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# Detection of methicillin-resistant staphylococcus aureus bacteria using liquid crystals

## *Sıvı kristaller kullanılarak metisiline dirençli staphylococcus aureus bakterisinin tespiti*

*Yazar(lar) (Author(s)): Emine KEMİKLİOĞLU<sup>1</sup>, Ebru Büşra TUNÇGÖVDE<sup>2</sup>*

*ORCID<sup>1</sup>: 0000-0002-7827-2423*

*ORCID<sup>2</sup>: 0000-0002-0167-765X*

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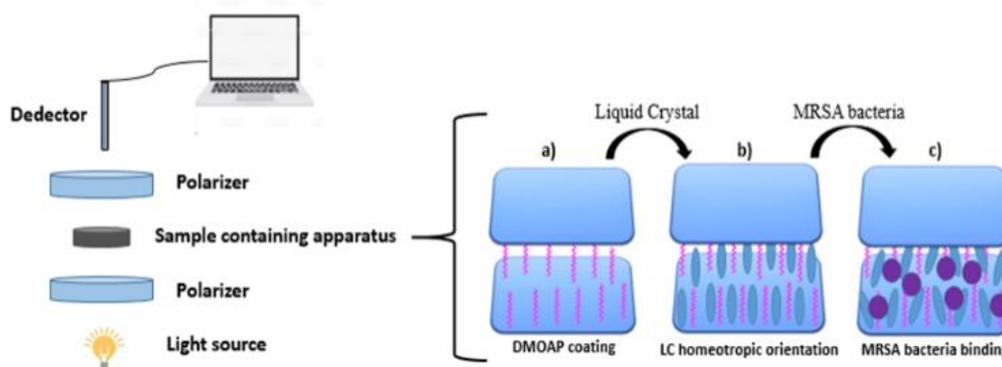
# Detection of Methicillin-Resistant Staphylococcus Aureus Bacteria Using Liquid Crystals

## Highlights

- ❖ Liquid crystals(LCs) are excellent biosensing products.
- ❖ MRSA bacteria cause severe infections which can lead to sepsis or death.
- ❖ MRSA bacteria was detected using an LC-based biosensing system.

## Graphical Abstract

In this study, a new biosensing system was developed to detect the MRSA bacteria by using the orientational responses of a nematic LC.



**Figure.** Detection principle of LC-based sensing procedure

## Aim

The aim of this study is to detect MRSA bacteria which prepared within the isotonic water and PBS by using the orientational responses of LC.

## Design & Methodology

Bacteria solutions were prepared in isotonic water and PBS. Then glass slides were coated with the alignment molecule DMOAP. The homeotropic orientation of the LC molecules was achieved by assembling LCs on the surface of the DMOAP-coated glass slides. After the bacteria solutions were diluted serially, the solutions were dropped on the LC surface. The changing of the LC orientation caused by bacteria binding was observed using a POM and reflection spectra were determined under a spectrometer.

## Originality

MRSA bacteria were detected for the first time by using the LC-based biosensing system.

## Findings

MRSA bacteria prepared within the isotonic water was detected ranging from the  $9.2 \times 10^3$  CFU/mL to  $9.2 \times 10^7$  CFU/mL concentration by using this sensing mechanism. Moreover, MRSA bacteria prepared in PBS was detected in the concentration range of  $7.1 \times 10^4$  CFU/mL to  $7.1 \times 10^8$  CFU/mL was detected with the help of this sensing technique.

## Conclusion

According to these results, this developed biosensing system can be effectively used in the detection of MRSA bacteria that cause serious diseases.

## Declaration of Ethical Standards

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

# Detection of Methicillin-Resistant Staphylococcus Aureus Bacteria Using Liquid Crystals

Research Article / Araştırma Makalesi

Emine KEMİKLİOĞLU<sup>1</sup>, Ebru Büşra TUNÇGÖVDE<sup>2\*</sup>

<sup>1</sup>Faculty of Engineering, Bioengineering Department, Manisa Celal Bayar University, Manisa, Türkiye

