ORIGINAL ARTICLE

# Biofilm antimicrobial susceptibility pattern for selected antimicrobial agents against planktonic and sessile cells of clinical isolates of staphylococci using MICs, BICs and MBECs

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## Abstract

**Background and Objective:** The most important tool which helps bacteria to tolerate survival in unwanted conditions and resistance to new generations of antimicrobial agents is biofilm formation through quorum sensing. This study aimed to detect biofilm formation using study isolates of staphylococci quantitatively. Further, concerning minimal inhibitory concentration (MICs), biofilm inhibitory concentration (BICs), and minimum biofilm eradication concentrations (MBECs), to determine biofilm antimicrobial susceptibility test for selected antimicrobial agents against the study isolates. **Patients and Methods:** A total of 28 catheter urine specimens and wound swabs belonged to 32 patients admitted to Ramadi Teaching Hospital during the period from February to June 2009 were included in this study. Quantitative assay by a spectrophotometric method with ELISA reader was achieved. Planktonic and biofilm antimicrobial susceptibility tests for planktonic and sessile cells performed. **Results:** Out of 12 (37.5%) isolates of *Staphylococcus epidermidis* and 20 (62.5%) isolates of *Staphylococcus aureus*, biofilms were produced in 100% of all study isolates and produced biofilm actively in the glucose supplemented media. Our result revealed that MICs were 2.1 ± 1.2 µg/ml, 46.7 ± 18.6 µg/ml, and 3.25 ± 1.86 µg/ml for ciprofloxacin, piperacillin, and amikacin, respectively, against logarithmic phase planktonic cells of *Staphylococcus* spp. Furthermore, BICs and MBECs for the selected antimicrobial agents were reached ×50–100 folds higher than MICs to inhibit and eradicate staphylococcal biofilm. **Conclusion:** All study isolates of staphylococci produced biofilm quantitatively in glucose supplemented media. Furthermore, in biofilm antimicrobial susceptibility test, the biofilm producing isolate isolates of staphylococci required ×50–100 fold higher than those values for MICs for the same strains with the planktonic state to inhibit and remove bacterial biofilm from the surface of catheters.

**Key words:** Bacterial biofilm, indwelling bladder catheters, staphylococci

## INTRODUCTION

It is well recognized that biofilms are the aggregation of microorganisms embedded in a self-produced extracellular slime of exopolysaccharides in addition to proteins and some DNA. They can form on both animate and inanimate surfaces.[1] Staphylococcal biofilm is challenging to treat, often needing weeks of antimicrobial therapy. Severe infections, particularly those involving implants, require surgical removal of the implant for the successful cure. If not treated aggressively, the infections can recur and develop into chronic problems that require prolonged and even lifelong antimicrobial suppression.[2] Moreover, other processes may regulate detachment of organisms from the biofilm to transport *Staphylococcus aureus* to new sites of infection, another exciting area open to further investigation.

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The recognition that *S. aureus* forms biofilms on both native tissue and medical devices and implants has been a critical step toward understanding its pathogenesis. Furthermore, the clinicians are always facing the challenges against these severe infections. *Staphylococcus epidermidis* is a skin commensal bacterium which rarely causes disease without the presence of foreign material. This bacterium is attaching to explanted medical devices encased in slime.[3] This event provides early evidence that the biofilm concept applied to human disease.

With the passage of time, studies have also demonstrated *S. aureus* biofilms on intravascular catheters, explanted pacemaker leads, within the bone, and as vegetation is on heart valves.[4,5] Multidrug-resistant pathogens cause infections of indwelling medical devices in most cases. The implant surface provides the best environmental condition for microbial attachment and growth, with benefits to microorganisms including increased availability of nutrients, maturation, and potential for symbiotic relationships.[6] The researcher has shown that *S. aureus* can produce the same slime as *S. epidermidis*. The fact that *S. aureus*, unlike *S. epidermidis*, is virulent enough to routinely produce biofilms on native tissue alone without the presence of foreign material makes elucidating its particular biofilm physiology essential for devising strategies to combat its biofilms more effectively.[2]

This study has been undertaken to detect *in vitro* biofilm formation by *S. aureus* and *S. epidermidis* qualitatively by tube adhesion method and Foley catheter assay and quantitatively by spectrophotometric assay with ELISA reader under two experimental conditions. Further, in term of minimal inhibitory concentration (MICs) and sub-MICs, to determine antimicrobial susceptibility test for logarithmic phase planktonic cells of study isolates of staphylococci to selected antimicrobial agents. Furthermore, in the condition of biofilm inhibitory concentration (BICs) and minimum biofilm eradication concentrations (MBECs), biofilm antimicrobial susceptibility test for biofilm-producing isolates of staphylococci to selected antimicrobial agents.

