REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Detection of Virulence of *Staphylococcus Aureus* Isolated from Wounds

Hanan Raheem Hassooni[1]
Ali Shakir Al Ezee[2]
Esra F. Al-Azawi[3]
Maryam O. Abd Alaa[4]
[1]Bilad Alrafidain University College/Iraq
[2]Department of Biology, College of Education For Pure Science, Diyala University, Iraq.
[3]Diyala Health Department , Public Health Department ,public health laboratory
[4]Department of Biology, College of Education For Pure Science, Diyala University, Iraq.
*e-mail: ali.shakir@uodiyala.edu.iq

Abstract

The virulence factors are the degree of pathogenicity of the bacteria to the organism, which means the ability of the agent to cause disease. The virulence factors give specific characteristics of the pathogen that enable it to confront the natural defenses of the host and thus cause disease. The most important of these factors are bacterial toxins, antibiotic resistance, and the production of various enzymes, all of which help the pathogen to adapt in different environments.

The virulence factor tests showed that all the (50) isolates produced biofilm and hemolysin enzyme type beta, and that all the (50) MRSA isolates were stained by Gram stain with blue color. The results showed that 47 (90%) of the isolates were urease producers, while 32 (64%) of them were protease producers, 15 (30%) isolates were lipase producers, 12 (24%) isolates were bacteriocin producers and 39 (78%) isolates were stapylokinase producers.
Keywords: ***staphylococcus aureus;*** **; wounds;** virulence factor tests

1- Introduction

Staphylococci were first detected and cultured by Koch (1878) and Pasteur in 1880, but the two researchers Ogston and Rosenbach made their initial detailed studies. Ogston, in (1881), named the clustered micrococci "staphylococci," which means a bunch of grapes, and Ogston in (1883) introduced staphylococci in the micrococci group which causes inflammations and suppurations. In (1884), Rosenbach described the genus Staphylococcus formally by isolating two staphylococci strains. He named them according to their colonies pigments: *S. aureus*, for gold pigment and *Staphylococcus albus* (now called epidermidis) for white pigment (Ibrahem, 2010). *S. aureus* is an opportunistic pathogen that can cause various diseases in humans such as endocarditis, pneumonia, septicemia and toxic shock. Humans and animals can be infected and colonized by these bacteria (Garcia-Alvarez *et al*., 2011and Ito *et al*., 2012).

Many virulent agents of *S. aureus* increase its susceptibility including capsule, which helps it in resisting bacteria, phagocytosis and enables it to form other enzymes, such as protease, lipase and staphylocyanase, which helps the bacteria to invade and spread in tissues and to produce α-and-β type lipoproteins as well as the production of food poisonings (Ryan and Ray, 2004).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Bacteria are unicellular organisms with a cellular diameter ranging from (1-2) micrometers, however, bacterial cells cannot be directly classified according to their sizes (Barton, 2005).

The simplest and common basic classification depends upon the cell wall structure, hence dividing bacteria into two groups known as Gram positive and Gram negative bacteria, this eponymous classification was picked after Christian Gram (Venkatesh *et al.*, 2014).

S. aureus is normally found colonizing many parts of human's body such as nose, mouth, mammary gland, hair and upper respiratory tract. *S.aureus* usually colonizes these parts of the body without causing harm. Although it is considered as a normal flora in some parts of human's body, *S. aureus* causes a wide range of infections in the skin, wound and deep tissues to life-threatening conditions such as endocarditis, pneumonia, septic arthritis and septicemia. These bacteria also most commonly cause nosocomial infections. However, little information are available on the virulence factors which cause all these conditions. furthermore, *S. aureus* can also cause joint and bone infections, food poisoning, scalded skin syndrome and toxic shock syndrome, through the production of many toxins (Winn Washington 2006; Akindele *et al.*, 2010).

1-1 Virulence factors

The remarkable ability of *S. aureus* for causing a wide range of serious infections in humans is attributed to the ability to form multiple virulence factors that contribute to its pathogenicity and capability to colonize the host, which allows these bacteria to adhere to tissue surfaces, avoiding or invading the immune system, and causing harmful toxic effects to the host (Costa *et al*., 2013). This process is a complex, multi-factorial and requires activity coordination between many bacterial gene products. *S.aureus* produces multiple virulence factors placing them into a group of accessory gene products which are unnecessary for cell division and normal growth . These factors can be classified into cell-surface - associated (adherence) factors and secreted (exotoxin) factors (Costa *et al.*, 2013).

