

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ  
رَبَّنَا افْتَحْ بَيْنَنَا وَبَيْنَ قَوْمِنَا بِالْحَقِّ  
وَأَنْتَ خَيْرُ الْفَاتِحِينَ

صدق الله العظيم

**Efficient inhibition of methicillin resistant -  
vancomycin intermediate *Staphylococcus aureus* (MRSA-VISA) by soybean glycinin basic subunit**

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# *Introduction*

- ***S. aureus* causes :**
  - **septicaemia,**
  - **skin and wound infections,**
  - **pharyngitis, Otitis media, Tonsillitis,**
  - **osteomyelitis,**
  - **cystitis and others.**
- **MRSA strains are spread**
  - **MRSA with Vancomycin MIC 2mg/L called MIC-Creep.**
  - **MRSA with Vancomycin MIC 8mg/L called VISA.**
  - **MRSA with Vancomycin MIC  $\geq 16$ mg/L called VRSA.**
- **MRSA, VISA and VRSA were isolated from different foods and showed currently high incidence in meat and dairy products.**

- **Daptomycin , Linezolid , telavancin , tigecycline are used for treatment of VISA , MRSA and VRSA , but unfortunately they are → inactivated by pulmonary surfactant → cause muscle toxicity .**

**New agents are necessary .**

**We used soybean protein**



**We extracted basic subunit and modify it biochemically.**

**→ Acidic subunit —S— S— basic subunit**

**Disulfide bond**

**→ Basic and modified basic subunit (GBasic) is used as an inhibitory for VISA strain herein.**

*Aim of the work*

Isolation and identification a VISA strain by both phenotypic and molecular techniques to be a model target for the potential inhibitory action of glycinin basic subunit (GBS) either *in vitro* or in minced beef meat .

# *Material and method*



- **Extraction of glycinin basic subunit (GBasic)**
- *Glycine max* (soybean seeds)

↓ **Grinding**

Soybean flour

↓ **Defatted by solvents**

**Defatted soybean syrup**

↓ **Nungo et al.(1992)**

J. Agric & Food Chemist .5:452-456.

Glycinin protein

Dissolve in 30mM Tris buffer (pH 8.0) containing  
15mM  $\beta$  – mercaptoethanol (0.5% w/v).

↓

- **Protein syrup → heating to 90°**

**for 30min → centrifugation**

**at 10000xG for 20 min.**



**ppt is the basic subunit**



**suspend in dist . Water**



**Freeze dried.**

Acute toxicity of GBasic:

*20 Rattus norveicus* (Male white albino rats)



**Four groups  
(5 rats for each)**

**First was treated  
with distilled  
water**

**2 nd treated with  
2000 mg/Kg**

**Third and fourth  
treated with 2500 &  
5000 mg/Kg  
respectively**



**After 24 hr  
symptoms wear read  
(no death).**

- *S .aureus* strains were isolated from post-operative pus.  
Antibiotic susceptibility by discs .  
Identification of MRSA was carried out .  
MIC & MBC was don in Muller Hinton broth .  
mec A , van A, B were detected by PCR .  
GBasic activity was conducted in *Vitro* and in food.

# *Results*

## **1- Isolation of S.aureus :**

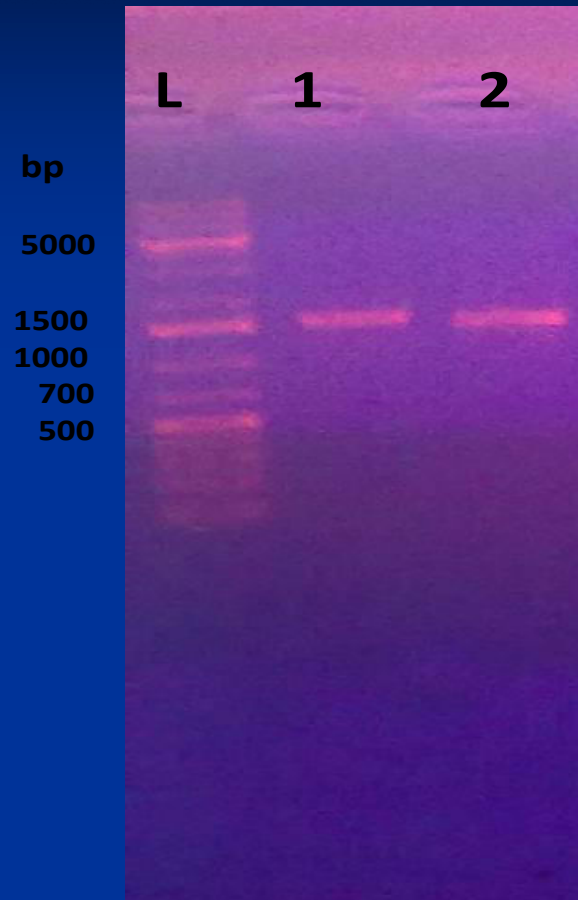
A total of 150 bacterial isolates were obtained from 100 post-operative pus samples (80 isolates), 100 urine samples (40 isolates) and 80 sputum samples (30 isolates) from patients admitted to Zagazig University Hospitals , Zagazig , Egypt in the period from January / 2010 until December / 2013. All the obtained isolates grew well on Baird Parker agar and were Gram positive cocci . They exhibited positive results with the following tests; catalase, coagulase, voges Proskaner test, urease, gelatin liquefaction, fermentation of glucose, lactose, mannitol, sucrose and salicin At the same time, they manifested negative results with the following tests; oxidase, indole production, H<sub>2</sub>S production, and utilization of both L-arabinose and D-sorbitol. As a consequence they can be classified as belonging to *S. aureus* bacterium.