## PATIENTS AND METHODS

A total of 28 catheter urine specimens and wound swabs obtained from 32 patients admitted to the Departments of Urology and Surgery in Ramadi General Hospital were studied during the period from February to June 2009. 21 (65.6%) were male and one (34.4%) was female with a male to female ratio of 1:1.9. The age of the patients is ranging from 19 to 76 years old with a mean value (31.14 years). Complete history had been taken directly from the patient. The information of the patients is arranging in an informative, detailed formula sheet. The clinical data regarding the distribution of isolates, type of specimens and type of infection are presenting in Table 1.

Under aseptic conditions, catheter specimens of urine obtained by withdrawing the sample with a syringe and needle from the junction area of a catheter. Catheter urine specimens are transporting to the laboratory with minimum delay. First, urine specimens were cultured as soon as possible by a semi-quantitative culture technique. Under aseptic conditions, a standardized of uncentrifuged urine was cultured on the sectors of nutrient, blood, and MacConkey agar plates. Then, overnight incubation for the plates at 37°C for 1 day was achieved. After incubation, the total number of colonies per ml was counted.

Isolates were well bacteriologically identified as staphylococci and differentiated either *S. aureus* or *S. epidermidis* depending on tube coagulase test and growing on coagulase Mannitol Salt Agar (HiMedia) and DNase agar (Oxoid) and confirmed by biochemical test.[7,8] The isolated microorganisms were stored in Brain Heart Infusion (BHI) broth medium containing 20% glycerol.

### Quantitative biofilm formation assays

#### *Spectrophotometric method*

Working cultures were prepared by inoculation on BHI agar in addition to Columbia Agar which supplemented with 5% blood and incubated aerobically at 37°C for 24 h. The cultures were used to prepare a bacterial suspension in sterile D.W. adjusted to a 0.5 McFarland turbidity standard. The yielded suspension is inoculating into a Brain Heart Infusion Broth, and the inoculated suspension is pouring into the wells of plastic microplates.[9]

The microtiter plate test which modified and improved was used for the quantification of biofilm. Sterile 96-well

**Table 1:** The distribution of clinical isolates of *Staphylococcus* spp. according to the type of specimens and type of infection

| Number (%) of isolates | Type of specimens | Type of infections |
|---|---|---|
| *Staphylococcus aureus* 20 (62.5%) | | |
| 13 (65) | Catheter urine | Catheterized urinary tract infection |
| 7 (35) | Wound swab | Diabetic foot infection |
| *Staphylococcus epidermidis* 12 (37.5%) | | |
| 7 (58.33) | Catheter urine | Catheterized urinary tract infection |
| 5 (41.67) | Wound swab | Diabetic foot infection |

flat-bottomed plastic microplates was filled with 250 µL of the BHI broth. Negative control wells contained the broth only. 20 µL of bacterial suspension was then added to each well. Incubation at 37°C for 24 h for the plates was achieved. After overnight incubation, the contents of each well were aspirated. Then, each well was washed for 3 times with 300 µL of sterile distilled water. The remaining attached bacteria were fixed with 200 µL of methanol per well. After that, the plates were emptied and left to air dry after waiting for 15 min. Then, staining technique for the plates was performed for 5 min with 160 µL per well with crystal violet used for Gram stain and rinsing off for the excessive stains was achieved by exposing the microplates for running tap water. After that, the plates were air-dried, the dye which was bound to the adherent cells was re-solubilized with 160 µL of 33% (v/v) of glacial acetic acid per each well. The optical density for each well was detected at 570 nm.[9,10]

### *Planktonic antimicrobial susceptibility test*

In this section of the study, antimicrobial susceptibility test for selected antimicrobial agents against logarithmic phase planktonic cells of *Staphylococcus* spp. was achieved. MICs and sub-MICs for ciprofloxacin, piperacillin, and amikacin were detected. This test was made according to the criteria mentioned by the National Committee for Clinical Laboratory Standard using an international quality isolate of *S. aureus* American Type Culture Collection (ATCC) 25923.[11,12]

### *Biofilm antimicrobial susceptibility test*

Detection, each of BIC and MBEC, performed antimicrobial susceptibility test for the sessile cell of *Staphylococcus* spp. The determination of different concentrations of antimicrobial agents used based on the MICs obtained by broth microdilution technique which was performed by logarithmic phase planktonic cells. Each study isolate was determined quantitatively by counting the cells of *Staphylococcus* spp. After 1-day incubation of 1 cm catheter segments with $10^6$ CFU/ml, the control for some colonies after vortexing catheter segments involved in broth media. It compares latterly to the total number of colonies for these isolates after exposure to the wanted concentration of antimicrobials. The concentrations of 1, 10, 50, 100, and 500 XMIC were selected to detect the BICs and MBECs.[13]

## RESULTS

The distribution of clinical isolates of *Staphylococcus* spp. was achieved according to the type of specimens and type of infection are represented in Table 1.

