

## Original Research Article

<https://doi.org/10.20546/ijcmas.2017.609.443>

## Modification of Glycan Expression in *Staphylococcus epidermidis* to Prevent Biofilm Formation

Ekpiken Solomon Ekpiken\*, Lawrence B. Etim and Mercy Ekong

Department of Biological Sciences Cross River University of Technology, Nigeria

\*Corresponding author

### ABSTRACT

*Staphylococcus epidermidis* is a common commensal of the skin and mucous membranes of humans. However, it is a common cause of nosocomial infection especially infection associated with the insertion of biomedical devices. Glycans have been shown to mediate vast majority of biological processes and its alterations have been reported to be used as biomarkers and applied in diagnosis of malignancies and infections. The absolute mean Optical Density (OD<sub>450nm</sub>) of the biofilm was calculated as 0.248 ± 0.044SD and the biofilm was observed to strongly adhere to the microtitre plate surface. The biofilm growth curve peaks at 2hrs with a mean OD<sub>450nm</sub> of 0.385; at 30 minutes and 4hrs growth, mean OD<sub>450nm</sub> were 0.365 and 0.383 respectively and drastically drops to 0.248 at 24hrs. A panel of 11 biotinylated lectins was used to detect any alterations in glycosylation of the glycans expressed by of *S. epidermidis* RP62A (ATCC 35984). Glycans' expression varies with time and most glycans showed strong expression at 2hrs of incubation except N-acetyl galactosamine (GalNAc), which showed strong expression at 30 minutes. Glycosylation inhibitors such as Neuraminidase, Cytidine 5'-monophospho-N-acetylneuraminic acid sodium salt (CMP-NANA), Sialic acid aldolase and 1M Hydrochloric acid were used to prevent biofilm establishment and development and their effects evaluated using toluidine blue stain and lectin screening. The glycosylation inhibitors showed remarkable 80% reduction in Extracellular Polysaccharides (EPS) production and the expression of glycans in comparison with the control, and were most effective at 2hrs relative to 0hr and 4hrs of incubation except 1M HCl, which was effective at 0hr compared to other times of incubation. This study reveals new strategies of using glycosylation inhibitors to prevent biofilm formation during implant of medical

#### Keywords

Glycosylation inhibitors, *Staphylococcus epidermidis*, Desialylation; Glycans; enzyme-linked lectin assay (ELLA) and Lectins.

#### Article Info

Accepted:  
10 July 2017  
Available Online:  
10 September

### Introduction

Biofilms are complex multicellular microbial communities immobilised by extracellular polysaccharides (EPS) that is produced by microorganisms, and can be attached to biotic and abiotic surfaces (Otto, 2009). It occurs in response to adverse environmental condition or stress and involves multiple regulatory signals that re-programme gene expression (Monds and O'Toole

2009). This gene expression reprogramming alters the expression of virulence factors, nutrient utilisation, surface molecules and equips the bacteria with the arsenals which enable their survival in adverse environmental conditions (Klebensberger *et al.*, 2009). Dapa *et al.* (2013) showed that lower concentrations of antibiotics can cause stress in bacteria thereby inducing biofilm formation. The structure of EPS keeps bacteria in close proximity, which enables them to

interact closely and exchange genetic materials (Flemming and Wingender, 2010; Koo, *et al.*, 2010) The intimate cell-to-cell interactions promote the spread of antibiotic resistance genes and other virulence factors among bacteria within the biofilm (Vuong *et al.*, 2004).

Medical device infections constitute a major cause of morbidity and mortality in hospitalised patients, and biofilms readily develop on all types of medical devices inserted in more than 25% of hospitalised patients (Hazan *et al.*, 2006). Approximately 60-70% of nosocomial infections are associated with implanted medical devices (Bryers, 2008). Catheter associated urinary tract infections account for 43% of nosocomial infections in UK (Hopkins *et al.*, 2011). Bacterial infections in patients with urinary catheters occur at 5-10% per day in patients with long term catheters ( $\geq 28$  days) (Maki and Tambyah 2001).

*S. epidermidis* infections rarely develop into life threatening diseases, however, they are extremely difficult to treat and recurrently posing a serious public health burden (Otto, 2009). Although *S. epidermidis* is a normal flora of the skin, it is a common cause of nosocomial infection especially infections associated with the implant of medical devices such as urinary catheters (Cogen *et al.*, 2008).

Lectins are carbohydrate binding proteins that can recognise and bind to specific glycan molecules (Sharon and Lis 2002). Altered glycan molecules have been reported as indicators for the development of malignant cells (Rosenfeld *et al.*, 2007); and can be applied as biomarkers for diagnosis of infections (Chen *et al.*, 2007).

Previous study had focused on the inhibition of bacterial attachment or colonisation on implanted medical devices like urinary catheters, by using physiochemical modifications on the device surface to create anti-adhesive surfaces; coating catheters with antimicrobial agents, silver salts (Hazan *et al.*, 2006), however, these approaches provide minimal effort in reducing biofilm associated infection. Glycan modification occurs during the later stages of biofilm development and sialylation of glycan molecules are involved in biofilm establishment. This research therefore

focuses on the desialylation of these glycan molecules by treating with specific glycosylation inhibitors to prevent biofilm formation and development.

## Materials and Methods

### Materials

*S. epidermidis* RP62A (ATCC 35984) cultured in blood agar plate (Oxoid, England) was sub-cultured in 10ml tryptone soy broth (TSB) (Oxoid, England) and incubated for 24hrs at 37°C.