<sup>2</sup>Biotechnology Department, Ege University, Izmir, Türkiye

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## ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is an essential pathogen for public health and this bacteria commonly cause serious infectious in humans. In recent years, MRSA bacteria are detected by the bacterial culture and nucleic acid-based methods which are time-consuming and labor-intensive. In this study, a novel liquid crystal (LC)-based biosensing system was developed to overcome these limitations. The objective of this study was to detect the presence of MRSA bacteria which prepared within the isotonic water and phosphate buffer saline (PBS). In this system, the binding of MRSA bacteria to the dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride (DMOAP)-coated surface disrupted the orientation of LCs, triggering a transition from a homeotropic orientation to a random one. This transition in the orientation of the LCs was observed as a change from a dark optical LC image to a bright one under a polarized light microscope (POM) and the reflection values of LC molecules were determined by using a spectrometer. Through this sensing mechanism, MRSA bacteria prepared within the isotonic water was detected ranging from the  $9.2 \times 10^3$  CFU/mL to  $9.2 \times 10^7$  CFU/mL concentration. Furthermore, MRSA bacteria prepared in PBS was detected in the concentration range of  $7.1 \times 10^4$  CFU/mL to  $7.1 \times 10^8$  CFU/mL by using this system.

**Keywords:** Biosensor, MRSA bacteria, liquid crystal.

# Sıvı Kristaller Kullanılarak Metisiline Dirençli Staphylococcus Aureus Bakterisinin Tespiti

## ÖZ

Metisiline dirençli Staphylococcus aureus (MRSA), halk sağlığı için temel bir patojendir ve bu bakteri genellikle insanlarda ciddi enfeksiyonlara neden olur. Son yıllarda MRSA bakterileri, zaman alıcı ve emek yoğun olan bakteri kültür ve nükleik asit esaslı yöntemlerle tespit edilmektedir. Bu çalışmada, bu sınırlamaların üstesinden gelmek için yeni bir sıvı kristal tabanlı biyoalgılama sistemi geliştirilmiştir. Bu çalışmanın birincil amacı, izotonik su ve fosfat tamponlu salin (PBS) içinde hazırlanan MRSA bakterilerinin varlığını tespit etmektir. Bu sistemde, MRSA bakterilerinin dimetiloktadesil [3-(trimetoksisilil) propil] amonyum klorür (DMOAP) kaplı yüzeye bağlanması, LC'lerin oryantasyonunu bozarak homeotropik bir yönelimden rasgele olana geçişi tetikledi. LC'lerin yönelimindeki bu geçiş, polarize ışık mikroskobu (POM) altında karanlık bir optik LC görüntüsünden parlak bir görünüme bir değişim olarak gözlemlendi ve LC moleküllerinin yansıma değerleri bir spektrometre kullanılarak belirlendi. Bu algılama mekanizması sayesinde, izotonik su içerisinde hazırlanan MRSA bakterileri  $9,2 \times 10^3$  CFU/mL ila  $9,2 \times 10^7$  CFU/mL konsantrasyon aralığında tespit edildi. Ayrıca bu sistem kullanılarak  $7.1 \times 10^4$  CFU/mL ile  $7.1 \times 10^8$  CFU/mL konsantrasyon aralığında PBS'de hazırlanan MRSA bakterileri tespit edildi.

**Anahtar Kelimeler:** Biyosensör, MRSA bakterileri, sıvı kristal.

## 1. INTRODUCTION

LCs are stable anisotropic fluids that show characteristics between isotropic liquids and solid crystals [1]. Additionally, LCs are outstanding detection materials in which the intrinsic properties of long-range orientational behavior and short-range molecular interactions can be used to transform biological binding events into observable macroscopic optical signals [2]-[4]. Nowadays, they are considered to be one of the most promising materials for biological and chemical

biosensing due to their excellent properties [1]. This LCs biosensing system includes detection of toxins, antigens, antibodies, enzymes [5], and bacteria or viruses [6]. Staphylococcus aureus (S. aureus) is a gram-positive bacterium which has facultatively anaerobic [7]. S. aureus, which is naturally found in skin and nose flora, is known for its severe infective nature [8],[9]. This bacterium is responsible for many diseases from skin infection to mortal toxemia resulting in multi-organ failures, so S. aureus bacterium attracted much attention, connected with healthcare-related infections [7],[8]