In the quantitative biofilm formation assay, the spectrophotometric method was achieved under two set of experimental conditions (with and without glucose). Our results showed that out of 20 (62.5%) isolates of *S. aureus*, all strains were produced biofilm actively (OD was >0.25) in the glucose supplemented media while 18 (90%) of them were produced biofilm actively in the glucose supplemented media and 2 (10%) isolates were weak biofilm producers in the glucose supplemented media. The most striking result is that no significant differences were detected in the optical densities at a wavelength of 570 nm with the presence and absence of glucose among isolates of *S. aureus* (0.536 ± 0.259) and (0.365 ± 0.17), respectively ($P = 0.03$) [Table 2].

Regarding *S. epidermidis*, our results showed that all *S. epidermidis* isolates which were submitted to this study produced biofilm actively in the glucose supplemented and in the absence of glucose 11 (91.67%) strains were produced biofilm actively and 1(8.33%) isolates were weak biofilm producers. As well as observed in *S. aureus* biofilm; furthermore, there are no significant differences detected in the values of optical density at 570 nm with the presence and absence of glucose among isolates of *S. epidermidis* (0.450 ± 0.18) and (0.387 ± 0.11), respectively ($P = 0.278$) [Table 3].

The study results revealed that MICs were 2.1 ± 1.2 µg/ml, 46.7 ± 18.6 µg/ml, and 3.25 ± 1.86 µg/ml for ciprofloxacin, piperacillin, and amikacin, respectively, in logarithmic phase planktonic cells of *Staphylococcus* spp. while the sub-MICs were 1.06 ± 0.62 µg/ml, 23.3 µg/ml ± 9.3, and 1.63 ± 0.93 µg/ml for the same antimicrobial agents, respectively [Table 4].

With regard to ciprofloxacin, the BIC for SA1, SA4, SA7, SA9, SA 10, SA11, SA18,SE5, SE6, SE8, SE10, and SE12 study isolates were 50 (100 XMIC), 200 (100 XMIC), 1000 (500 XMIC), 100 (100 XMIC), 1000 (500 XMIC), 1000 (500 XMIC), 100 (100 XMIC), 1000 (500 XMIC), 500 (500 XMIC), 2000 (500 XMIC), 1000 (500 XMIC), and 1000 (500 XMIC) µg/ml consequently. The

**Table 2:** The results of the spectrophotometric assay among 20 isolate of *Staphylococcus aureus* under two experimental conditions

| Experimental condition | $O.D_{570}$ m Mean±SD | Number biofilm producer (%) | SP (%) | WP (%) | NP (%) | *P* value | Number of biofilm producer by tube method (%) | Number of biofilm producer by catheter method (%) |
|---|---|---|---|---|---|---|---|---|
| With glucose | 0.536±0.259 | 20 (62.5) | 18 (90) | 2 (10) | - | 0.03 | 17 (85) | 19 (95) |
| Without glucose | 0.365±0.17 | 19 (95) | 16 (80) | 3 (15) | 1 (5) | | | |

**Table 3:** The results of the spectrophotometric assay among 12 isolates of *Staphylococcus epidermidis* submitted for two experimental conditions

| Experimental condition | $O.D_{570}$ m Mean±SD | Number biofilm producer (%) | SP (%) | WP (%) | NP (%) | *P* value | Number of biofilm producer by tube method (%) | Number of biofilm producer by catheter method (%) |
|---|---|---|---|---|---|---|---|---|
| With glucose | 0.450±0.18 | 12 (37.5) | 12 (100) | - | - | 0.278 | 10 (83.33) | 12 (100) |
| Without glucose | 0.387±0.11 | 12 (37.5) | 11 (91.67) | 1 (8.33) | - | | | |