### Biofilm growth in a microtitre plate

*S. epidermidis* biofilms were grown according to the procedure described by Deighton *et al.* (2001) with slight modifications. Approximately 50 $\mu$ l of fresh TSB culture containing 3.0x10<sup>6</sup>cfu/ml was inoculated into 8 wells of a flat bottomed microtitre plate (Greiner bio-one, Germany) and incubated for 24hrs at 37°C. After incubation, excess medium containing planktonic cells were removed and the plate washed with 1xTris-buffered saline (TBS, pH 7.6) for three times using ELISA washer (bioTek, USA) and dried for at least 2hrs at room temperature. The optical density (OD<sub>450nm</sub>) was measured using a microplate reader (Titertek Multiskan MCC/340, Finland).

### Crystal violet staining and time-course analysis

*S. epidermidis* biofilms were grown according to the procedure described by Deighton *et al.* (2001) with slight modifications and incubated for 30 minutes, 2hrs, 4hrs and 24hrs at 37°C respectively. The microtitre plates were stained using 0.4% (w/v) ammonium crystal violet stain and incubated for 10 minutes at room temperature to evaluate the presence of bacterial cells in the biofilms. Excess stain was removed and the plates rinsed twice with tap water and dried for at least 2hrs at room temperature, and the OD<sub>450nm</sub> was measured using a microplate reader (Titertek Multiskan MCC/340, Finland).

### Lectins screening and time-course analysis

Approximately 50 $\mu$ l of fresh TSB culture containing 3.0x10<sup>6</sup>cfu/ml was inoculated into each

well of 4 microplates and incubated for 30 minutes, 2hrs, 4hrs and 2hrs at 37°C respectively. Planktonic cells were discarded in a disinfectant and the plates dried for at least 2hrs at room temperature. Enzyme-linked lectin assay (ELLA) was performed according to manufacturers' instructions using a panel of 11 different biotinylated lectins (Vector Laboratories), with different specificity which include: *Sambucus nigra* agglutinin (SNA), Wheatgerm agglutinin (WGA), succinylated wheatgerm agglutinin (sWGA), *Galanthus nivalis* agglutinin (GNA), *Maackia amurensis* agglutinin II (MAA- II), Soybean agglutinin (SBA), *Solanum tuberosum* agglutinin (STA), *Erythrina cristagalli* agglutinin (ECA), *Lens culinaris* agglutinin (LCA), *L-Phaseolus vulgaris* (IPHA) and Concanavalin A (ConA). This analysis was performed in triplicate. Wells without biotinylated lectin served as negative control while wells without biofilm served as blank wells. The OD<sub>450nm</sub> of each well was measured using a microplate reader.

### **Biofilm time-dependent inhibition Assay**

Approximately 50µl of fresh TSB culture containing 3.0x10<sup>6</sup>cfu/ml was inoculated into each well of 4 microplates and 1µl each of Neuraminidase (from *Clostridium perfringens*) (1U/ml), CMP-NANA (cytidine 5'-monophospho-N- acetylneuraminic acid sodium salt) (100µg/ml), Sialic acid aldolase (from *E. coli* K-12) (200µg/ml) (Sigma Life Science) and 1M Hydrochloric acid (HCl) were added to each plate at 0hr, 2hrs and 4hrs respectively; and incubated for 24hrs at 37°C. After incubation, the plates were washed with 1xTris- buffered saline (TBS, pH 7.6) thrice using ELISA washer and dried for at least 2hrs at room temperature. Enzyme-linked lectin assay (ELLA) was repeated according to manufacturer's instruction using 6 different biotinylated lectins including SNA, WGA, sWGA, GNA, ECA and ConA. The lectins discontinued were those with similar glycan specificity with the ones selected and those that showed no obvious interaction with the glycans, therefore they would provide no additional information. This analysis was performed in triplicate. Wells without the treatment served as the positive control while wells without biofilm served as the negative control. The OD<sub>414nm</sub> of each well was measured

using a microplate reader.

### **Toluidine blue staining**

The effect of the treatments to prevent biofilm development at different incubation times was evaluated using 0.25% (w/v) toluidine blue stain and incubated for 30 minutes at room temperature. The OD<sub>450nm</sub> of each well was measured using a microplate reader.

## **Results and Discussion**

### **Biofilm growth in a microtitre plate**

The detection of *in vitro* biofilm formation of *S. epidermidis* RP62A (ATCC 35984) in a microtitre plate after 24 hours of incubation was evaluated using crystal violet stain, which is usually used as the gold standard. The absolute mean OD<sub>450nm</sub> of the biofilm was calculated as 0.248± 0.044SD and the biofilm was observed to strongly adhere to the microtitre plate surface.

### **Biofilm growth and time-course analysis**

*S. epidermidis* biofilm growth curve stained with crystal violet after 24hrs incubation is presented in figure 1. The biofilm growth curve shows a downward curve due to EPS formation. This result indicates that *S. epidermidis* grows optimally at 2hrs and biofilm develops and decreases at 4hrs due to EPS formation. The growth curve peaks at 2hrs with a mean OD<sub>450nm</sub> of 0.385; at 30 minutes and 4hrs growth mean OD<sub>450nm</sub> were 0.365 and 0.383 respectively and drastically drops to 0.248 at 24hrs.