\*Sorumlu Yazar (Corresponding Author)  
e-posta : emine.kemiklioglu@cbu.edu.tr

MRSA is known as one of the most common *S. aureus* resistant strains because MRSA easily spread over the hospitals through patients who have weakened immune systems [7],[10]-[12]. MRSA-related infections generally cause severe antibiotic-resistant diseases and resulting in a constant increment in death rate [7],[13]. Recent reports show that MRSA develops resistance rapidly and creates many essential problems in the clinic world [8]. This bacterium can survive in harsh environmental conditions, grow and produce virulence proteases, leading to staphylococcal food poisoning that causes serious gastrointestinal problems such as diarrhea, vomiting, and stomach pain [7]. Therefore, specific and viable diagnostic biosensing mechanisms are needed to detect MRSA bacteria and combating the spread of MRSA infections [7],[12]. Conventionally, the detection of MRSA was performed using the bacterial culture method [7],[14]. However, this process has some advantages such as time-consuming, labor-intensive [7]. These disadvantages delay the identification of bacteria in emergency and serious diseases such as toxemia, thereby restricting its practical application for fast diagnosis [7],[15].

Nowadays, methods that provide early diagnosis of besides important cancer diseases [16] and infections caused by viruses and bacteria are gaining importance day by day. Ataş et al. and Eren et al. the diagnostic systems they developed also contributed to new methods used in the detection of viruses [17,18]. Investigators are consistently looking forward to developing new biosensing mechanisms to detect bacteria. Regarding that, biosensing technologies are developing day by day as an alternative to conventional methods for the detection of MRSA.

In this study, a new sensing method was developed to detect the presence of MRSA bacteria which prepared within the isotonic water and PBS by using the orientational responses of a nematic LC. The detection principle of this biosensing mechanism is based on the change in orientation of the LC molecules caused by the binding of MRSA bacteria to the DMOAP coated surface. In this system, DMOAP molecules immobilized on glass slides form long alkyl chains (Figure 1a) which enable the LC molecules in a homeotropic direction as seen in Figure 1b. The binding of MRSA bacteria disrupts the LC's homeotropic orientation, thereby causes a change in the LCs orientation from the homeotropic to the random one as shown in Figure 1c. This change of orientation can be observed as a transition from a dark optical LC image to a brighter optical LC image under a POM, providing the detection of MRSA. Additionally, the reflection values of LC molecules were determined by using a spectrometer.



**Figure 1.** Detection principle of LC-based sensing procedure: a) coating of glass slides with DMOAP alignment agent, b) assembling of the LC molecules on the DMOAP coated glass slide, c) binding of MRSA bacteria to DMOAP-coated surface.

## 2. MATERIAL AND METHODS

Bacterial culture was supplied from Microbiologics and purchased in KWIK-STIK format. Each KWIK-STIK (0360U) unit contains a lyophilized microorganism pellet, an ampoule of hydrating fluid, and an inoculating swab. KWIK-STIK microorganisms are 3 passages or fewer from the reference culture. KWIK STIK bacterial collection is a product derived from American Type Culture Collection (ATCC®) cultures.

### 2.1. Methods

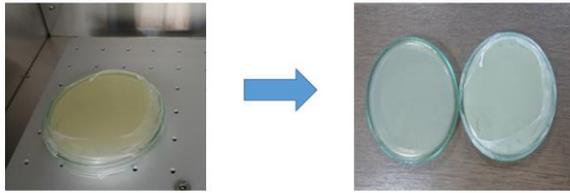
Infection by methicillin-resistant *Staphylococcus aureus* (MRSA) has been a global threat to public health for many years. Vancomycin has been one of the drugs used to treat MRSA infections for decades [19]. However, clinical isolates of *S. aureus* moderately and completely resistant to vancomycin have emerged over the past two decades and have fewer adverse public health effects than MRSA. Therefore, MRSA, which has become a global threat to public health, was chosen instead of vancomycin-resistant *S. aureus* (VRSA) in this study.

#### 2.1.1. Preparation of Bacteria Solution

As a first step, a solid medium was prepared for the bacterial inoculation procedure. 11.2 g of Nutrient agar was suspended in 400 mL of distilled water and boiled at 100°C to obtain a homogeneous mixture. Then, the medium which was sterilized by autoclaving for 20 min. at 1 atm pressure (at 121°C) was poured into petri dishes and left on a laminar flow bench overnight.