**Table 4:** The results of minimal and subminimal inhibitory concentrations for antimicrobial agents against selected isolates of staphylococci

| Isolate number | Ciprofloxacin | | Piperacillin | | Amikacin | |
|---|---|---|---|---|---|---|
| | MIC µg/ml | Sub-MIC µg/ml | MIC µg/ml | Sub-MIC µg/ml | MIC µg/ml | Sub-MIC µg/ml |
| SA1 | 0.5 | 0.25 | 64 | 32 | 8 | 4 |
| SA 4 | 2 | 1 | 64 | 32 | 2 | 1 |
| SA 7 | 4 | 2 | 64 | 32 | 4 | 2 |
| SA 9 | 1 | 0.5 | 32 | 16 | 2 | 1 |
| SA10 | 2 | 1 | 16 | 8 | 4 | 2 |
| SA11 | 2 | 1 | 32 | 16 | 2 | 1 |
| SA12 | 1 | 0.5 | 64 | 32 | 4 | 2 |
| SE5 | 2 | 1 | 32 | 16 | 1 | 0.5 |
| SE6 | 1 | 0.5 | 32 | 16 | 2 | 1 |
| SE8 | 4 | 2 | 64 | 32 | 2 | 1 |
| SE10 | 4 | 2 | 64 | 32 | 4 | 2 |
| SE12 | 2 | 1 | 32 | 16 | 4 | 2 |
| *Staphylococcus aureus* 25922 | 0.5 | 0.25 | 0.25 | 0.125 | 1 | 0.5 |
| Mean±SD | 2.1±1.2 | 1.06±0.62 | 46.7±18.6 | 23.3±9.3 | 3.25±1.86 | 1.63±0.93 |

MIC: Minimal inhibitory concentration

above study isolates have been yielded that clearance of broth (biofilm inhibition) and reduced the viable count of bacterial biofilm from 35 × $10^5$, 33 × $10^5$, 30 × $10^4$, 40 × $10^5$, 30 × $10^2$, 65 × $10^5$, 70 × $10^4$ to 63 × $10^4$, 60 × $10^5$, 200 × $10^4$, 55 × $10^4$, and 73 × $10^5$, respectively. On the other hand, the concentrations of 500 (100 XMIC), 1000 (500 XMIC), 1000 (500 XMIC), 500 (500 XMIC), 1000 (500 XMIC), 2000 (1000 XMIC), 500 (500 XMIC), 2000 (1000 XMIC),1000 (1000 XMIC), 2000 (500 XMIC), 4000 (1000 XMIC), and 2000 (1000 XMIC) µg/ml were enough to eradicate Staphylococcus spp. biofilm from the catheters with high significant difference, MIC 2.1 ± 1.2), BICs (829.2 ± 678.4), and MBECs (1458.3 ± 1010.4) µg/ml [Table 5 and Figure 1].

Furthermore, our results revealed that the member of aminoglycosides and amikacin inhibits Staphylococcus biofilm at the following inhibitory concentrations: 800, 200, 400, 1000, 1000, 200, 2000, 100, 1000, 1000, 400, and 1000 µg/ml. It was reduced the viable counts for these isolates markedly as observed in Table 6, but these concentrations were not enough to remove bacterial adherence from the surfaces of catheters. Furthermore, the MBECs for amikacin

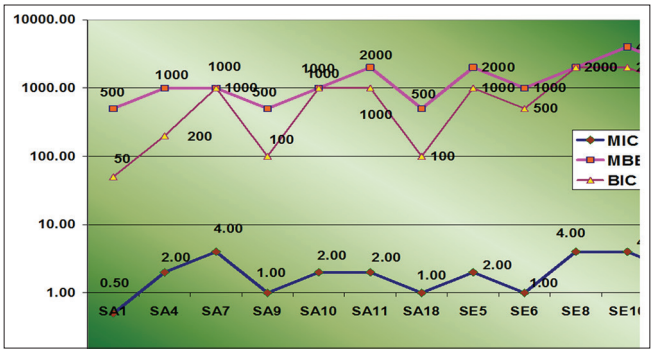


**Figure 1:** The comparison of susceptibility parameters for ciprofloxacin against logarithmic phase planktonic and sessile cells of study isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*

against these study isolates were 4000, 1000, 2000, 2000, 1000, 1000, 4000, 500, 2000, 2000, 4000, and 2000 µg/ml, respectively, and no colony appeared on the culture counting plate. Statistically, there is the highly significant difference ($P < 0.05$) with MICs (3.25 ± 1.86), BICs (758.3 ± 535.1), and MBECs (2125 ± 1245.4) µg/ml as represented in Table 6 and Figure 2.

