

Phenotypic Assessment of Exoenzyme Activity by Clinical Isolates of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is a common causative agent of hospital infection and community acquired infection as the cell possesses a wide armamentarium of virulence factor that include different exoenzyme and toxins. The main aim of this study was to evaluate the virulence factor from clinical isolates of *Staphylococcus aureus*. In this study total ten isolates were used for the screening of catalase, coagulase, lipase, thermonuclease, beta-lactamase and Dnase. The present study showed that 100% strains demonstrate catalase enzyme, coagulase enzyme, beta-lactamase. Out of 10 strains only 2 (20%) strains produced Dnase and thermonuclease enzyme while only 30% strains showed positive result for lipase production which was detected by phenol red test. Based on the result of this study almost all the strains showed positive result for various enzymatic tests but 3 strains considered to be more virulent as they showed the production of Dnase, lipase and thermonuclease. The enzymes make *Staphylococcus aureus* more pathogenic and give rise to diverse spectrum of diseases ranging from minor to life threatening infections. Phenotypic tests offers an alternative method for simultaneous detection of the clinically important virulence factors of *Staphylococcus aureus* strains for diagnostic purposes as well as research studies.

Keywords: Enzyme, Phenotypic test, *Staphylococcus aureus*, Virulence

INTRODUCTION

Staphylococcus aureus is both a human commensal and frequent cause of clinically important infections, including bacteremia, septic arthritis, pneumonia, osteomyelitis, gastrointestinal tract, and wound infections (Lowy, 1998). It has been estimated that about 20-30% of the population are permanently colonized by this bacterium. One of the reasons that *S.aureus* is a frequent cause of infection is that it can survive for month on any type of surface. *Staphylococcus aureus* possess different virulence factors that promote tissue colonization, tissue damage, and distant diseases (Foster & Hook, 1998; Dinges *et al.*, 2000). *Staphylococcus aureus* has the ability to live inside host cells and can invade in-vitro

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a variety of phagocytes, including fibroblasts (Sinha *et al.*, 1999), osteoblasts (Jevon *et al.*, 1999), endothelial (Ogawa *et al.*, 1985), and epithelial cells (Dziewanowska *et al.*, 1999;). After internalization, *Staphylococcus aureus* may either persist, escaping host defenses and antibacterial agents, or multiply and further disseminate. *Staphylococcus aureus* produces an array of virulence factors to facilitate its pathogenesis. The most important virulence factor is the cell-wall associated factor, including capsular polysaccharide, and a group of protein called microbial surface components recognizing adhesive matrix molecules. All these proteins play important roles in microbial adhesion on host proteins. One important feature of *S.aureus* is the ability to secrete toxins which were Staphylococcal Enterotoxins, TSS Toxin-1 and cytolytic toxins

that formed β -barrel pores in the cytoplasmic membranes of target cells and cause leakage of the cell's content and cell lysis. These were α , β , γ , δ and panton valentine leukocidin. The virulence of the bacteria is further regulated by extracellular enzymes that are produced during different stages of infection for example during growth, cell division and during avoidance of host defense and spread of the bacteria. *Staphylococcus aureus* is responsible to produce various exoenzymes such as catalase, coagulase (free and bound), lipase, nuclease, staphlokinase, thermonuclease and beta-lactamase. Much interest has been shown in reports that a number of enzymes produced by staphylococci are linked with enterotoxin production, such as heat-stable nuclease (Chesbro and Auburn, 1967; Victor *et al.*, 1969). The purpose of our study was to evaluate the virulence factors of *Staphylococcus aureus* using different phenotypic tests.

MATERIAL AND METHODS

Strains: Total ten strains of *Staphylococcus aureus* were used for enzymatic detection. Strains were derived from lesions in hospital patients.

Coagulase Test: For slide coagulase tests, drop of human plasma was placed and mix well. For tube method, heavy suspension of organism was added in sterile tube containing plasma and incubate it.

Lipase Test: The production of lipase was detected by using (0.01% olive oil) phenol red media. The composition of phenol red media: 0.01%, Lipidic substrate (Olive oil): 1%, Calcium chloride : 10mM, Agar: 2%, Distilled water: 50ml), the pH was adjusted using 0.1 N NaOH, filter paper disc with lipase sample was placed over the agar and incubated for 20 mins.