In the next step, liquid medium was prepared by adding 4 g tryptone, 4 g NaCl, 2 g yeast extract into 400 mL of distilled water. After stirring the mixture in a magnetic stirrer to obtain a homogeneous mixture, it was sterilized by autoclaving for 20 min. at 1 atm pressure and the temperature of 121°C.

After the solid and liquid medium were prepared, the bacterial inoculation procedure was carried out. Bacteria were inoculated on agar plates using swab by streaking method and incubated at 35°C for 30 hours as seen in Figure 2. The bacterial inoculation procedure was performed according to the KWIK-STIK format [20].



**Figure 2.** Bacteria inoculation process.

A solid medium was prepared with the same procedure for the bacteria counting step, and it was sterilized and poured into petri dishes as seen in Figure 3.



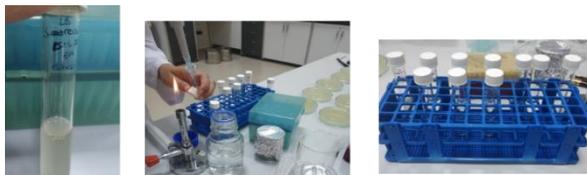
**Figure 3.** Solid medium preparation procedure for bacteria counting.

Subsequently, 5 mL of liquid medium was added to a screw cap test tube to prepare the bacteria stock solution. After sterilizing the loop over the fire, the bacteria were transferred from the solid medium to the test tube involving a 5 mL liquid medium by using the loop. The test tube was incubated overnight at 35°C and 100 rpm as shown in Figure 4.

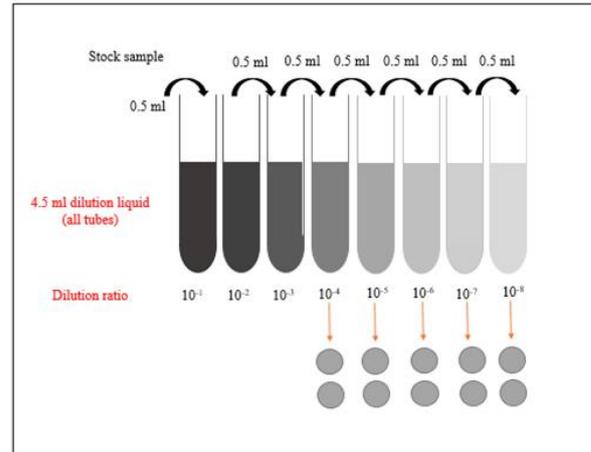


**Figure 4.** Inoculation process from solid medium to liquid medium.

The bacterial culture was serially diluted to obtain the bacterial colony forming units (CFU/ml) of the original stocks. 0.9% NaCl as a dilution liquid was prepared to be used in the dilution process. Initially, 4.5 mL of dilution liquid (Isotonic water) was put into each sterile tube. Then, 0.5 mL of liquid was taken from the stock solution and transferred to the 1st tube. At the end of the transfer to the 1st tube, the total volume in the 1st tube became 5 mL (0.5 mL sample+4.5 mL dilution liquid). At the end of this process, dilution was made 10 times (1/10). The same procedures were continued and dilution was made up to  $10^{-8}$  as seen in Figure 5 and Figure 6.



**Figure 5.** Dilution process of bacteria solution.



**Figure 6.** Demonstration of the gradual dilution process.

In the first stage of the spreading plate method, 0.1 mL of diluted samples were transferred to the agar petri dish. Immediately after sample transfer, the sample was homogeneously spread over the surface of the medium with the help of a T spatula. Agar plates were incubated at 35°C for 20 hours as shown in Figure 7.



**Figure 7.** Spreading plate method.

At the end of all the procedures, the colony counting process was carried out as shown in Figure 8. Counting process was performed according to the formulation given in Eq. 1 on two plates that were inoculated from the same dilution. Eq. 1 can be expressed as

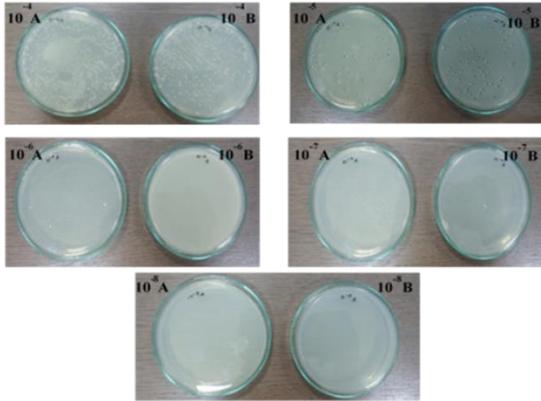
$$\text{Average number of colonies of two parallel} = \frac{100 + 84}{2} = 92 \frac{\text{CFU}}{\text{mL}} \quad (1)$$