1-1-1 Catalase

Staphylococci is a catalase enzyme producer, and this enzyme converts hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Catalase test is important to distinguish staphylococci, which give positive results from streptococci, which are catalase negative bacteria (Jawetz *et al.*, 2010).

1-1-2 Lipase

Different lipases, phospholipases, lipoprotein lipases, esterases and lyases are produced by *Staphylococcus aureus* bacteria (Arvidson, 2000). The ability of *S.aureus* strain to clear egg yolk by phospholipoprotein lipases which split the lipid moiety from lipovitellin is the most accepted diagnostic property (Dinges *et at*., 2000).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

1-1-3 Biofilm

A biofilm is a mucous layer of bacterial origin composed of polypeptide and an outer substance e.g minerals, nucleic acids, proteins and cell wall content. Biofilm is formed due to bacterial cell exposure to physical conditions, or high density of the cell, etc.. (Burke *et al*., 2010).

1-1-4 Protease

It is an enzyme that plays a crucial role in converting the local host tissues into nutrients necessary for bacterial growth, and it is an important factor in bacterial pathogenicity as it works on cracking and destruction of the host proteins, including heavy chains immunoglobulin, in addition to preventing protease inhibitors from working (Stevens, 2007).

1-1-5 staphylokinase

Staphylokinase or fibrinolysin is produced by all *S. aureus* strains and is able to dissolve fibrin clots which helps bacterial ease and quick movement in the body, and then the dissemination of infection rapidly. Staphylokinase enzyme differs from streptococcal fibrinolytic enzymes (Hava and Camilli, 2002).

2- Materials and Methods

Use of Multiple Culture Media According to the manufacturer's instructions, all culture media used in this study were sterilized by autoclave at 121 °C under 1 bar for 15 min. All glassware that needs to be dried and sterilized in an oven at 180°C for two hours were also sterilized. On the other hand, solutions that were damaged by high temperatures were sterilized by filtration using 0.22 mm diameter Millipore filters (MacFaddin , 2000).

2-1 Samples collection:

In the current study, Swab samples from (70) different sources wounds were included. They were collected from patients who attended to Baaquba Teaching Hospital during the period from June 2020 to the end of August 2020 by means of sterile swabs with media and were cultured on blood agar and brain heart infusion agar.

2-2 Detection of virulence factors:

1- Haemolycin production
2- Urease production
3- Biofilm production
4- Detection of protease production
5- Staphylokinase production
6- Protease production

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

3- Results and Discussion

3-1 Detection of some virulence factors

Some virulence factors were investigated and the production of Haemolysin ,Urease ,Biofilm ,Protease ,Lipase , Staphylokinase and Bacteriocin were detected in all isolates of MRSA in this study, table (3-1).

Table (3-1): Detection of some virulence factors

virulence factors	**isolates (50)**	
	NO	**%**
Heamolysin	**50**	**100**
Urease	**47**	**90**
Lipase	**15**	**30**
Staphylokinase	**39**	**78**
Biofilm	**50**	**100**
Bacteriocin	**12**	**24**
Protease	**32**	**64**

- Haemolysin: production was detected by culturing the isolates on blood agar. All Positive results appeared as a hemolytic zone around the colonies. This occurs because Hemolysin, an exotoxin, is the enzyme produced by the *S. aureus* which causes the complete lysis of red blood cells.
- **Urease** production test was done on urea agar slants. It was used to detect the isolates ability to produce an enzyme, called urease, which breaks down urea into ammonia and carbon dioxide changing the color of indicator (phenol red) from yellow to pink considered a positive result. The results showed that all 50 isolates were 47(94%) Urease producers.
- **Biofilm** production was done on Congo red agar. It was used to detect the isolates ability to produce biofilm. The results showed that all 50 isolates were 43 (84%) Biofilm producers.
- **Protease** production It was used to detect the isolates ability to produce an enzyme, called protease. The results showed that 32(64%) isolates protease producer.
- **Lipase** production It was used to detect the isolates ability to produce an enzyme, called Lipase. The results showed that 15(30%) isolates lipase producer.
- **Staphylokinase** production It was used to detect the isolates ability to produce an enzyme, called Staphylokinase. The results showed that 39(78%) isolates Staphylokinase produce.
- **Bacteriocin** production It was used to detect the isolates ability to produce an enzyme, called Bacteriocin. The results showed that 12(24%) isolates Bacteriocin producer.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