## **2- Susceptibility of the isolated bacteria to different antibiotics :**

Antibiotic sensitivity test was conducted on the different 150 *S. aureus* isolates using 10 antibiotics, indicating that 30 bacterial isolates were methicillin resistant ( MRSA) . One isolate among this group (isolated from post-operative pus) showed positive  $\beta$ - lactamase activity and recorded 8 and 10  $\mu\text{g}/\text{mL}$  MIC and MBC for vancomycin. So, it was considered as VISA strain and designated as VISA P59.

### 3- Molecular characterisation and identification of the VISA isolate (P59).

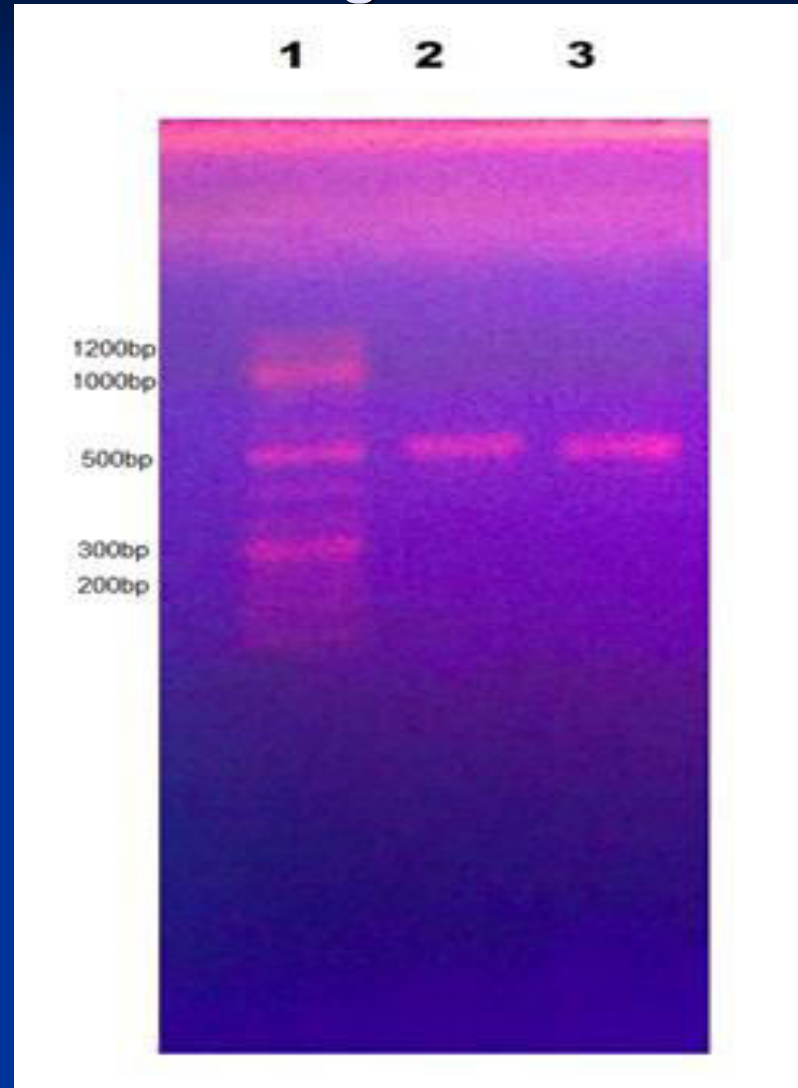
#### a- confirmed identification by 16S rRNA