The bacterial colony-forming unit (CFU/ml) value can be described by Eq. 2:

$$\frac{\text{CFU Average number of colonies of two parallel plates} \times \text{Dilution factor}}{\text{mL Volume transferred from dilution tube to the petri dish}} \quad (2)$$

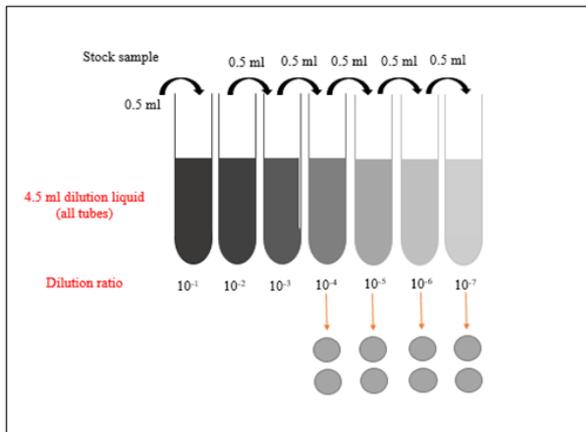
As a result of the calculation performed according to Eq. 2, the bacteria concentration was found to be

$$\frac{92 \times 10^5}{10^{-1}} = 9.2 \times 10^7 \frac{\text{CFU}}{\text{mL}}$$



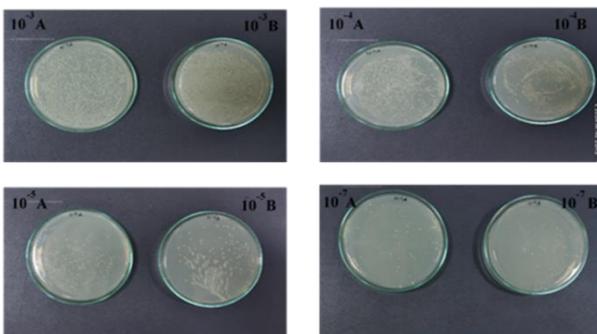
**Figure 8.** Images of bacterial colony plate.

The same steps in the bacterial solution prepared in isotonic water were also applied in bacterial solutions prepared with PBS. In the first step of the PBS preparation procedure, the powdered PBS was mixed in 1L of purified water until homogeneous (pH=7.4) (Including BSA). Then, PBS sterilization was performed with the edge of a 0.22  $\mu\text{m}$  syringe filter [21]. Dilution procedures for PBS were performed in the same way as for isotonic water. The stock solution was diluted to  $10^{-7}$  as shown in Figure 9.



**Figure 9.** Demonstration of the gradual dilution process.

The counting procedure was carried out on two plates that were inoculated from the same dilution and averaged (Figure x). The bacteria concentration was calculated as  $\frac{71 \times 10^6}{10^{-1}} = 7.1 \times 10^8 \frac{\text{CFU}}{\text{mL}}$



**Figure 10.** Images of bacterial colony plate.

## 2.1.2. Preparation of DMOAP-coated Glass Slides

The glass slides were thoroughly cleaned out prior to the DMOAP-coating step. Glass slides were sequentially washed in deionized (DI) water, Isopropyl Alcohol (IPA), and ethanol for 20 min. in an ultrasonic bath. Glass slides were dried in an incubator at 70°C for 20 min. Following the cleaning process, the glass slides were immersed in 1% (v/v) aqueous DMOAP solution for 15 min., then rinsed with DI water to remove excess DMOAP. The glass slides were then dried at 70°C for 15 min. and then allowed to cool to room temperature. After all these processes, the glass layers were coated with DMOAP and surface modification was achieved.

## 2.1.3. Fabrication of LC cells

As the first step to fabricate the LC cell, LC heated at 45°C was dropped onto the DMOAP coated glass slide. Then, *S. aureus* solutions prepared with isotonic water and PBS dropped onto this LC. LC cell was fabricated by sandwiching two glass slides (DMAOP-coated slide and DMOAP coated slide involving bacteria).