### **Lectins screening and time-course analysis**

A panel of lectins was screened at different time as shown in Figure 2. The selection of the lectins was based on the degree of specificity with glycans expressed during biofilm formation. Eleven lectins were analysed after 30 minutes, 2hrs, 4hrs and 24hrs of incubation. At 30 minutes of incubation, it was observed that N- acetyl glucosamine (GlcNAc) binding lectins: STA and sWGA showed very strong interaction with the sugar, GlcNAc. It was equally observed that N- acetyl galactosamine (GalNAc) binding lectin, SBA

showed strong interaction with terminal  $\alpha$ -GalNAc. However, GNA and ConA were observed to have strong interaction with mannose than LCA. At 2hrs of incubation, mannose was strongly expressed and interacted with GNA, LCA and ConA, Also, sialic acid was strongly expressed and interacted with SNA and WGA (which also interact with GlcNAc); terminal sialic acid attached to  $\alpha$ -2,3 Gal or on N-glycan cores was equally expressed and interacted with MAA-II. However, STA and sWGA interacted with GlcNAc and was also expressed along with galactose which interacted with ECA. At 4hrs it was observed that SNA and WGA showed strong interaction with sialic acid and sWGA also interacted with GlcNAc while other sugars were either masked or modified, particularly mannose, galactose and N- acetyl galactosamine. At 24hrs of incubation, sialic acid, mannose and GalNAc were expressed and interacted with SNA, WGA, GNA and SBA respectively. Galactose and GlcNAc were also expressed and interacted with ECA, STA and sWGA. Other glycans like terminal mannose and complex glycan structure were either masked or modified and showed weak interaction with their lectins.

### **Biofilm time-dependent inhibition assay**

The glycosylation inhibitors used include neuraminidase, CMP-NANA, sialic acid aldolase and hydrochloric acid (HCl). The effect of neuraminidase on EPS production in *S. epidermidis* biofilm development at different incubation times was analysed using toluidine blue stain as shown in figure 3a. There was a 90% reduction in EPS production when the biofilm was treated with neuraminidase in comparison with the control. However, treatment with neuraminidase was most effective at 2hrs of treatment in comparison with 0hr and 4hrs. Also, the effect of neuraminidase on sialylation in *S. epidermidis* biofilm development at different incubation times was analysed using lectin staining as shown in figure 3b. There was a remarkable reduction in glycan expression compared to the control. Neuraminidase cleavage was most effective at 0hr and 2hrs, compared to 4hrs of treatment so a weak and decreased interaction with SNA and WGA was observed. Also, GlcNAc expression was reduced at 0hr and 2hrs in comparison to 4hrs of

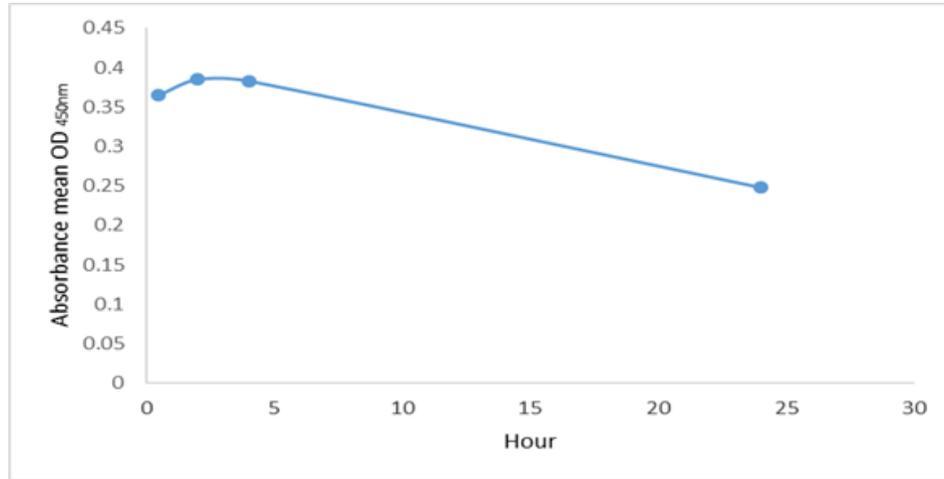
incubation. Generally speaking, sialic acid cleavage dramatically influenced the expression of other glycans. High mannose reduction was most effective at 2hrs compared to 0hr and 4hrs as shown with GNA interaction. Galactose and terminal mannose reduction were most effective at 0hr and 2hrs compared to 4hrs as shown with ECA interaction; and 2hrs and 4hrs compared to 0hr as shown with ConA interaction respectively.

The effect of CMP-NANA on EPS production in *S. epidermidis* biofilm development at different incubation time was analysed using toluidine blue stain and presented in figure 4a. There was a remarkable reduction in EPS production when treated with CMP-NANA in comparison to the control; however, treatment was most effective at 2hrs compared with 0hrs and 4hrs. Figure 4b shows the effect of CMP-NANA on sialylation of *S. epidermidis* biofilm development at different incubation time analysed using lectin staining. It was observed that CMP-NANA treatment remarkably reduced sialylation as compared to the control. Overall effectiveness of CMP-NANA was observed at 0hr except in galactose, which was effective at 4hrs. Sialic acid reduction was observed to be effective at 0hr though not remarkable as compared to 2hrs and 4hrs as observed with SNA and WGA interactions. Galactose and GlcNAc reduction was also observed most effective at 4hrs and 0hr as observed with ECA and sWGA interaction respectively. However, mannose was reduced at 0hr and 4hrs as observed with GNA and ConA interaction.

