INTRODUCTION

Staphylococcus aureus is a major pathogen responsible for various infections including bacteremia, pneumonia, skin and soft tissue infections, and osteomyelitis. Over the last two decades epidemic strains of methicillin-resistant *S. aureus* (MRSA) have disseminated widely in acute-care and long-term care facilities. Risk factors for MRSA acquisition in outpatients include history of past hospitalization or surgery, residence in chronic care facilities and injection drug use ***(Zetola et al., 2005)***.

More recently, MRSA infections have been reported from Australia, USA and Europe, in populations lacking previous contact with healthcare facilities **(Denis*et al*, 2005)**These community-acquired MRSA (CA-MRSA) strains have been associated with skin and soft tissue infections and necrotizing pneumonia**(Denis*et al*, 2005)**

The pathogenicity of *Staphylococcus aureus* infections is related to various bacterial surface components (*e.g.*, capsular polysaccharide and protein A), including those recognizing adhesive matrix molecules (*e.g.*, clumping factor and fibronectin binding protein), and to extracellular proteins

(*e.g*., coagulase, hemolysins, enterotoxins, toxic-shock syndrome [TSS] toxin exfoliatins, and Panton-Valentine leukocidin [PVL]) **(Lina *et al,* 1999)**.

It is worth to be mentioned that no study have been carried out for detectingPanton-Valentine leukocidin (PVL) gene insome clinical isolatesin Egypt since 2007 in the study of **Rizk *et al., 2007.***

Therefore, this work is carried out for detection of PVL gene 'which clearly needed' to reduce the diseases caused by this virulence gene.

Staphylococcus aureus Panton-Valentine leukocidin (PVL) is a pore-forming toxin and *S. aureus*PVL-positive strains are oftenmethicillin-resistant (MRSA) ***(Labandeira-Rey et al., 2007)***.

In general, the precise roles of individual staphylococcal factors in invasive infections are difficult to assess, but PVL production has been preferentially linked to furuncles, cutaneous abscesses, and severe necrotic skin infections **(Lina *et al,* 1999)**

PVL genes were detected in 93% of strains associated with furunculosis and in 85% of those associated with severe necrotic hemorrhagic pneumonia (all community-acquired). They were detected in 55% of cellulitis strains, 50% of cutaneous abscess strains, 23% of osteomyelitis strains, and 13% of finger-pulp–infection strains **(Lina *et al.*, 1999)**.**Tristan *et al., (2007)*** also reported that, in particular, the leukotoxic action of PVL is responsible for the high mortality rate associated with necrotizing pneumonia.

Purified PVL induces severe inflammatory lesions leading to capillary dilation, chemotaxis, polymorphonuclear cells (PMNs) infiltration, PMN karyorrhexis, and skin necrosis **(Lina *et al,* 1999).**

Labandeira-Rey *et al., (2007)* showed not only that PVL is a key virulence factor in pulmonary infections but also that expression of the luk-PV genes interferes with global regulatory networks, which may also enhance virulence. A detailed analysis of such dysregulation will be useful to identify targets for the potential development of novel therapies to treat *S. aureus* infections.

AIM OF THE WORK

- Detection of Panton Valentine Leukocidin (PVL) genes prevalence among MRSA isolates from some Egyptian hospitals.
- Detecting of MRSA isolates association with the site of infection.
- Detecting PVL gene association with the site of infection.

Review of Literature

I) *Staphylococcus aureus*

- **Description**

Staphylococcus aureus is a gram positive coccus about 1 µm in diameter. The cocci are usually arranged in grape-like clusters**(David *et al.,* 2012)**.The organisms are non-sporing, non-motile, and usually non-capsulate **(David *et al.,* 2012).**

- **Culture characteristics**

They grow readily on ordinary media within a temperature range 10 - 42˚c, the optimum being 37 ˚c and pH 7.4 -7.6. They are aerobes and facultative anaerobes **(Ananthanarayan and Paniker's, 2005).**

- **Pathogenicity and virulence**

Staphylococcus aureus is one of the most common causes of life threatening. *S. aureus* lives on a skin and mucous membranes of warm blooded animals; human is one of the primary carriers **(Lisa and Kevin, 2005).**

S. aureus is present in the nose of 30% of healthy people, may be found on the skin. It causes infection most commonly at sites of lowered host resistance, such as

damaged skin (*e.g*. surgical site infection) or mucous membranes (*e.g*. ventilator associated pneumonia) **(David *et al*., 2012,).**

Most strains possess a large number of cell-associated and extracellular factors, some of which contribute to the ability of the organism to overcome the body's defense and to invade, survive and to colonize the tissue. These factors are responsible for the establishment of infection, enabling the organism to bind to connective tissue, opposing destruction by the bactericidal activities of humoral factors such as complement, and overcoming uptake and intracellular killing by phagocytes **(David *et al*., 2012).**