Discussion

- A high incidence of isolation for MRSA isolates in clinical samples were from wound infection.
- S. aureus has the ability to produce numerous virulence factors (enzymes and toxins), and has the ability to produce a slime layer in different amounts (biofilm) causing a wide variety of diseases.
- This is due to the redundancy of most of the above-mentioned virulence factors, as well as their complex regulatory mechanisms , Targeting the regulatory factors
- one needs to keep S. aureus virulence factors might have a lower ecacy against HA-MRSA, despite the use of multicomponent vaccines.

References

[1]. Akindele, A.A. ; Adewuyi, I.K. ; Adefioye, O.A. ; Adedokun S.A. and Olaolu, A.O. (2010). Antibiogram and Beta-Lactamase Production of *Staphylococcus aureus* Isolates from Different Human Clinical Specimens in a Tertiary Health Institution in life, Nigeria. American-Eurasian Journal of Scientific Research; 5(4):230-233.

[2]. Arvidson, S. (2000). Extracellular ezymes. In Fischetti, V., A., (eds). Gram-positive pathogens. ASM Press, Washington DC.

[3]. Barton, L. L.(2005). Structural and Functional Relationships in Prokaryotes. New York, Springer:820 ISBN 0-38-720708-2.

[4]. Burke, F.M.; McCormack, N.; Rindi, S.; Speziale, P. and Foster, T.J.(2010).Fibronectin-binding protein B variation in Staphylococcus aureus.BMC Microbiology.10:160.

[5]. Costa, A. R.; Batistão, D. W. F.; Ribas, R. M.; Sousa, A. M.; Pereira, M. O. and Botelho, C. M. (2013). Staphylococcus aureus virulence factors and disease. In A. Mendez-Vilas, Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education. 1:702-710.

[6]. Dinges, M.M.; Orwin, P.M. and Schlievert, P.M. (2000). Exotoxins of *Staphylococcus aureus.* Clin. Microbiol. Rev. 13:16-34.

[7]. Garcia-Alvarez, L.; Holden, M.T.; Lindsay, H.; Webb, C.R.; Brown, D.F.; Curran, M.D.; Walpole, E.; Brooks, K.; Pickard, D.J. and Teale, C. et al. (2011). Methicillin- resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11: 595- 603.

[8]. Hava, D.L. and Camilli, A. (2002). Large-scale identification of serotype 4 *Streptococcus pneumoniae* virulence factors. Mol. Microbiol. 45: 1389-1405.

[9]. Ibrahem, S. (2010). Methicillin Resistance in Staphylococci : Horizontal Transfer of Mobile Genetic Element(SCC*mec*) between Staphylococcal Species National Institute for Health and Welfare. University of Helsinki, Finland

[10]. Ito, T.; Hiramatsu, K. and Tomasz, A.(2012). Guidelines for reporting novel mecA gene homologues. Antimicrob Agents Chemothe . 56: 4997–9.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 25 March 2022; Revised: 15 April 2022; Accepted: 17 May 2022; Publication: 02 July 2022

[11]. Jawetz, Melnick, and Adelbergs. (2010).Medical microbiology.25 th edition. Mc Graw-Hill companies.USA. Chapter 13, page 185-193.

[12]. Stevens, D. (2007). Genetics of MRSA: The United States and Worldwide. In: J.A. Weigelt (ed.). MRSA. Informa Healthcare, New York. P. 31-41.

[13]. Venkatesh, V. N.; Shwetha, D. C.; Ravish Kumar, M.; Sudhindra, K. S. and Srinivasa settee.(2014). Study of antibiotic susceptibility pattern of methicillin resistant Staphylococcus aureus at tertiary care unit hospital in South India. J.of Biomedical and Pharmaceutical Research. 3(2) .25-28.

[14]. Winn Washington , A.S., Janda William , Koneman Elmer , Procop Gary , Schreckenberger Paul , Woods Gail 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology Lippincott Williams & Wilkins, Philadelphia.