Slime Forming Staphylococcus epidermidis Isolated From Orthopedic Prosthesis Infections And Its Sensitivity To Antibiotics

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Staphylococcus epidermidis (S.epidermidis) is a frequent cause of infections of indwelling medical devices especially those with orthopedic implants. S.epidermidis grows on medical devices as an adherent biofilm consisting of cells enmeshed in a sticky, extracellular slime that is firmly attached to the underlying surface. The slim matrix makes S.epidermidis biofilm highly resistant to antibiotics and host defenses and nearly impossible to eradicate. The aim of the study is to determine importance of slime formation in S. epidermidis orthopedic prosthesis infections and to investigate if slime formation has an effect on its antibiotics sensitivity. 80 coagulase negative staphylococcus strains (CoNS) were isolated from 200 tissue specimens of patients with orthopedic prothesis infections. Out of these 80 CoNS, 52 (65%) strains were S.epidermidis. Isolated S. epidermidis were plated on Congo red agar and subjected to PCR to detect icaA & icaD genes to identify and confirm slime producing strains respectively. All biofilm producing strains were subjected to MIC and MBEC using Calgary Biofilm Device(CBD). 36 (69%) S. epidermidis strains were slime (biofilm) producers and 16 (31%) strains were non slime (non biofilm) producers by CRA, while by PCR 39(75%) strains of S. epidermidis were biofilm producers and 13 (25%) strains were non biofilm producers. The results also revealed that the minimal biofilm eradication concentrations (MBECs) were higher than the corresponding conventionally determined MICs for all antibiotics tested. MIC 50 and MBEC 50 for vancomycin, were 2 µg/ml versus 8 µg/ml, gentamycin, 1 µg/ml versus 32 µg/ml, oxacillin, 4 µg/ml versus 16 µg/ml, erythromycin, 8 µg/ml versus 64 µg/ml, ciprofloxacin, 0.5 µg/ml versus 2 µg/ml and cephalothin 4 µg/ml versus 16 µg/ml. MIC90 and MBEC90 for vancomycin were 4 µg/ml versus 16 µg/ml, gentamycin, 16 µg/ml versus 128 µg/ml, oxacillin, 8 µg/ml versus 128 µg/ml, erythromycin, 16 µg/ml versus 128 µg/ml, ciprofloxacin, 4 µg/ml versus 8 µg/ml and cephalothin 32 µg/ml and 128 µg/ml. The results of the present study confirm that ica genes can be considered a virulence marker in the pathogenesis of implant associated orthopedic infection by S. epidermidis. This study also demonstrates marked differences between the results of susceptibility testing performed according to standard NCCLS guidelines and testing based on biofilm susceptibility testing.

INTRODUCTION

The growing use of indwelling medical devices such as intravascular catheters, artificial heart valve and orthopedic implants has resulted in a significant increase in the number of device related infections. Coagulase negative staphylococci especially Staphylococcus epidermidis (S epidermidis) are recognized as a major cause of these infections especially in orthopedics (41). Staphylococcus epidermidis has the ability to colonize the artificial surface in a self-generated viscous biofilm composed of polysaccharides called slime (16). This phenomena is now regularly referred to as biofilm formation. Biofilm formation may be divided into two phases. Frist, a complex process involving multiple physicochemical, protein and polysaccaride factors leads to primary attachment of bacterial cells to a polymer surface (25). Second, the attached bacteria proliferate and accumulate in a multilayered biofilm known as sessile bacteria(20). The structure of this polysaccharide is unique and according to its function in intercellular aggregation, it was referred to as polysaccharide intercellular adhesion (PIA) and is essential for biofilm accumulation of most clinical S. epidermidis (15). Formation of biofilm has been claimed to be the mechanism of producing infections associated with biomaterials (28,36). Recently the genetic control of the biofilm formation has been determined, where the synthesis of capsular polysaccharide is mediated by ica operon (14,8,27). Sole expression of icaA induces only low trasferase enzymatic activity, but co-expression of icaA with icaD significantly increases the activity and is related to the phenotypic expression of the capsular polysaccharide (14). Biofilm formation is a major concern in nosocomial infections because it protects microorganisms from phagocytosis and antibiotics agents leading to chronic infection and sepsis particularly in immuno-compromised patients.

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(9). The difficulty in eradication of a chronic infection associated with microcolony and biofilm formation (sessile bacteria) lies in the fact that biofilm bacteria are able to resist higher antibiotic concentrations than that bacteria in suspension (Planktonic bacteria). So, The aim of this study is to determine the importance of biofilm formation in *S. epidermidis* orthopedic prosthesis infections and to investigate the susceptibility of the biofilm forming strains to the antibiotics.

**MATERIALS AND METHODS**

This study was done in the Microbiology and Orthopedics departments, Faculty of Medicine, Zagazig University. 200 infected tissue specimens were obtained from 200 patients with infected orthopedic prosthesis (Table:1). These specimens were subjected to routine standard methods for isolation of the responsible organisms(1,5). Isolated Staphylococci were subjected to Api Staph (bioMe´rieux) and Coagulase test(5). Isolates were kept frozen at -20 ºC in trypticase soy broth containing 20 % gylecrol (vol/vol).

Table 1: Origin of the isolates.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total joint replacements (hip or knee)</td>
<td>76(40)</td>
</tr>
<tr>
<td>Plates, pins, screws (for fixation of tibia, femer, ulna, etc)</td>
<td>61(21)</td>
</tr>
<tr>
<td>Ligaments or tendon reconstruction (comprising suture threads)</td>
<td>46(12)</td>
</tr>
<tr>
<td>Others: associations of 2 or more prosthesis types</td>
<td>17(7)</td>
</tr>
<tr>
<td>Total</td>
<td>200(80)</td>
</tr>
</tbody>
</table>

Number in parenthesis indicates coagulase negative strains.

