gauge the capacity of MRSA isolates to create static biofilms. The crystal violet dye used in the research had absorbance at 570 nm that ranged from 2.41 0.18 (MRSA 5/255) to 2.89 0.24 (MRSA 25/224), which indicated that it had a high capacity to produce bioflms (OD>0.5) (Figure 4). MRSA 9/265, MRSA 25/214, and MRSA 12/206 showed the three highest absorbances (2.890.24, 2.820.14, and 2.690.33, respectively). Similarly, the control strain (2.260.09) showed robust bioflm-forming capabilities and showed no significant change in its ability to generate biofilms.

Testing for Mucin Binding

The proportion of mucin-adherent cells tapered between 68.930.61 and also 86.621.96 as shown in (Figure 4) & (Table 3), it demonstrates how MRSA isolates adhere to porcine stomach mucus on the temporal mucosa.

Table (3): The proportion of mucin-adherent cells tapered between 68.930.61 and also 86.621.96

S. aureus isolates	Adhesion (%)
25923	75
12/242	70
12/208	85
25/214	70
5/255	90

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9/265	79
25/224	84

While compared to the reference strain (72.613.90%), the percentages of adhering cells for MRSA isolates 5/255 and 12/206 were 86.621.96 (P0.01) and 84.252.10 (P0.05), respectively and indicating a considerable affinity for mucin.

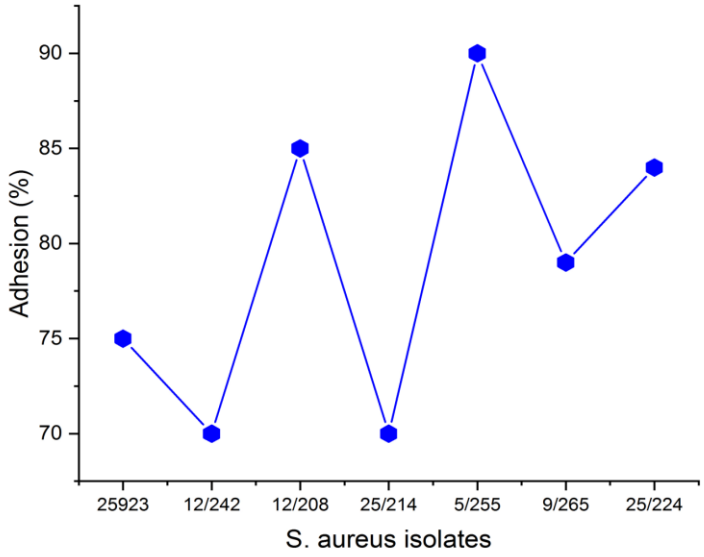

Figure (4) : Capacity of MRSA isolates to bind mucin.

Discussion

In medical specimens, wound infections accounted for a significant portion of the MRSA isolates (28). S. aureus may generate a number of virulence factors (enzymes and toxins) and a slime layer (biofilm) in varying levels, which can lead to a broad range of illnesses (29). The majority of the virulence factors outlined above have redundant functions, and their intricate regulation systems are to blame for this. Despite the use of multicomponent vaccines, targeting the regulatory elements may be less effective in protecting against MRSA (30). The virulence factors offer the virus certain qualities that allow it to fight the host's natural defences and so produce illness.

Conclusion

Despite the fact that just small subsets of isolates were examined in this investigation, those associated with cattle had more genetic diversity than their caprine or ovine counterparts.

A more varied repertoire among the bovine isolates was also indicative of this. This study's isolates showed very little evidence of antibiotic resistance. These findings underline the need of preventing temperature misuse while minimizing any danger associated with SE production. They also imply that preserving the milk cold chain will do so. It has been investigated whether probiotics and postbiotics may act as antagonists in the treatment of gut-mediated MRSA

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infections. In conclusion, this study demonstrates how principal component analysis and heatmap analysis may be used to identify the drug-resistant and most dangerous staphylococcal isolates.

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