[**Effects Of Vancomycin On Staphylococcus aureus**](http://www.google.com/url?sa=t&rct=j&q=effects%20of%20sub-mics%20of%20cell-wall%20active%20and%20protein-synthesis%20inhibitor%20antibiotics%20on%20the%20quantity%20of%20biofilm%20genes%20and%20the%20exoproteins%20expression%20of%20%20staphylococcus%20aureu%20clinical%20isolates%20%20&source=web&cd=8&cad=rja&ved=0CF8QFjAH&url=http%3A%2F%2Flib.bioinfo.pl%2Fpaper%3A15616280&ei=zCfmUYSDKourrAfN9YHYBw&usg=AFQjCNEjtKBC5l38Rc53_GF-XLN4DnaO3g)

**Biofilm Genes Expression**

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 خلاصة البحث يهتم بدراسة تاثير قدرة التراكيز الدنيا لبعض المضادات الحياتية ذات الطيف الواسع على بعض المكورات العنقودية الموجبة لصبغة كرام وعيوب تلك المضادات فى تحفيز البكتيريا فى المقاومة وازدياد الامراضية بواسطة الطرق الجزئية الحديثة المتقدمة

**Abstract.** *Staphylococcus aureus* is well known as a major human pathogen, since microbial adherence is the initial step of many infectious processes, the ability of antibiotics to affect this property may be an important criterion in selecting an antibiotic for therapy. However, the clinical efficacy of antibiotics is not only determined by their respective bactericidal or bacteriostatic activity, but also by their action on bacterial virulence factor release, especially when present at suboptimal concentrations.

**Objective:** The main objective was to study the effect of vancomycin on steady state levels of mRNA of biofilm target genes *ica*A, B.

**Introduction**

 *Staphylococcus aureus* is well evidenced as a major human pathogen of strong clinical significance due to increasing infections with multi-resistant isolates (Zetola *et al*., 2005). Since microbial adherence is the initial step of many infectious processes, the ability of antibiotics to affect this property may be an important criterion in selecting an antibiotic for therapy (Shibl, 1987). Currently *S. aureus* has developed resistance to the most of β-lactam antibiotics, clindamycin, tobramycin, erythromycin and linezolid (Lindqvist *et al.*, 2009; Crawford *et al*., 2007; Tsiodras *et al.*, 2001). In principle antibiotics can both up and down modulate the synthesis and release of virulence factors (Bernardo *et al*., 2004). However, there have been very few reports on the effect of sub-MIC of cell wall active inhibitor antibiotics on the expression of virulence function such as, biofilm formation in different *S. aureus* clinical isolates. Therefore, the main objective was to determine changes in the quantity of these virulence factors in the presence of cell wall inhibitor antibiotic.

**Material and Methods:** Three different clinical isolates of *S. aureus* (**Table** **1.**) weretreated with sub-inhibitory concentrations of vancomycin. The isolates were received in the form of stock culture from the Medical Microbiology Laboratory/UPM/Malaysia. The vancomycin powder was purchased commercially from (BOIRON, Malaysia). To study the effect of cell wall inhibitors on steady state levels of mRNA of two biofilm genes expression*.* Cells of 3 different *S. aureus* isolates were grown with and without vancomycin for 24 h post antibiotic additions. RNA extraction was carried out using the same method that previously mentioned (Salman, 2010). Purified RNA was immediately converted to cDNA using RevertAidTM first strand cDNA synthesis kit. All primer sets for biofilm genes were ready to use in the laboratory. The relative expression of target genes was calculated by relative standard curve method. The data were then subjected to analysis the target gene expression using the Relative Expression Software Tool (REST) program.

**Table 1: Characteristics of clinical isolates used in this study.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Strain/No.** | **Isolation site** | ***spa* types** |  **MLST** | **SCCmec** |
|  **ST** |  **CC** |
| 1 (**MRSA**)/88 | Blood | t413 | ST239 | CC8 | IIIA |
| 2 (**MRSA**)/340 | Pus swab | t217 | ST22 | CC22 | IVh |
| 3 (**MRSA**)/20 | Abscess swab | t185 | ST188 | CC1 | V |

**Results**

**MIC Determination**

Overall MIC determination showed different vancomycin MICs (range 2.0 - 4.0 *µ*g/ml) were observed amongst the different isolates. No vancomycin resistance was found among the isolates according to CLSI, (2011) break points (Table 2).