Staphylococci produce two types of diseases; infection and intoxication. In the former the cocci gain access to damaged skin, mucosal tissue sites, colonies by adhering to cells or extracellular matrix, evade host defense mechanism, multiply and cause tissue damage. In intoxications, the disease is caused by the bacterial toxins produced either in the infected host or performed *in vitro***(Ananthanarayan and Painker's, 2005).**

Staphylococcus aureus produce a number of exoproteins and enzymes that contribute to its ability to

invade and colonize tissues. The toxins and virulence factors of the organism can affect immune system of infected individuals directly by lysing white blood cells or indirectly by acting as superantigens. As an example, the leukotoxins can lyse white blood corpuscles (WBCs), whereas enterotoxins and Toxic Shock Syndrome Toxin (TSST) can act as Superantigens **(Mertz *et al.*, 2007).**

Some of the most common virulence factors of *S.aureus* are teichoic acid and fibronectin binding protein, which enable the bacteria to gain entry into the host and colonize the tissues. The enzymes, hyaluronidase and coagulase, aid in spreading of infection. The organism also produces leuckocidins, catalase, and protein A which help in multiplication of the organism in the host tissues. Damage to the host tissues is caused by hemolysins, exofoliatines, enterotoxins, and toxic shock syndrome toxin (TSST) produced by the microorganism. These enzymes and exoproteines enable the organism to cause acute and chronic infections **(Archer, 1998).**

S.aureus is able to invade host cells by producing a number of membrane damaging toxins **(Krull *et al.*, 1996).** Alpha toxin is also involved in the reduction of the activity of

macrophages **(Marshal *et al.*, 2000 and Huseby *et al.*, 2007),** it has a lethal effects on a wide variety of cell types and lyses erythrocytes of several animal species **(Parija, 2009).** The ß-toxins of *S.aureus* can affect the function of both lymphocytes and neutrophils **(Marshal *et al.*, 2000 and Huseby *et al.*, 2007),** can lyse erythrocytes thereby evading the host immune system and scavenge nutrients **(Huseby *et al.*, 2007),** it is a hot-cold hemolysin; i.e., its hemolytic properties are increased by exposure of the RBCs to cold temperature **(Parija, 2009).** The delta toxin is responsible for the pathological changes that occur during an infection **(Alouf *et al.*, 1988). Parija, 2009,** mentioned that Leukocidines include (a) alpha lysine, (b) Panton Valentine Leukocidin, and (c) Leucolysin.

1- The alpha-lysine is the most important leukocidin. It causes marked necrosis of the skin and hemolysis by damaging the cell membrane, leading to release of low molecular weight substances from the damaged cells.

2- PV-leukocidins are 6 in number, each consisting of two components. These toxins cause death of human leukocytes and macrophages without causing any lysis.

3- Leukolysin is thermo stable and cause lysis of leukocytes and necrosis of tissues *in vivo.*

- **Epidemiology**

Patients and carriers are the common source of infection. In about 10-30% of the healthy persons; organisms are found in the nose, skin, axilla, and throat. More than 50% of the hospital workers carry *Staph. aureus*and they are the commonest cause of hospital cross- infection **(Vasanthakumari,2007).**

- **Sources and acquisition of infection**

Infected lesion

Large numbers of Staphylococci are disseminated in pus and dried exudate discharged from large infected wounds, burns and secondarily infected skin lesions, and in sputum coughed of patient with pneumonia. Direct contact is the most important mode of spread, but air born dissemination may also occur. Cross-infection is an important method of spread of Staphylococcal disease, particularly in hospitals, and scrupulous hand hygiene is essential in preventing spread. Food handlers may similarly introduce enterotoxin- producing food poisoning strains into food **(David *et al.*, 2012).**

Healthy carriers

*Staph.areus*grows harmlessly on the moist skinof the nostrils in about 30% of healthy persons, and perineum is also colonized. Organisms are spreading from the sites into the environment by the hands, clothing and dust consisting of skin squamous and cloth fibers **(David *et al.*, 2012).**

During the first day or two of life most babies become colonized in the nose and skin by Staphylococciand transmission from babies to nursing mothers who then develop mastitis, is well described **(David *et al.*, 2012).**

Animals

Animals may disseminate *Staph. aureus*and so cause humane infection, *e.g*. milk from dairy cow with mastitis, causing staphylococcal food poisoning **(David *et al.*, 2012).**