**Table 5:** The biofilm inhibitory concentrations and minimal biofilm eradication concentration for ciprofloxacin against biofilm producing isolates of *Staphylococcus* spp.

| Isolate number | Colony count for control (CFU/ml) | BIC | | | MBEC | | |
|---|---|---|---|---|---|---|---|
| | | Number of folds higher than MIC | Concentration µg/ml | Colony count CFU/ml | Number of folds higher than MIC | Concentration µg/ml | Colony count CFU/ml |
| SA1 | $35\times10^5$ | ×100 | 50 | $41\times10^2$ | ×100 | 500 | Zero |
| SA4 | $33\times10^5$ | ×100 | 200 | 98 | ×500 | 1000 | Zero |
| SA7 | $30\times10^4$ | ×500 | 1000 | 75 | ×500 | 1000 | Zero |
| SA9 | $40\times10^5$ | ×100 | 100 | $33\times10^2$ | ×500 | 500 | 3 |
| SA10 | $30\times10^2$ | ×500 | 1000 | 100 | ×500 | 1000 | 5 |
| SA11 | $65\times10^5$ | ×500 | 1000 | $35\times10^2$ | ×1000 | 2000 | Zero |
| SA12 | $70\times10^4$ | ×100 | 100 | $40\times10^2$ | ×500 | 500 | Zero |
| SE5 | $63\times10^4$ | ×500 | 1000 | $30\times10^2$ | ×1000 | 2000 | Zero |
| SE6 | $60\times10^5$ | ×500 | 500 | 72 | ×1000 | 1000 | 2 |
| SE8 | $200\times10^4$ | ×500 | 2000 | 150 | ×500 | 2000 | zero |
| SE10 | $55\times10^4$ | ×500 | 2000 | 87 | ×1000 | 4000 | 5 |
| SE12 | $73\times10^5$ | ×500 | 1000 | $33\times10^2$ | ×1000 | 2000 | Zero |
| Mean±SD | | | 829.2±678.4 | | | 1458.3±1010.4 | |

BIC: Biofilm inhibitory concentration, MBEC: Minimal biofilm eradication concentration

**Table 6:** The biofilm inhibitory concentrations and minimal biofilm eradication concentration for amikacin against biofilm-producing isolates of *Staphylococcus* spp.

| Isolate number | Colony count for control (CFU/ml) | BIC | | | MBEC | | |
|---|---|---|---|---|---|---|---|
| | | Number of folds higher than MIC | Concentration µg/ml | Colony count CFU/ml | Number of folds higher than MIC | Concentration µg/ml | Colony count CFU/ml |
| SA1 | $112\times10^5$ | ×100 | 800 | $32\times10^2$ | ×500 | 4000 | Zero |
| SA4 | $100\times10^5$ | ×100 | 200 | $61\times10^2$ | ×500 | 1000 | Zero |
| SA7 | $35\times10^4$ | ×100 | 400 | 73 | ×500 | 2000 | Zero |
| SA9 | $83\times10^4$ | ×500 | 1000 | 85 | ×1000 | 2000 | 8 |
| SA10 | $30\times10^4$ | ×500 | 1000 | 100 | ×500 | 1000 | Zero |
| SA11 | $135\times10^5$ | ×100 | 200 | $41\times10^2$ | ×500 | 1000 | Zero |
| SA12 | $95\times10^5$ | ×500 | 2000 | $33\times10^2$ | ×1000 | 4000 | 3 |
| SE5 | $63\times10^4$ | ×100 | 100 | 53 | ×500 | 500 | Zero |
| SE6 | $32\times10^5$ | ×500 | 1000 | 47 | ×1000 | 2000 | Zero |
| SE8 | $200\times10^4$ | ×500 | 1000 | 180 | ×1000 | 2000 | 7 |
| SE10 | $180\times10^4$ | ×100 | 400 | 34 | ×1000 | 4000 | Zero |
| SE12 | $75\times10^5$ | ×500 | 1000 | $34\times10^2$ | ×1000 | 2000 | Zero |
| Mean±SD | | | 758.3±535.1 | | | 2125±1245.4 | |

BIC: Biofilm inhibitory concentration, MBEC: Minimal biofilm eradication concentration

Third-generation cephalosporins, piperacillin inhibited *Staphylococcus* spp. biofilm at the concentrations range between 100 and 500 folds higher than MIC at the following inhibitory concentrations: 6400, 6400, 32000, 3200, 8000, 3200, 6400, 3200, 3200, 32000, 6400, and 3200 µg/ml for the isolates as mentioned earlier of *Staphylococcus* spp., respectively. Piperacillin decreased the viable counts for these isolates to the levels at which inhibit the growth of bacteria but not eradicate it [Table 7]. Biofilm-producing isolates of *Staphylococcus* spp. were killed at the following cidal concentrations 64000, 32000, 32000, 32000, 16000, 16000, 16000, 16000, 32000, 64000, 64000, and 32000, respectively, with high significant difference $P < 0.05$ with MICs (46.7 ± 18.6), BICs (9466.7 ± 10666.9), and MBECs (34666.7 ± 19094.7) µg/ml [Table 7 and Figure 3].