The effect of sialic acid aldolase on EPS production in *S. epidermidis* biofilm development was analysed at different incubation time using toluidine blue stain and is shown in figure 5a. A dramatic reduction in EPS production was observed with sialic acid aldolase treatment as compared with the control. A remarkable reduction was observed at 2hrs in comparison with 0hr and 4hrs due to degradation of sialic acid into its component parts. The effect of the treatment of *S. epidermidis* biofilm with sialic acid aldolase was analysed using lectin staining and is shown in figure 5b; a remarkable reduction in the expression of glycans as compared to the control was observed. Sialic acid reduction was effective at 0hr

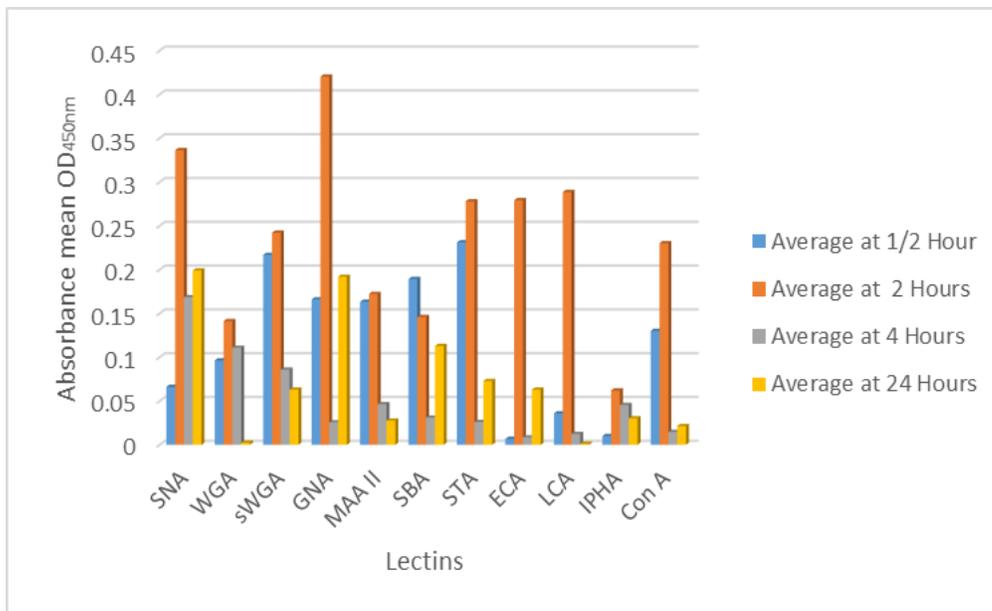
relative to 2hrs and 4hrs as detected by SNA, However, both sialic acid and GlcNAc expression showed dramatic reduction over time as detected by WGA. GlcNAc expression was also reduced

and effective at 2hrs as detected by sWGA. Galactose reduction was effective at 0hr; however mannose reduction was effective at 0hr and 4hrs as observed with ConA and GNA.



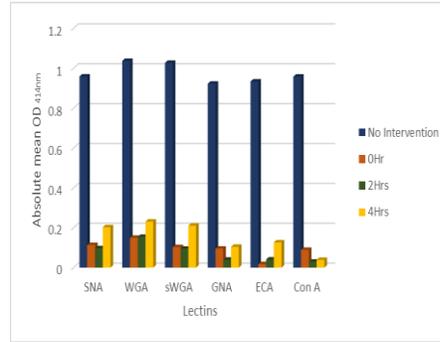
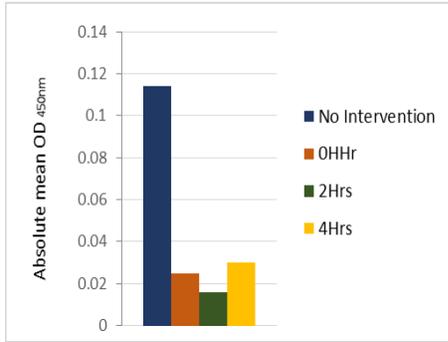
**Figure 1: Biofilm growth curve**

Crystal violet stain of a biofilm growth at 30 minutes, 2hrs, 4hrs and 24hrs. The curve peaks at 2hrs and start decreasing. This result indicates that the *S. epidermidis* RP62A grows optimally at 2hrs and as EPS start forming, bacterial cells were not detected by the stain as they were enveloped in the EPS.

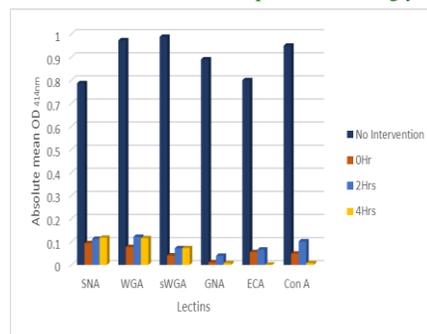
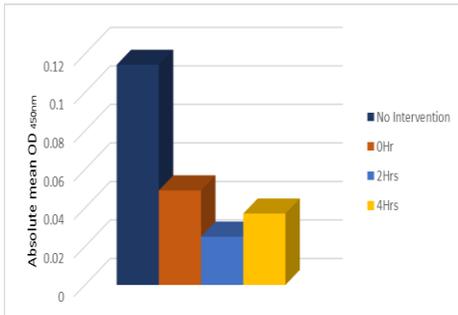