Environment

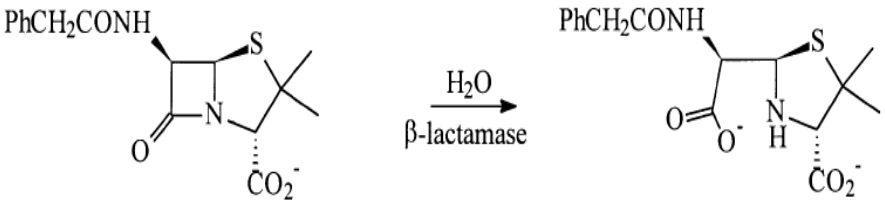
Although not spore forming staphylococci may remain alive in a dormant state for several months when dried in pus, sputum, bed cloths, or dust, or inanimate surfaces such as floors. Environmental reservoirs are therefore increasingly recognized as important in hospitals in contributing to endemic MRSA. Staphylococci are fairly readily killed by

exposure to light and by common disinfectants, hence the emphasis on regular and effective environmental decontamination in controlling MRSA **(David *et al.*, 2012).**

The acquisition of *Staph.aureus*may be exogenous (from external source such as environment) and more theoretically preventable and endogenous (from a carriage site, or minor lesion elsewhere in the patient's own body). It is important to remember that body surfaces of human beings and animals are the main reservoir **(David *et al.*, 2012).**

- **Susceptibility to antibiotics**

*Staph.aureus*and other staphylococci are inherently susceptible to many antimicrobial agents. About of 90% of strains found in hospitals are now resistant to benzylpenicillin due to the production of the enzyme penicillinase, a ß-lactamase that open a ß- lactam ring **(David *et al.*, 2012).**



(Bounaga *et al.*, 1998)

The first bacterium which is penicillin resistant was found in 1947, just for years after the drug started begin mass produced. Mecithillin was then the antibiotic of choice, but has been replaced by oxicillin due to significant kidney toxicity. MRSA (Methicillin- Resistant *S. aureus*) was first detected in Britain in 1961 and is now “quite common” in hospitals. MRSA was responsible for 37% of fatal cases of sepsis in the UK in 1999, up from 4% in 1991. Half of all *S. aureus* in the US are resistant to penicillin, mathicillin, tetracycline and erythromycin **(Sabra and Farag, 2012).**

II) Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is major pathogen causing nosocomial infection and is an emerging cause of community-associated infection **(Fridkin*et al,* 2005).**

MRSA is a significant cause of both health care–associated and community-associated infections **(Liu *et al,* 2011).**

- **History**

The first case of methicillin - resistant *Staphylococcus aureus* (MRSA) were described in the United Kingdom in 1961, shortly after the advent of methicillin **(Jevons, 1961).** Then increasing rates of MRSA amongst all *S.aureus* infectionsin the United States have risen from 2.4% in 1975 to 29% in 1991, with overall rate of methicillin resistance for all *S. aureus*infections reported to be 43.2% in 1999-2000 **(Hill *et al,* 2005).**

Following the introduction of methicillin into clinical use, methicillin-resistant *S. aureus*(MRSA) has emerged as a major nosocomial problem worldwide. Today MRSA continues to be a significant burden in hospitals but has also emerged as a problem in the community **(Shore*et al,***

2008). Methicillin resistance in *S.aureus* is encoded by the *mecA* gene, which is located within amobile staphylococcal cassette chromosome (SCC) elementknown as SCC*mec* **(Shore *et al,* 2008).**

- **Overview**

Staphylococcus aureus is a major cause of community and healthcare infections, and methicillin-resistant *S. aureus*(MRSA) is currently the most commonly identified antibiotic resistant pathogen in many parts of the world. Treatment of infection caused by *S. aureus*has become more problematic since the occurrence of methicillin resistance, as MRSA strains are resistant to all β-lactam antibiotics thereby significantly limiting the treatment options. Concerning Africa, several countries reported MRSA as a problem **(Shah *et al,* 2011).**

Epidemiologically MRSA infections have been divided into HA-MRSA (health care associated MRSA) and CA-MRSA (community – acquired MRSA) **(Legato, 2009).**

- **Health - care associated MRSA (HA-MRSA)**

HA-MRSA has been growing problem worldwide in hospitalization patients since the 1960s and often causes severe, invasive disease. Between 1995 and 2001in the

United States the proportion of MRSA increased from 22% to 57% in over 24000 cases of nosocomial *S.aureus* bacteremia. Nosocomial MRSA infections are responsible for longer hospital stays, higher mortality, and higher costs than patients with methicillin- sensitive *S.aureus* (MSSA) **(Legato, 2009).**

Risk factors of HA-MRSA

Risk factors of HA-MRSA infections include antibiotic use, surgery, intravenous devices, prosthetic devices such as artificial joints and heart valves intensive care unit stays, hemodialysis, and exposure to other patients with MRSA **(Legato, 2009).**

Transmission of HA-MRSA

The most common mode of transmission of HA-MRSA is contaminated hands of health care workers. However, fomites such as stethoscope ear tips and surfaces proximate to infected patients can also serves as reservoirs. In one study, environmental surfaces had an MRSA contamination rate of 59% in the hospital rooms of patients with heavy gastrointestinal MRSA colonization and diarrhea **(Legato, 2009).**