## DISCUSSION

It is well realized that biofilm growth is governed by common factors of physical, biological, and chemical processes. Adherence of a cell to a substrate is termed adhesion, and cell-to-cell attachment is defined cohesion. It refers to the mechanisms affiliated to biofilm, which collectively determine the adhesive and cohesive properties of a biofilm, will exhibit.[14] The aggregation of microorganisms on a surface is represented by consequence of the following steps: (1) Adsorption of an organism on a collector surface. The second stage represented by an attachment, or the integration of the interface between an organism and a collector which leads to form polymer bridges between the organism and collector.

Further, the third step includes colonization (growth and division) of an organism on the collector's surface.[14] Since the process of biofilm formation starts with bacterial adhesion, one might expect that the physiology of adherent and planktonic (non-adherent) forms of *S. aureus* differs considerably. Investigators have studied *S. aureus* in the early stages of attachment to silicone surfaces and shown that differences in physiology between adherent and planktonic cells can be detected even before cellular aggregation and production of the polysaccharide matrix. The stationary phase of growth describes a stage where the number of viable bacterial cells is equal to that observed or detected with the number of declined or dead cells. At high cell load, a series of cell signaling mechanisms are employed by the biofilm, and this is collectively termed quorum sensing.[15] Quorum sensing describes a process where many auto-inducers (chemical and peptide signals in high concentrations, e.g., homoserine lactones) are used to stimulate gene expression of alginates enzymatic and mechanical processors, which composes a fundamental part of the extracellular matrix. Of particular note is a significant increase in antimicrobial resistance evident within 2 h of initial adhesion and increasing over 7 days. Some of the resistance, particularly to the cell wall active β lactam and glycopeptide antimicrobials, can be attributed to the decreased growth rate of the adherent bacteria. Taken together, these differences in physiology between the adherent and planktonic *S. aureus* support the existence of two different phenotypes: Biofilm and planktonic.[15,16]

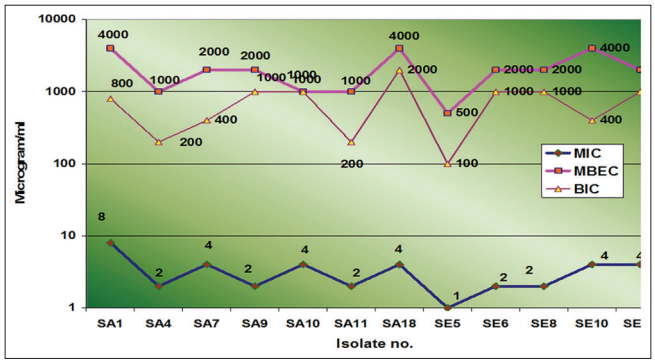


**Figure 2:** Differentiation of susceptibility tools for amikacin against logarithmic phase planktonic and sessile (adherent) cells of study isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*

With regard to the antimicrobial susceptibility test for planktonic cells, our result showed that MICs were 2.1 ±1.2 µg/ml, 46.7 ± 18.6 µg/ml, and 3.25 ± 1.86 µg/ml for ciprofloxacin, piperacillin, and amikacin, respectively, against logarithmic phase planktonic cells of *Staphylococcus* spp. while the sub-MICs were 1.06 ± 0.62 µg/ml, 23.3+9.3, µg/ml, and 1.63 ± 0.93 µg/ml. Routinely, the

**Table 7:** The biofilm inhibitory concentrations and minimal biofilm eradication concentration for piperacillin against biofilm-producing isolates of *Staphylococcus* spp.

| Isolate number | Colony count for control (CFU/ml) | BIC | | | MBEC | | |
|---|---|---|---|---|---|---|---|
| | | Number of folds higher than MIC | Concentration µg/ml | Colony count CFU/ml | Number of folds higher than MIC | Concentration µg/ml | Colony countCFU/ml |
| SA1 | $33\times10^5$ | ×100 | 6400 | 100 | ×1000 | 64000 | Zero |
| SA 4 | $38\times10^5$ | ×100 | 6400 | 95 | ×500 | 32000 | Zero |
| SA 7 | $35\times10^4$ | ×500 | 32000 | 33 | ×500 | 32000 | Zero |
| SA 9 | $116\times10^5$ | ×100 | 3200 | $37\times10^2$ | ×1000 | 32000 | 8 |
| SA10 | $40\times10^5$ | ×500 | 8000 | 69 | ×1000 | 16000 | Zero |
| SA11 | $53\times10^5$ | ×100 | 3200 | $41\times10^2$ | ×1000 | 16000 | Zero |
| SA12 | $67\times10^5$ | ×100 | 6400 | 85 | ×500 | 16000 | Zero |
| SE5 | $63\times10^4$ | ×100 | 3200 | 37 | ×500 | 16000 | 9 |
| SE6 | $88\times10^5$ | ×100 | 3200 | $39\times10^2$ | ×1000 | 32000 | Zero |
| SE8 | $200\times10^4$ | ×500 | 32000 | 66 | ×1000 | 64000 | Zero |
| SE10 | $79\times10^5$ | ×100 | 6400 | $33\times10^2$ | ×1000 | 64000 | 7 |
| SE12 | $81\times10^5$ | ×100 | 3200 | 75 | ×1000 | 32000 | Zero |
| Mean±SD | | | 9466.7±10666.9 | | | 34666.7±19094.7 | |