**Figure.2** Lectin screening at different time-course

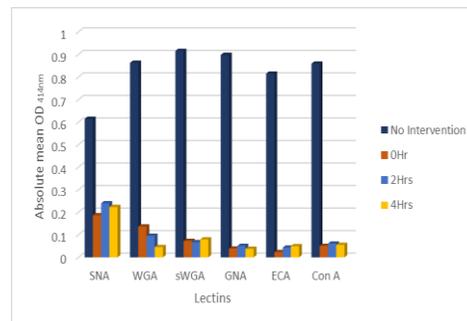
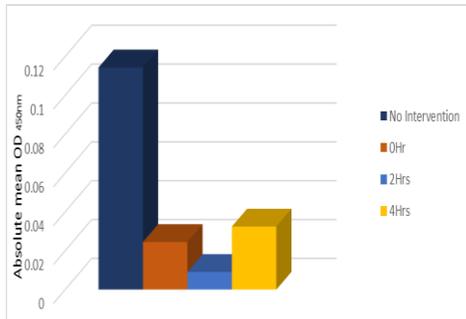
Most of the glycans were observed to be mostly expressed at 2hrs, however, GlcNAc, mannose, terminal sialic acid  $\alpha$ -2,3 Gal, terminal  $\alpha$ -GalNAc were expressed at 30 minutes though Gal  $\beta$  1,4 GlcNAc and highly branched non-bisected complex N-glycans were not expressed at this time. Those glycans that were not expressed were either masked or modified as such the lectins cannot bind.



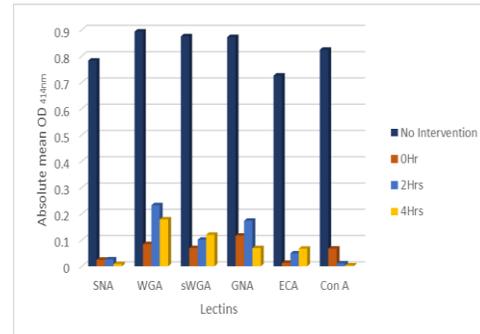
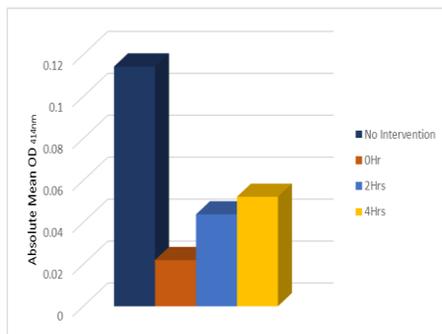
**Figure.3** Treatment of biofilm with neuraminidase (a) The effect of neuraminidase on EPS at different incubation time stained with toluidine blue. (b) The effect of neuraminidase on EPS at different incubation times with lectin staining. A dramatic decrease in the expression of the glycans



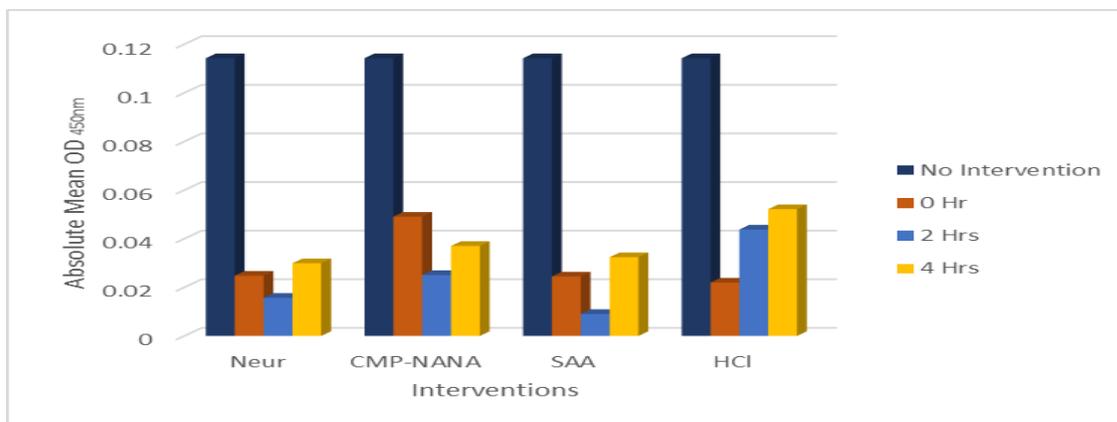
**Figure.4** Treatment of biofilm with CMP-NANA (a) The effect of NANA in EPS production to prevent biofilm development at different incubation times. (b) The effect of CMP-NANA on sialylation at different incubation times with lectin staining.



**Figure.5** Treatment of biofilm with sialic acid aldolase The effect of sialic acid aldolase in EPS to prevent biofilm development at different incubation time. (b) The effect of CMP-NANA on sialylation at different incubation times with lectin staining.



**Figure.6** Treatment of biofilm with 1M Hydrochloric acid (a)The effect of change in pH in EPS production at different incubation time. (b) Sialic acid  $\alpha$ -2,3 Gal, terminal mannose and Gal  $\beta$ -1,4 GlcNac were observed to be inhibited as pH changed, however, GlcNac and high mannose **were not inhibited**.



**Figure.7** Toluidine blue staining

Neur= Neuraminidase, CMP-NANA= Cytidine 5'- monophospho-N- acetylneuraminic acid Na salt, SAA= Sialic acid aldolase, HCl= Hydrochloric acid.