**Figure 1. Agarose gel electrophoresis of amplified DNA (PCR product) obtained from 16S r RNA gene of *S. aureus* P59 (VISA P59). Lane 1, DNA marker of known molecular sizes, Lanes 2 & 3, PCR products of the amplified 16S r RNA gene KR270348**

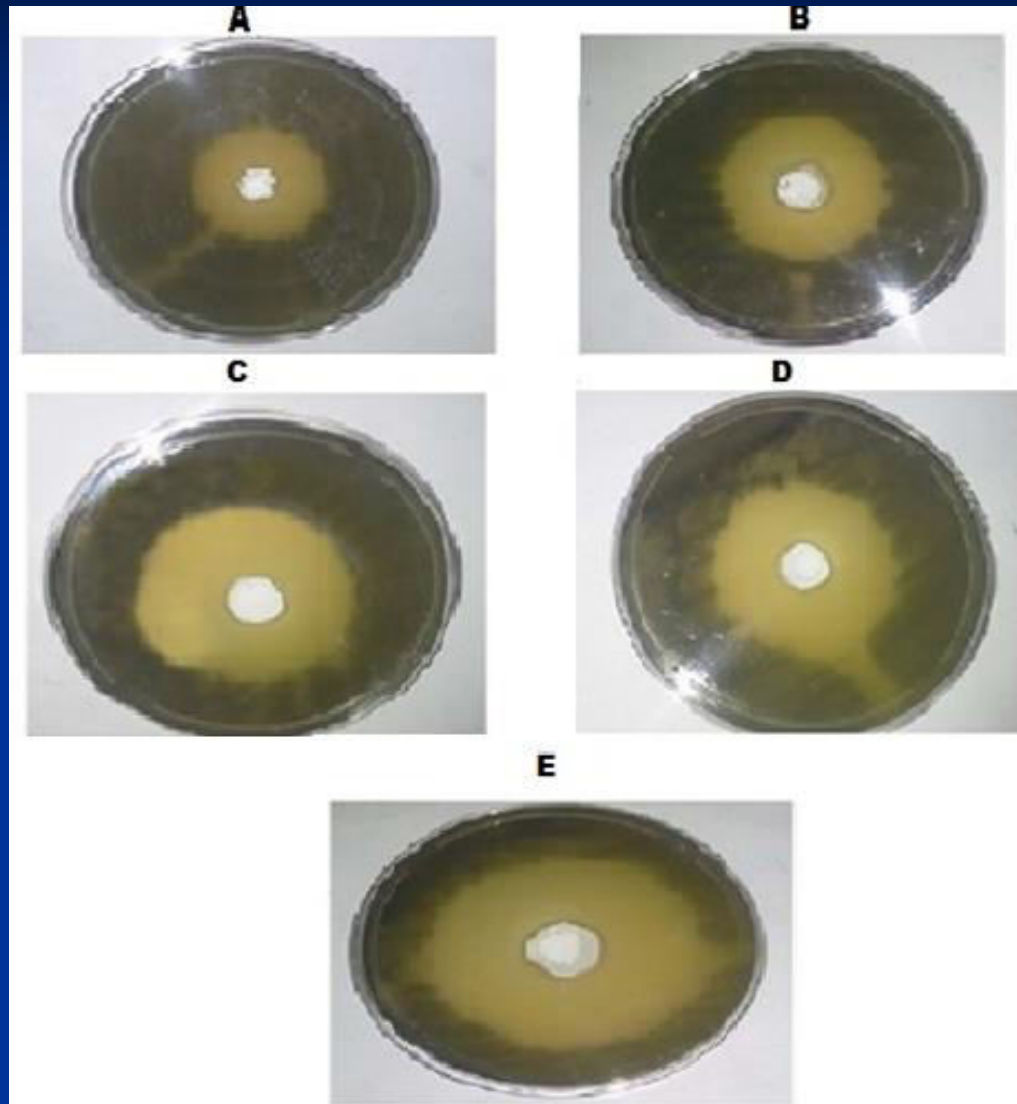


## b- Detection of MRSA *mecA* gene



**Figure 4. Agarose gel electrophoresis of the PCR products of *mecA* gene of VISA P5. Lane 1 , DNA marker of known molecular size , Lanes 2 & 3 , PCR products of the amplified *mecA* gene.**