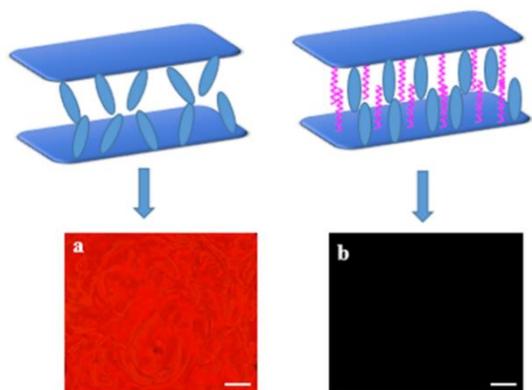
## 2.1.4. POM and Spectrometric Analysis

The changing of the LC orientation caused by bacteria binding was observed using a POM and reflection spectra were determined under a spectrometer.

## 3. RESULTS AND DISCUSSION

### 3.1. DMOAP- coating of Glass Slides

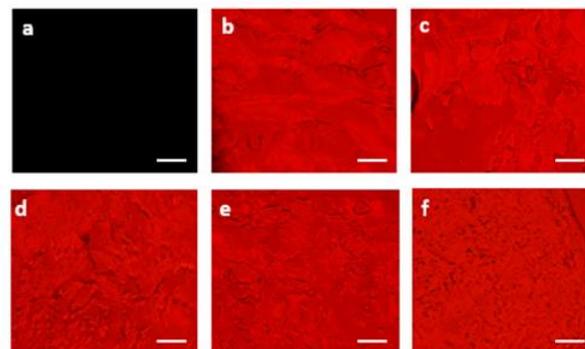
As the first step of the study, LC molecules were assembled on a DMOAP-coated glass slide to understand better the effect of bacteria concentration on LC orientation. Then, the optical image of the LC molecules under POM was observed. For that purpose, optical textures of LCs on DMOAP-coated and uncoated glass slides were both examined to recognize the effect of DMOAP coating by dropping 5  $\mu\text{l}$  of 5CB LC onto a DMOAP-coated glass slide [21,22]. Figure 11a showed the optical texture of the LCs on the non-coated DMOAP glass slide, whereas Figure 11b showed the optical texture of the LCs on DMOAP-coated glass slide. In Figure 11a, the optical texture of the LC was observed as bright images. This is because the LCs were randomly aligned on the glass surface. As a result, birefringence of light occurred on the surface of the LCs. In Figure 11b, the LC optical images were dark because LC molecules were induced into the homeotropic orientation via the DMOAP coating process. As a result, the transmitted light was not diffracted on the LC surface.



**Figure 11.** The POM images of LC a) non-coated DMOAP glass slide b) DMOAP-coated glass slide. Scale bar is 10 $\mu$ m.

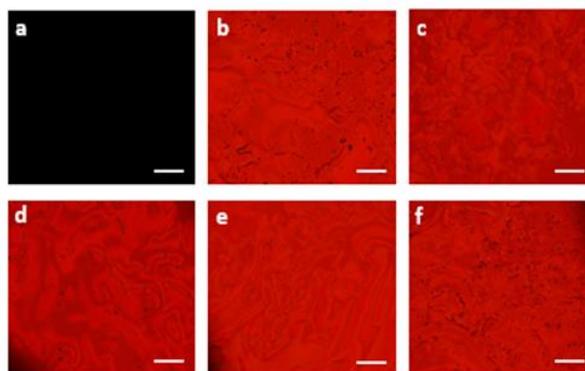
### 3.2. Detection of MRSA Bacteria Concentration