The effect of change in pH on EPS production in *S. epidermidis* biofilm was analysed at different incubation time using toluidine blue stain and is shown in figure 6a. A remarkable overall reduction in EPS production in comparison with the control was observed, however, treatment was most effective at 0hr, relative to 2hrs and 4hrs. The effect of change in pH on sialylation of *S. epidermidis* biofilm development at different incubation times was analysed using lectin staining and shown in figure 6b. It was hypothesised that change in pH may change the surface charge of the wells thus preventing attachment and possibly prevent biofilm formation. It was observed that the acid remarkably reduced the expression of sialic acid as observed with weak interaction with SNA, however, GlcNAc was most effective at 0hr as detected by WGA and sWGA. High mannose reduction was observed to be most effective at 4hrs relative to 0hr and 2hrs as detected by GNA. Galactose reduction was observed to be most effective at 0hr compared to 2hrs and 4hrs as detected by ECA and terminal mannose reduction was most effective at 2hrs and 4hrs as detected by ConA.

### Toluidine blue staining

The effect of the interventions to prevent biofilm development by intervening with glycosylation at different incubation time is shown in figure 7. A remarkable reduction in EPS production was observed when compared with the control. However, neuraminidase, CMP-NANA and sialic acid aldolase were most effective at 2hrs of

intervention while HCl was most effective at 0hr of intervention.

The absolute mean OD<sub>450nm</sub> value of *S. epidermidis* biofilm at 24hrs of incubation,  $0.248 \pm 0.044SD$  indicates that there is biofilm growth, which strongly adheres to the plate surface. This is consistent with previous studies by Deighton *et al.* (2001), and also confirms that *S. epidermidis* RP62A (ATCC 35984) is a strong biofilm producer. The crystal violet stained cell optical density confirmed the biofilm thickness that is expressed as an index of adherence to the surface of the plate and not the presence of EPS. However, according to Crémet *et al.* (2013) EPS represent the cell biomass and not its viability.

Decrease in *S. epidermidis* biofilm growth curve stained with crystal violet corresponds with increase in EPS production which in turn cocooned the cells from staining. Hence, *S. epidermidis* strong adherence ability as earlier reported by (Deighton *et al.*, 2001).

High specificity of lectins for glycan molecule ensures high sensitivity to detect alterations in glycosylation due to malignancies or other immune disease (Rosenfeld *et al.*, 2007). N-acetyl glucosamine was expressed at 30 minutes because it is a major component of EPS in addition to bacterial cell wall (Khan *et al.*, 2013), which was synthesised as the bacteria grow. N-acetyl galactosamine expression may be involved in cell-to-cell communication, which is very important in biofilm formation. N-acetyl galactosamine is also

commonly found in mucins (Varki *et al.*, 2009). Gal  $\beta$ 1,4 GlcNAc, which is the second or third most abundant glycan in *S. epidermidis* biofilm (Bales *et al.*, 2013), also constitutes the core of blood group carbohydrates that is expressed in abundance in normal cells but reduced in malignant cells (Ghazarian *et al.*, 2011). Mannose is the predominant glycan in *S. epidermidis* biofilm (Bales *et al.*, 2013), and is associated with inflammatory response (Yates 2005). Bales *et al.* (2013) reported that microorganisms use mannose as component of surface antigens or capsule. Expression of terminal sialic acid was expected because it is found at the cell surface and secreted molecules (Varki, 2007), and first comes in contact with the lectin. Many carbohydrate structures such as terminal sialic acid linked to  $\alpha$ -2,3 Gal have been reported to be expressed in various human malignant cells (Shida *et al.*, 2009). Sialic acid has been observed to either act as a biological masking (antirecognition) agent, by shielding recognition sites or as a biological recognition agent for a wide variety of molecules such as lectins and antibodies (Schauer, 2009). ELLA is a useful method for studying qualitative and quantitative variations in the polysaccharides components of a developing biofilm (Leriche *et al.*, 2000).

The remarkable reduction in EPS production at different time of incubation relative to the control shows that neuraminidase reduced sialylation by cleaving sialic acid from the glycoprotein, which is crucial for biofilm formation thereby inhibiting biofilm formation, however, it was most effective at 2hrs, which depicts that treatment should be initiated before 2hrs of biofilm formation. In comparison of the effect of neuraminidase on sialylation stained with lectins, a remarkable reduction in sialic acid interaction with SNA and WGA at 0hr and 2hrs also confirms that neuraminidase desialylated sialic acid from the glycan moieties. Neuraminidase has been reported to cleave cell surface sialic acid (Suzuki and Abe 2014). This study, confirmed that neuraminidase cleaved sialic acid at the early stage of biofilm development, within 2hrs of biofilm growth. This may be because sialic acid is typically attached to the terminal positions of several glycan molecules. The remarkable increase at 4hrs compared to 0hr and 2hrs was expected because at this time the

biofilm has developed and matured therefore treatment with neuraminidase was ineffective. The use of neuraminidase is of medical importance in treating biofilm associated infections at the early stage before biofilm development. Most neuraminidases are observed to cleave specific sialic acid linkages. In this study, neuraminidase from *Clostridium perferingens* rapidly cleaved  $\alpha$ -2,6 linkages. However, neuraminidase from *Arthrobacter ureafaciens*, exhibits high cleavage potential for  $\alpha$ -2,6 linkages; while some linkages may be completely or partially resistant to neuraminidase. In contrast,  $\alpha$ -2,3 linkages are cleaved more rapidly and easily than  $\alpha$ -2,6 linkages (Varki *et al.*, 2009). This phenomenon may be the reason for the increased expression of sialic acid linked with  $\alpha$ -2,6 linkage at 4hrs relative to 0hr and 2hrs. Also, modification of the sialic acid molecules (methylation, sulfation, succinylation and acetylation) can prevent cleavage (Varki *et al.*, 2009), and modification of glycan molecules enable microorganisms to evade immune defence (Schauer, 2009).