BIC: Biofilm inhibitory concentration, MBEC: Minimal biofilm eradication concentration

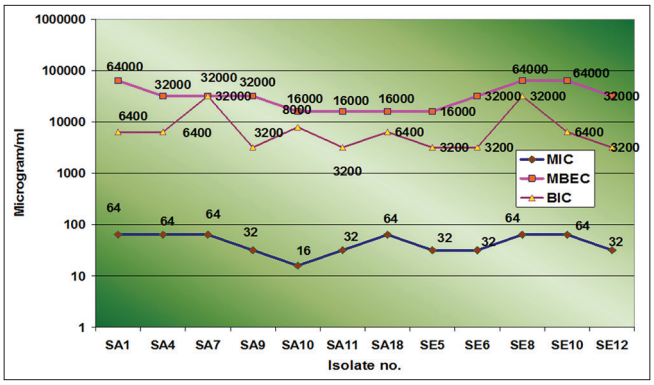


**Figure 3:** The juxtaposition of susceptibility parameters for piperacillin against logarithmic phase planktonic and adherent cells of study isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*

laboratory man has been detected the efficacy and potency of selected antimicrobial agents by determining the lowest concentration of drugs which inhibits microbial growth after 24 h of incubation.[17] The assigned measurements are performed on freely floating; planktonic laboratory phenotypes in all well established and reference diagnostic laboratories. The assays are measure only the concentration of chemotherapeutic agents which required inhibiting the growth or killing planktonic state bacteria. The concentration necessary for some antimicrobial agents to kill sessile or adherent bacteria may be higher than a 1000 times that needed for killing planktonic state of bacteria of the same strain.[18] Therefore, the use of standard laboratory planktonic bacteria for selection of chemotherapeutics may be inappropriate under some circumstances.[16] In this study, the inhibitory concentrations for specific antimicrobial agents (ciprofloxacin, cefotaxime, and amikacin) against logarithmic phase-planktonic cells were determined using macrobroth dilution technique with the presence of international standard isolates (*S. aureus* ATCC 25923). The MICs were 2.1 ± 1.2 µg/ml, 46.7 ± 18.6 µg/ml, and 3.25 ± 1.86 µg/ml for the above-mentioned antimicrobial agents, respectively.

In spite of MIC assay remain the standard golden test and the best way to select potentially the active antimicrobials, MBEC was created for the rapid and more accurate antimicrobial susceptibility assay for bacterial biofilm in the expectation that the MBEC would be more reliable for detection of clinically effective antimicrobials in such infections.[17] Thus, our study has been aimed to clarify which concentrations are required to remove permanently pseudomonal biofilm which was stuck on an indwelling urinary catheter depending on MICs and sub-MICs for study antimicrobial agents.

Antimicrobial susceptibility pattern for the sessile cell of *Staphylococcus* spp. was performed by detection the minimal biofilm eradication concentration (MBEC). The computation of a variety of antimicrobials concentration used depended on the minimum inhibitory concentrations obtained by broth microdilution technique achieved against logarithmic phase planktonic cells of *S. aureus*. The study result showed that the biofilm cells were needed 50-100 times the MIC values for ciprofloxacin detected for the same isolates in the logarithmic phase of planktonic cells with MIC mean (2.1 ± 1.2) µg/ml and MBEC mean (1.06 ±0.62) µg/ml with the highly considerable difference ($P < 0.05$). Yassien and Khardori[18] documented that the high concentrations of fluoroquinolones were used to treat the performed biofilms. This may be due to that: First, these concentrations would be expected to reach the biofilms when therapeutic doses of fluoroquinolone (ciprofloxacin is one of the fluoroquinolones) are spread through the vascular catheters.

Further, second using such high concentrations would minimize the exposure of very large inoculum of bacteria in the biofilms to sub-inhibitory concentrations of the fluoroquinolones. The effect is concentration dependent. On the other hand, *in vitro* effect of ciprofloxacin for microbial biofilm in dental caries, Al-Ouqaili *et al*.[19] concluded that that the biofilm-producing isolates of oral streptococci were required 10–50 XMICs of ciprofloxacin to inhibit bacterial biofilm and 100–1000 XMICs to remove of bacterial biofilm in patients with dental caries.