Nuclease Test: The production of nuclease enzyme was detected by using DNase agar. The supernatant of broth culture was inoculated in

the 6 mm wells bored in media and media was incubated at 370C for 24 hours.

Thermonuclease Test: The production of thermonuclease enzyme was also detected by using DNase agar. The heated supernatant of broth culture (1000C for 15 min) was inoculated in the 6 mm wells bored in media and incubated at 370 C for 3-4 hours.

Beta-Lactamase Test: The production of beta-lactamase was detected by two iodometric methods that were tube method and filter paper method. In filter method, the filter was dipped in the solution of 1% soluble starch in penicillin at concentration of 10,000 U/ml, drained and allow to air dry for approximately 2 hours at room temperature. The strips were inoculated with a loop full of freshly isolated test organism by rubbing the colonies onto the paper strip in a circular manner to cover an area approximately 5mm in diameter. The inoculated filter paper was covered with a Petri dish and incubated for 30 minutes at 370C. The paper was flooded with iodine solution and then incubates at 370 C for half hour. In tube method, penicillin solution was dispensed in 0.5ml volume in small test tubes. Test bacteria were removed with a loop from overnight culture on solid medium and suspended in the penicillin solution. After one hour at room temperature two drops of starch indicator was added to the suspension, followed by one drop of iodine reagent.

RESULTS & DISCUSSION

Staphylococcus aureus is a virulent bacterium and a major human pathogen, which produces a variety of virulence determinants. In order to find out some virulence factors of *Staphylococcus aureus*, some laboratory tests were performed for the enzyme detection in which ten strains of *Staphylococcus aureus* were used. Prevalence of different virulence factors among clinical strains of S. aureus can be summarized in Table 1. Illustrations of some phenotypic tests for enzyme detection are also given (Figure 2-5).

Table I: Virulence Determinants produced by Clinical Strains of *Staphylococcus aureus*.

Virulence Determinants	1	2	3	4	5	6	7	8	9	10
Catalase	+	+	+	+	+	+	+	+	+	+
Coagulase	+	+	+	+	+	+	+	+	+	+
Thermonuclease	-	+	+	-	-	-	-	-	-	-
Lipase	-	+	+	+	-	-	-	-	-	-
Dnase	-	+	+	-	-	-	-	-	-	-
Beta-Lactamase	+	+	+	+	+	+	+	+	+	+

100% strains showed the production of both bound and free coagulase as in human host. Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood (Kloos and Bannermen, 1994). 100% strains were catalase positive which indicate that our isolates has the ability to decompose the H₂O₂. *Staphylococcus aureus* catalase enzyme protects intraphagocytic microbes by destroying hydrogen peroxide produce by phagocyte. Thus catalase may be a significant bacterial virulence factor. 100% strains showed the production of beta-lactamase which means all strains are drug resistant as they break penicillin into penicillonic acid. The production is done by the iodometric method which was most reliable and inexpensive method for detection. Only 20%strains showed the production of Dnase & thermonuclease. Dnase and thermonuclease activity could be used to identify potentially pathogenic Staphylococci (Jeffries *et al.*, 1956). Thermonuclease is known as a specific virulence factor in *Staphylococcus aureus* as it was responsible for many food-borne diseases that are nausea, vomiting, and abdominal cramps with or without diarrhea (Kadariya *et al.*, 2014). 30% strains shows the production of lipase by phenol red test. In the study we use olive oil as a substrate in the medium which increased the activity of lipase which hydrolyzed the triglycerides into free fatty acid and glycerol. The rest of the strains that did not

showed the lipase activity that may be due that they need more optimal conditions as we study that lipase production mainly depends on pH and substrate or they did not have the ability to breakdown the lipids. Lipase play an important role in pathogenesis of *S.aureus* as their role is readily seen in skin and food-borne infections (Nath and Hindumathy, 2012).

From the present study, we concluded that we should focus on these phenotypic detections as they are most reliable, fast and inexpensive techniques as compared to different molecular techniques in the detection of virulence determinants as these laboratory techniques are useful to control the strategies to limit the bacterial spread & detects the diseases caused by *Staphylococcus aureus*.

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