The remarkable decrease in EPS production when treated with CMP-NANA compared to the control depicts that CMP-NANA reduced sialylation. Treatment was most effective at 2hrs relative to 0hr and 4hrs. Comparing this result with lectin staining in figure 4b, CMP-NANA reduced sialylation at 0hr as detected by SNA and WGA. It was hypothesised that CMP-NANA is a substrate for sialylation; however, the result obtained was the opposite. CMP-NANA reduction in sialylation caused a remarkable reduction in other sugars like mannose and GlcNAc. Weak interaction of other glycans with their respective lectins suggests that they were either modified by methylation, sulfation, succinylation and acetylation processes or the anomer conformation was changed.

The reduction in EPS production of *S. epidermidis* biofilm due to treatment with sialic acid aldolase confirms the degradation of sialic acid; and was most effective at 2hrs of treatment. However, in comparison with lectin screening (figure 5b), it was most effective at 0hr and a remarkable reduction in sialylation was seen when compared with the control. This result suggests that sialic acid aldolase reversibly degrades sialic acid, which

confirms previous study, which reported that sialic acid aldolase degrades sialic acid into pyruvate and N- acetyl mannosamine; (Yu *et al.*, 2006). Sialic acid aldolase has been widely used for the chemoenzymatic synthesis of sialic acids and their analogs (Huang *et al.*, 2007).

Alteration of pH remarkably reduced EPS production and was most effective at 0hr compared to 2hrs and 4hrs of incubation. It was hypothesised that pH alteration changes the surface charge of the plate which invariably prevents bacterial attachment, which is critical in biofilm formation (Hazan *et al.*, 2006). The lectin staining showed a remarkable reduction in sialic acid expression as sialylation was reduced due to change in pH. This result is consistent with previous study by Varki *et al.* (2009), which reported that change in pH by using a stronger acid can destroy sialic acid moieties. However, GlcNAc and high mannose expression were reduced at 0hr and 4hrs respectively. High mannose and GlcNAc are important components of the bacterial cell wall. Due to increased resistance of bacterial biofilms to conventional antimicrobial agents and the increasing spread and rise in antibiotic resistant bacterial strains, it is therefore necessary to develop other effective preventative strategies such as incorporating these glycosylation inhibitors in catheters to treat and prevent biofilm associated infections. Sialic acids are critical in biofilm formation, therefore, its detection, modification and cleavage is of significant biomedical importance. The expression of glycans present in *S. epidermidis* biofilms at different incubation time varies; however, future work will use a wider panel of lectins to detect all the glycans expressed and prevent masking of some glycans observed in this study.

## References

- Bales, P. M., Renke, E. M., May, S. L., Shen, Y., & Nelson, D. C. (2013). Purification and Characterization of Biofilm-Associated EPS Exopolysaccharides from ESKAPE Organisms and Other Pathogens. *PloS One*, 8 (6): 1–8.
- Bryers, J. D. (2008). Medical biofilms. *Biotechnology and Bioengineering*, 100 (1): 1–18.
- Chen, S., Laroche, T., Hamelinck, D., Bergsma, D., Brenner, D., Simeone, D. Brand, R., Haab, B. B. (2007). Multiplexed analysis of glycan variation on native proteins captured by antibody microarrays. *Nature Methods*, 4 (5): 437–444.
- Cogen, A. L., Nizet, V., & Gallo, R. L. (2008). From bench to bedside skin microbiota: a source of disease or defence? *British Journal of Dermatology*, 158: 442–455.
- Crémet, L., Corvec, S., Batard, E., Auger, M., Lopez, I., Pagniez, F., Dauvergne, S. and Caroff, N. (2013). Comparison of three methods to study biofilm formation by clinical strains of *Escherichia coli*. *Diagnostic Microbiology and Infectious Disease*, 75 (3): 252–255.
- Dapa, T., Dapa, T., Leuzzi, R., Ng, Y. K., Baban, S. T., Adamo, R., Kuehne, S., Scarselli, M., Minton, N., Serruto, D. and Unnikrishnan, M. (2013). Multiple factors modulate biofilm formation by the anaerobic pathogen *Clostridium difficile*. *Journal of Bacteriology*, 195 (3): 545–555.
- Deighton, M. a, Capstick, J., Domalewski, E., & van Nguyen, T. (2001). Methods for studying biofilms produced by *Staphylococcus epidermidis*. *Methods in Enzymology*, 336 (1996): 177–195.
- Ghazarian, H., Idoni, B., & Oppenheimer, S. B. (2011). A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics. *Acta Histochemica*, 113(3), 236–47. doi:10.1016/j.acthis.2010.02.004
- Hazan, Z., Zumeris, J., Jacob, H., Raskin, H., Kratysh, G., Vishnia, M., ... Lavie, G. (2006). Effective prevention of microbial biofilm formation on medical devices by low-energy surface acoustic waves. *Antimicrobial Agents and Chemotherapy*, 50(12), 4144–52. doi:10.1128/AAC.00418-06
- Hopkins, S., Cookson, B., & Kessel, A. (2011). English National Point Prevalence Survey on Healthcare-associated Infections and Antimicrobial Use , 2011, 1–140. Retrieved from <http://www.hpa.org.uk/>
- Khan, M. M. T., Takizawa, S., Lewandowski, Z., Habibur Rahman, M., Komatsu, K., Nelson, S. E., ... Ohgaki, S. (2013). Combined effects of EPS and HRT enhanced biofouling on a submerged and hybrid PAC-MF membrane bioreactor. *Water Research*, 47(2), 747–57. doi:10.1016/j.watres.2012.10.048
- Klebensberger, J., Birkenmaier, A., Geffers, R., Kjelleberg, S., & Philipp, B. (2009). SiaA and SiaD are essential for inducing autoaggregation as a specific response to detergent stress in *Pseudomonas aeruginosa*. *Environmental Microbiology*, 11(12), 3073–86. doi:10.1111/j.1462-2920.2009.02012.x
- Koo, H., Xiao, J., Klein, M. I., & Jeon, J. G. (2010). Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the