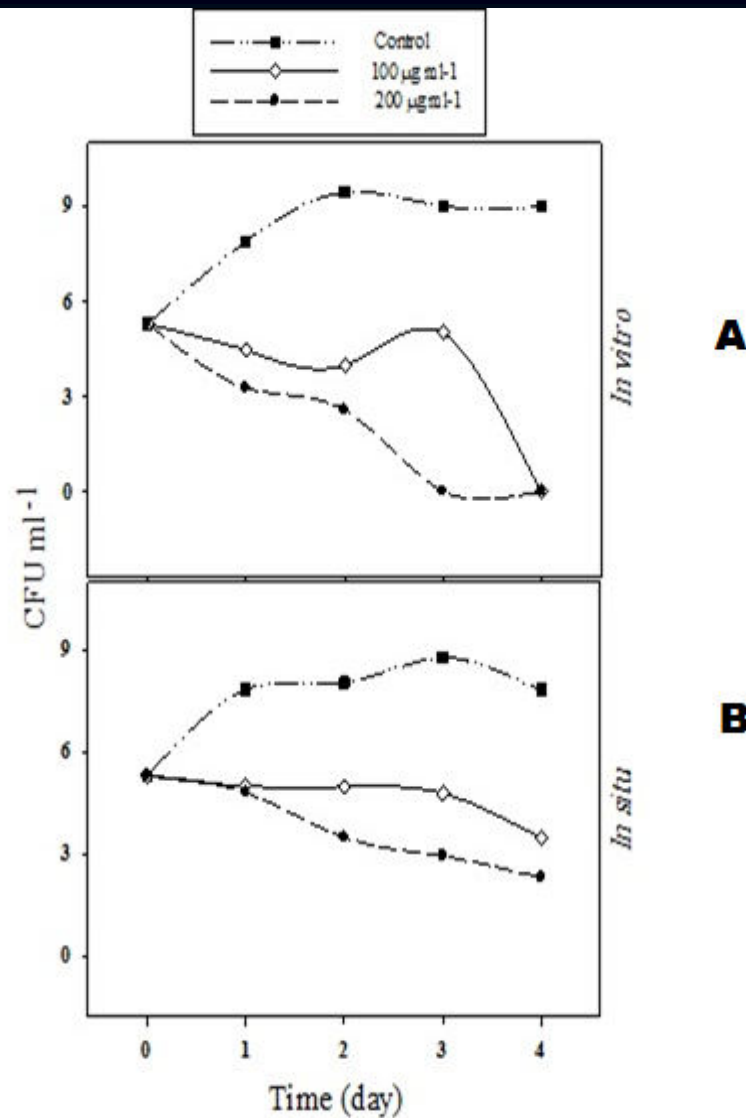
**4- Effect of different concentration of glycinin basic subunit(GBS) on *S.aureus* VISA P59 strains (3.125, 6.25, 12.5, 25, 50 $\mu$ g/mL)**



**Figure 5. Inhibition zones on *S.aureus* VISA P59 induced by different concentrations of glycinin basic subunit (GBS).**

	<b>Diameter of inhibition zone (mm)</b>				
<b>Temperature</b>			<b>pH</b>		
<b>(C°)</b>	<b>4.4</b>	<b>5.4</b>	<b>6.4</b>	<b>7.2</b>	<b>7.4</b>
<b>-2</b>	<b>44</b>	<b>35</b>	<b>40</b>	<b>41</b>	<b>45</b>
<b>3</b>	<b>43</b>	<b>41</b>	<b>41</b>	<b>40</b>	<b>24</b>
<b>25</b>	<b>48</b>	<b>49</b>	<b>50</b>	<b>49</b>	<b>48</b>
<b>37</b>	<b>48</b>	<b>49</b>	<b>50</b>	<b>50</b>	<b>48</b>
<b>45</b>	<b>11</b>	<b>12</b>	<b>12</b>	<b>15</b>	<b>14</b>

**Table 1. Inhibition zones (mm) induced by glycinin basic subunit (6.25µg/mL) at different pH values(4.4-7.4) and different incubation temperatures (-2-45 °C) in VISA P59.**



**Figure 6. Effect of glycinin basic subunit (GBS) on growth and viability of VISA P59 *in vitro* (A) and *in situ* (minced meat).**

*Conclusion*

VISA P59 was characterized by biochemical and molecular techniques and is resistant to most classes of antibiotics . Its genome contains methicillin resistance gene (mec A), but did not contain vancomycin resistance genes (van A and van B). GBS is a safe glycinin basic subunit and showed no toxicity to experimental rats. Its antimicrobial activity was stable over a wide range of pH values and temperature degrees. It could efficiently inhibit VISA P59 either *in vitro* or *in situ* ( in minced beef meat).

A detailed oil painting of a vibrant red rose in full bloom, accompanied by a smaller bud and several dark green leaves. The rose is the central focus, with intricate petal details. The background is a light, textured surface with faint, elegant scrollwork patterns. The overall style is classic and romantic.

*Thanks*

1602  
L. Franks