The primary aim of this study is to detect MRSA bacteria by disrupting the LC orientation. To verify the probability of detecting MRSA bacteria with this developed LC-based sensing method, DMOAP-coated glass slides were incubated in serial dilutions of MRSA bacteria solutions within the isotonic water ranging from 0 CFU/mL to  $9.2 \times 10^7$  CFU/mL. After the incubation process, the binding of MRSA bacteria to the DMOAP-coated surface was detected by observing the LCs optical responses under POM. We assumed here that the LCs homeotropic orientation would be disrupted after MRSA bacteria binding to DMOAP-coated surfaces that involve the LC molecules resulting in the obtainment of random orientation of the LCs. As seen in Figure 12, a graded change from dark optical images of LC to the bright optical images ones was observed with increasing concentrations of MRSA bacteria from 0 CFU/mL to  $9.2 \times 10^7$  CFU/mL. This indicated that the orientation of LC molecules changed from a homeotropic to a random one after binding of MRSA bacteria. The density of the bound MRSA bacteria molecules correlates with the extent of the corresponding LC molecules orientation changing. As shown in Figure 12a LCs had homeotropic alignment on the DMOAP coated surface in the absence of MRSA bacteria and the optical image of LC molecules under the microscope was uniform dark. As the concentration of MRSA bacteria increased from 0 CFU/mL to  $9.2 \times 10^7$  CFU/mL, increased bright optical images were recorded as seen in Figure 12 (a–f). The low MRSA bacteria concentration did not induce an apparent orientational changes of LCs because the amount of MRSA bacteria binding was too low as seen in Figure 12 (b-c). Figure 12 (d-f) demonstrated that there is an essential orientational transition in LC molecules due to the increase in the concentration of this MRSA bacteria.



**Figure 12.** POM images of samples including MRSA bacteria concentration within the isotonic water of a) 0 CFU/mL, b)  $9.2 \times 10^3$  CFU/mL, c)  $9.2 \times 10^4$  CFU/mL, d)  $9.2 \times 10^5$  CFU/mL, e)  $9.2 \times 10^6$  CFU/mL, f)  $9.2 \times 10^7$  CFU/mL. Scale bar is 10 $\mu$ m.

Immediately after the detection of MRSA bacteria which prepared in isotonic water, the presence of MRSA bacteria prepared in PBS was also detected. DMOAP-coated glass slides were incubated in serial dilutions of MRSA bacteria solutions within the PBS ranging from 0 CFU/mL to  $7.1 \times 10^8$  CFU/mL. The growth rate was high in samples prepared with PBS. Therefore, the bacterial concentrations had increased. LCs had a homeotropic orientation on the DMOAP coated surface in the absence of MRSA bacteria and the optical image of LC molecules under the microscope was dark as seen in Figure 13a. As the concentration of MRSA bacteria increased from 0 CFU/mL to  $7.1 \times 10^8$  CFU/mL, increased bright optical images were obtained under the POM as shown in Figure 13 (a–f). At the low bacterial concentration, the change in LC orientation was not distinct because the amount of MRSA bacterial binding events was low as seen in Figure 13 (b-c). Figure 13 (d-f) indicated that significant changes in the orientational transition were observed with increasing concentration of MRSA bacteria.



**Figure 13.** POM images of samples including MRSA bacteria concentration within the PBS of a) 0 CFU/mL, b)  $7.1 \times 10^4$  CFU/mL, c)  $7.1 \times 10^5$  CFU/mL, d)  $7.1 \times 10^6$  CFU/mL, e)  $7.1 \times 10^7$  CFU/mL, f)  $7.1 \times 10^8$  CFU/mL. Scale bar is 10 $\mu$ m.

As bacterial solutions prepared with isotonic water and PBS are compared, the growth rate of the solution prepared with PBS was higher. This because bacteria may have used the BSA protein in PBS as a nutrient source [20]. Therefore, the concentration of bacteria was higher. Since the concentrations of the samples prepared with PBS were higher, the binding event occurred more. As a result, the change in LC orientation was more distinct.

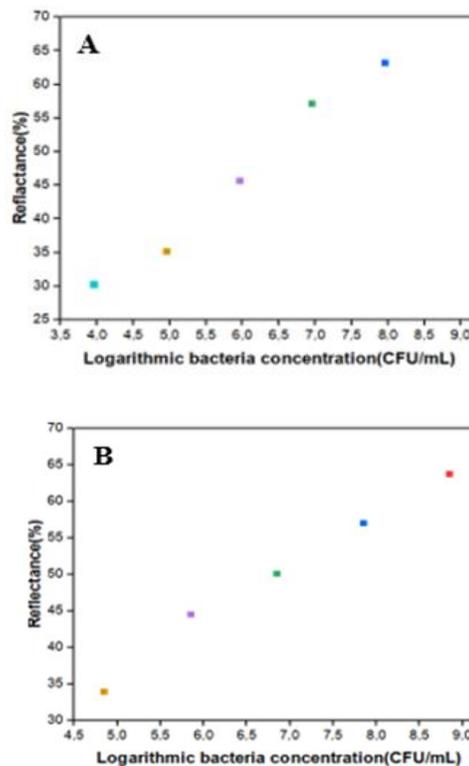
Following the POM analysis, the reflection changes caused by the LC orientation change depending on the amount of bound MRSA bacteria were determined at right angles with an Ocean Optics spectrometer (USB2000). These reflection spectrum were attained as a function of the increasing MRSA bacteria concentration as seen in Figure 14A and Figure 14B.