It is well reported that the mode of action of ciprofloxacin in the biofilm eradication is included: First, electrostatic interference with the adhesion of bacteria and/or glycocalyx to the substrate; second, the activation or release of enzymes to disrupt the exopolysaccharide (glycocalyx) in the biofilm; and third, inhibition of the formation of new glycocalyx, the observed effects of the ciprofloxacin may improve the pharmacodynamics effect of the antibiotics used to manage and treat prosthetic device associated infections.[16] The molecular basis and genetic background for biofilm formation in staphylococci are multifaceted. The capability for forming a biofilm affords at least two characters: The adherence of cells to a surface and accumulation to create multilayered cell clusters. A trademark is the production of the slimy substance PIA, a polysaccharide which composed of beta-1, 6-linked N-acetylglucosamines with partly deacetylated residues, in which the cells are embedded and protected against the host's immune defense and antibiotic treatment.[20]

Furthermore, regarding piperacillin, the study yielded that the logarithmic phase of sessile cells was required 50–100 times the MIC with mean (46.7 ± 18.6) µg/ml and MBEC mean (23.3 ± 9.3) µg/ml. Mah *et al*.[21] explained the genetic basis for biofilm antibiotic resistance by identifying a chromosomal locus called "ndvB" which is requiring for the synthesis of periplasmic glucans. These periplasmic glucans interact physically with antimicrobials, and the formed glucose polymers may prevent antimicrobials from reaching their sites of action by remaining and capturing these antimicrobial agents in the periplasm. Further, the

same researchers indicated biofilm themselves are not only merely a physical barrier for drug penetration but also the microorganisms within these microbial population creates an unusual manipulation of the bacterial cell to resist the action of antimicrobial agents.

Further, regarding amikacin, the study revealed that the sessile or adherent cells were needed 50–100 fold greater than MICs detected for study isolates of *Pseudomonas aeruginosa* in logarithmic phase planktonic cells with MIC mean (3.25 ± 1.86) µg/ml and MBEC mean (1.63 ± 0.93) µg/ml high significant difference ($P < 0.0001$). Anderl *et al*.[22] documented that biofilm infection associated resistance is probably not encoded by genetic expression or may be due to the selection of resistant bacterial populations. Therefore, the resistance phenomenon disappears when bacteria are removing from the catheter. The cause of resistance is mainly due to the physiological state of the individual cells rather than a function of biofilm formation. Biofilm-producing bacteria will grow more slowly than planktonic bacteria as a result of the adhesion process rather than depletion of the nutrients. Furthermore, it has been hypothesized that only the surface of biofilm layers faced to a lethal dose of the antimicrobial agent, due to a reaction–diffusion barrier which limits the transportation of the antibiotic to the biofilm.

Two main aspects of the biofilm phenotype are particularly relevant to clinical disease. The first feature is the dramatic increase in antimicrobial resistance. For *S. aureus* biofilms, the *in vitro* minimum bactericidal concentrations of most antimicrobial agent's average 2–1000-fold higher than their identical planktonic forms.[23,24] The second feature of the biofilm phenotype is the inability of the host immune cells to kill the biofilm bacteria. For example, investigators recently showed that leukocytes were able to bind and penetrate an *S. aureus* biofilm, but were unable to phagocytose any of the bacteria within the biofilm structure.[25] These features explain why antimicrobial therapy alone for biofilm-related infections frequently fails. Successful surgical debridement or replacement of an implant provides the mechanism for the mechanical removal of the bacteria.

The conventional approach to management of *S. aureus* biofilm infections remains antimicrobial therapy and proper surgical resection and debridement. However, the emergence of new antimicrobial resistance typified by vancomycin-intermediate and resistant *S. aureus* strains is a driving force for developing alternative approaches, among them the surface modification of intravascular catheters with antimicrobial agents. While there have been indications that these catheters reduce *S. aureus* infections, their efficacy remains controversial.[26]

The study suggested that all study isolates of Staphylococci were produced biofilm qualitatively by a spectrophotometric method with ELISA reader which was an accurate method for detecting the bacterial adherence to the surface of microtiter plates. Further, under two set of experimental conditions, presence, and absence of glucose in the culture media, glucose serves as a potent enhancer for biofilm production for the study isolates. Further, in antibiogram assay of bacterial biofilm, the biofilm-producing staphylococci were required ×50–100 fold higher than MIC for the same strains at planktonic state to inhibit and eradicate bacterial biofilm from the surface of catheters.

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