- establishment of microcolonies within multispecies biofilms. *Journal of Bacteriology*, 192(12), 3024–32. doi:10.1128/JB.01649-09
- Leriche, V., Sibille, P., & Carpentier, B. (2000). Use of an enzyme-linked lectinsorbent assay to monitor the shift in polysaccharide composition in bacterial biofilms. *Applied and Environmental Microbiology*, 66(5), 1851–6. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=101422&tool=pmcentrez&rendertype=abstract>
- Mack, D., Fischer, W., Krokotsch, a, Leopold, K., Hartmann, R., Egge, H., & Laufs, R. (1996). The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis. *Journal of Bacteriology*, 178(1), 175–83. Retrieved from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=177636&tool=pmcentrez&rendertype=abstract>
- Maki, D. G., & Tambyah, P. a. (2001). Engineering out the risk for infection with urinary catheters. *Emerging Infectious Diseases*, 7(2), 342–7. doi:10.3201/eid0702.700342
- Mody, R., Joshi, S., & Chaney, W. (1995). Use of lectins as diagnostic and therapeutic tools for cancer. *Journal of Pharmacological and Toxicological Methods*, 33(1), 1–10. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7727802>
- Monds, R. D., & O'Toole, G. a. (2009). The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends in Microbiology*, 17(2), 73–87. doi:10.1016/j.tim.2008.11.001
- Otto, M. (2009). NIH Public Access. *Current Topic in Microbiology and Immunology*, 322, 207–228.
- Rosenfeld, R., Bangio, H., Gerwig, G. J., Rosenberg, R., Aloni, R., Cohen, Y., ... Maya, R. B.-Y. (2007). A lectin array-based methodology for the analysis of protein glycosylation. *Journal of Biochemical and Biophysical Methods*, 70(3), 415–26. doi:10.1016/j.jbbm.2006.09.008
- Schauer, R. (2009). Sialic acids as regulators of molecular and cellular interactions. *Current Opinion in Structural Biology*, 19, 507–514. doi:10.1016/j.sbi.2009.06.003
- Sharon, N., & Lis, H. (2002). How proteins bind carbohydrates: lessons from legume lectins. *Journal of Agricultural and Food Chemistry*, 50(22), 6586–91. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12381156>
- Shida, K., Misonou, Y., Korekane, H., Seki, Y., Noura, S., Ohue, M., ... Miyamoto, Y. (2009). Unusual accumulation of sulfated glycosphingolipids in colon cancer cells. *Glycobiology*, 19(9), 1018–33. doi:10.1093/glycob/cwp083
- Suzuki, O., & Abe, M. (2014). Galectin-1-mediated cell adhesion, invasion and cell death in human anaplastic large cell lymphoma: regulatory roles of cell surface glycans. *International Journal of Oncology*, 44(5), 1433–42. doi:10.3892/ijo.2014.2319
- Varki, A. (2007). Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. *Nature*, 446(7139), 1023–9. doi:10.1038/nature05816.
- Varki, A., Schauer, R. (2009). Sialic acids. In: Varki, A., Cummings, R., Esko, J. *et al.*, (eds). *Essentials of Glycobiology*. 2nd edn. Cold Spring Harbour (NY) Cold Spring Harbour Laboratory Press. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK1896/> [Accessed August 15, 2014].
- Vuong, C., Voyich, J. M., Fischer, E. R., Braughton, K. R., Whitney, A. R., Deleo, F. R., & Otto, M. (2004). Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cellular Microbiology*, 6(3), 269–275. doi:10.1111/j.1462-822.2004.00367.x.
- Yu, H., Chokhawala, H. a, Huang, S., & Chen, X. (2006). One-pot three-enzyme chemoenzymatic approach to the synthesis of sialosides containing natural and non-natural functionalities. *Nature Protocols*, 1(5), 2485–92. doi:10.1038/nprot.2006.401.

### How to cite this article:

Ekpiken Solomon Ekpiken, Lawrence B. Etim and Mercy Ekong. 2017. Modification of Glycan Expression in *Staphylococcus epidermidis* to Prevent Biofilm Formation. *Int.J.Curr.Microbiol.App.Sci*. 6(9): 3604-3613. doi: <https://doi.org/10.20546/ijcmas.2017.609.443>