Figure 14A showed the changes in the percentage of reflectance as a result of increasing MRSA bacteria concentration within the isotonic water from  $9.2 \times 10^3$  CFU/mL to  $9.2 \times 10^7$  CFU/mL. Figure 14B showed the changes in the percentage of reflectance as a result of increasing MRSA bacteria concentration within the PBS from  $7.1 \times 10^4$  CFU/mL to  $7.1 \times 10^8$  CFU/mL. MRSA bacteria concentrations were plotted at a logarithmic scale.

As shown in Figure 14A, the reflection percentage in the presence of  $9.2 \times 10^3$  CFU/mL MRSA bacteria concentration was attained as 30.18%. When the MRSA bacteria concentration was raised to  $9.2 \times 10^4$  CFU/mL, the reflection value was obtained as 35.15%. Reflectance values were obtained as 45.61% and 57.09% for  $9.2 \times 10^5$  CFU/mL and  $9.2 \times 10^6$  CFU / mL MRSA bacteria concentrations, respectively. The reflection value of 63.17% was obtained at the highest MRSA bacteria concentration ( $9.2 \times 10^7$  CFU/mL).

As seen in Figure 14B, the reflection value of 33.91% at the lowest MRSA bacteria concentration ( $7.1 \times 10^4$  CFU/mL). When the MRSA bacteria concentration was increased to  $7.1 \times 10^5$  CFU/mL, the reflection value was obtained as 44.44%. Reflectance values were obtained as 50.05% and 56.97% for  $7.1 \times 10^6$  CFU/mL and  $7.1 \times 10^7$  CFU/mL MRSA bacteria concentrations, respectively. The reflection value of 63.70% was obtained at the highest MRSA bacteria concentration ( $7.1 \times 10^8$  CFU/mL).

When the concentration of MRSA bacteria was increased, optical texture of LCs distinctly changed depending on the binding of MRSA bacteria and this change in the LC orientation led to a essential change in the sample reflectance value as seen in Figure 14A and Figure 14B. As all results were examined, the optical change of the LCs which was observed under POM and the change in the reflection values which was determined with the help of the spectrometer were consistent.



**Figure 14.** Reflection spectra of LC molecules as a result of the increasing concentration of MRSA bacteria; A) prepared in isotonic water, B) prepared in PBS.

#### 4. CONCLUSION

As a summary, we have designed an LC-based biosensing system which can detect the presence of MRSA bacteria that prepared within the isotonic water and PBS. The binding of MRSA bacteria to the DMOAP-coated surface induced a transition from the homeotropic orientation to random orientation of LCs. This transition was visualised as a change from a dark to bright optical image of LC under the POM. MRSA bacteria prepared within the isotonic water was detected ranging from the  $9.2 \times 10^3$  CFU/mL to  $9.2 \times 10^7$  CFU/mL concentration by help of this sensing mechanism. Moreover, MRSA bacteria prepared in PBS was detected in the concentration range of  $7.1 \times 10^4$  CFU/mL to  $7.1 \times 10^8$  CFU/mL was detected with the help of this sensing technique. Additionally, spectrometric analysis gave supported that the homeotropic to random change in LCs orientation was caused by the binding of MRSA bacteria to the DMOAP-coated surface. As a result, our new biosensing system has shown up as a favorable and easier option to detect MRSA bacteria in a faster and effective way.

**DECLARATION OF ETHICAL STANDARDS**

The authors of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

**AUTHORS' CONTRIBUTIONS**

**Ebru Büşra TUNÇGÖVDE:** Carried out the experimental studies. Wrote the manuscript.

**Emine KEMİKLİOĞLU:** Designed the experimental study. Wrote the manuscript.

**CONFLICT OF INTEREST**

There is no conflict of interest in this study.

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