**Table 2. Antibiotic concentrations of 1/2 MIC added to each isolates of *S.***

 ***aureus* used in this study.**

|  |  |
| --- | --- |
| No. of isolates |  Antibiotic concentration in µ/ml |
|  Vancomycin |
| MIC | 1/2MICa |
| 1 (**MRSA**)/88 | 2 | 1 |
| 2 (**MRSA**)/340 | 2 | 1 |
| 3 (**MRSA**)/20 | 4 | 2 |

**a Antibiotic concentrations added to each isolates of *S. aureus* used in this study**

**Biofilm Genes Expression in Cultures Exposed to Vancomycin for 24 hours**

In sub-1/2MIC, vancomycin treated 3 different isolates of MRSA, the steady-state mRNA transcription levels of biofilm target genes were showed the up and down irregular changes in mRNA transcription levels of two biofilm target genes (Table 3)

**Table 3. Fold change in mRNA levels of biofilm target genes in cultures of different *S. aureus*  isolates grown in the presence of Vancomycin.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No. of isolates** | **Gene** | **Type** | **Expression** | **P value** | **Result** |
| 1 (MRSA)/88 | 16s | REF | 1.000 |  |  |
| *ica*A | TRG | 1.943 | 0.000 | UP |
| *ica*B | TRG | 2.458 | 0.000 | UP |
| 2 (MRSA)/340 | *ica*A | TRG | 0.519 | 0.001 | DOWN |
| *ica*B | TRG | 2.868 | 0.001 | UP |
| 3 (MRSA)/20 | *ica*A | TRG | 1.155 | 0.431 | Not different |
| *ica*B | TRG | 1.987 | 0.003 | UP |

REF indicates reference gene, TRG target gene, UP to significantly increased, and DOWN to significantly decreased if P value is <0.05 (Target sample is different to control). Not different indicates to target sample is not different to control if P value is > 0.05.

**Discussion**

The effects of sub-inhibitory concentrations of certain antibiotics, especially those that inhibit bacterial cell wall, may have an impact in altering both bacterial surface components and interactions of bacteria with host tissues (Stevens *et al*., 2007). Since microbial adherence is the initial step of many infectious processes, the ability of antibiotics to affect this property may be an important consideration in the selection of an antimicrobial agent to combat staphylococcal infections.

In this study, analysis of the relative expression of *ica*A,BmRNA after 24 hours post vancomycin additions of sub-MIC reveals a reduction and induction in the amounts of *ica*AB target genes amongst different isolates of *S. aureus* and a general pattern that is not consistent across all isolates. This is particularly important in different isolates of *S. aureus*, in response to these antibiotics even at low concentrations could lead to worsening outcomes. It is probable that vancomycin treatment generates signals in diverse physiological pathways, which are recognized by multiple signal sensors that in turn activate multiple response regulators (Kuroda *et al*., 2003), or it can be speculated that vancomycin can induce stress conditions which interact on regulatory genes, in particular genes involved in two-component regulatory systems, which have been well described in *S. aureus.*. Similarly, other studies were found that sub-MIC of clindamycin stimulates synthesis of some Biofilm genes at transcription levels (Herbrt *et al*., 2001). It was also reported by several researchers, that exposure of staphylococci to cell wall inhibitors caused the rapid and extensive up-regulation of a unique set of genes which had a wide range of functions (Gardete *et al*., 2006).

**In this study, we conclude**, the up- and down-modulation of *ica*A,B target genes*,* may be explained by the different global transcription changes occurred in the presence of low antibiotic concentration, or according to the clonal gentic map variation of different *S. aureus* clinical isolates, therefore using this antibiotic to treat *S. aureus* infections may contribute to worse outcomes. Further studies are needed to answer the question of whether this antibiotic could prove beneficial for patients without their action on bacterial virulence factor release, especially when present at suboptimal concentrations.

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