**Slime production detection**

**Phenotypic screening:** biofilm producing strains were identified by culture of *S. epidermidis* strains on Congo red agar (CRA) plates. CRA is prepared by adding 0.8 g Congo red (Sigma) and 36g saccharose (Sigma) to 1L of brain heart infusion agar (Oxoid). The plates were incubated at 37 ºC for 24 h. and then over-night at room temperature. Biofilm producing strains form black colonies while non biofilm producing strains develop red colonies (12)

**Genotypic confirmation:**

**Bacterial DNA extraction:** DNA was extracted using the diatheva bacterial DNA extraction Kit (diatheva, Italy). Briefly, one ml of trypticase soy broth containing bacteria is first concentrated by centrifugation. The pelleted bacterial cells are lysed with lysozyme and enzymatically digested with Proteinase K. The lysate is then mixed with the SiliMag beads, which capture the DNA in presence of the specific binding buffer. The DNA remains bound while a rapid washing step removes contaminating bacterial components. Finally, the elution step removes the DNA from magnetic particles by adding 100 µl double-distilled water to the SiliMag beads-DNA complex and incubating for 10 min at 65°C. The SiliMag beads were immobilized on a magnetic stand and the supernatant was collected. DNA was stored at -20°C.

**DNA amplification:** Multiplex PCR was used for detection of *icaA* and *icaD*, the genes responsible for biofilm formation. A 3.5-µl aliquot of extracted DNA was processed in a 50 µl reaction volume. FailSafe PCR Enzyme Mix and FailSafe PCR 2X PreMix (Epicentre, USA) were used in a master mix formed of 19 µl sterile water, 0.5 µl each primer (0.5 µM final concentration), 3.5 µl template DNA, 0.5 µl FailSafe PCR Enzyme Mix (1.25 Units) and 25 µl of FailSafe PCR 2X PreMix. The primers for *icaA* were 5’-ACA GTC GCT ACG AAA AGA AA-3’ and 5’-GGA AAT GCC ATA ATG ACA AC-3’. The amplified product is 103bp. The primers for *icaD* were 5’-ATG GTC AAG CCC AGA CAG ACA GAG -3’ and 5’-CGT GTT TTC AAC ATT TAA TGC AA-3’ (3). The amplified product is 198bp. Amplifications were carried out in a thermal cycler (Perkin-Elmer cetus type (480). Forty amplification cycles of 1min. at 94°C, 1 min. at 60°C, and 2 min at 72°C. After the last cycle, samples were incubated for 10 min at 72°C. The products of amplification were checked by electrophoresis in 1.5% agarose gel at 100v. The bands were visualized by staining with ethidium bromide 10 mg/ml comparing it to bands of molecular weight marker the Hae digest of ØX174 (Finnzyme) which give bands ranging from 72-1353bp.
Minimum biofilm eradication concentration (MBEC):
Biofilm formation and measurement of antimicrobial sensitivity of bacterial biofilm were performed on the Calgary Biofilm Device (CBD) (MBEC Biofilm Technologies, Calgary, Alberta) according to previously described methods (4, 30, 33). The device features a microtiter plate lid with 96 pegs or projections distributed on the lid. Each peg provided the surface for bacteria to adhere, colonize and form a uniform biofilm. The pegs fit precisely into the wells of a standard 96-well microtiter plate. The lid was used in conjunction with special troughs for growing of bacteria, washing, and incubation.

Biofilm formation on the Calgary Biofilm Device: 4 ml tryptic soy broth (TSB) was put in the trough. The trough was inoculated with 100 µl of 0.5 McFarland suspension of the organism obtained from colonies selected from tryptic soy agar (TSA). The pegged lid was placed over the troughs and the unit incubated on a rocker, 10 rpm (2.5 X g) at 37°C and 95% relative humidity. The pegs were colonized for 20 h (30). The assessment of biofilm was determined by breaking several pegs from various points on the lid. The removed pegs were placed in microfuge tubes containing 200 µL of TSB, sonicated for 5 min and plate counts of viable bacterial cells were performed on TSA.

Antibiotic preparation: 6 antibiotics belong to the following group were used: aminoglycosides (gentamicin sulfate), penicillilnase resistant penicillin (oxacillin), macrolides (erythromycin), cephalosporins (cephalothin), glycopeptides (vancomycin) and quinolones (ciprofloxacin). Ciprofloxacin from Bayer (Barcelona, Spain) and the remaining antibiotics were from Sigma (St Louis, MO, USA), were prepared as stock solutions of 6.200 µg/ml and the stock solutions were stored at 70°C. Working solutions were prepared in Muller-Hinton broth (MHB) at a concentration of 1.024 µg/ml and from these working solutions serial twofold dilutions were made in MHB in the wells of the 96-well plate.

Biofilm susceptibility testing: Biofilms were formed on the lid of the CBD as described above and then transferred to a standard 96-well plate in which dilutions of the specified antibiotics were prepared in 100 µl MHB. Antibiotic plates were incubated overnight at 35°C, after which the lid was removed, rinsed in phosphate-buffered saline and placed in a second 96-well plate containing MHB. The biofilm was removed from the CBD lid by sonication as described above, a new plate cover was added and the viability of the biofilm was determined after 24 h of incubation at 35°C by obtaining plate counts. The minimal biofilm eradication concentration (MBEC) was defined as the minimal concentration of antibiotic required to eradicate the biofilm (4, 33, 35).

MIC assays: The minimal inhibitory concentration (MIC), which represents the concentration of antibiotic required to inhibit growth of a planktonic bacterial population, was determined using the CBD. The MIC was determined from the bacteria that were shed from the pegs of the CBD when it was placed in the differing concentrations of antibiotics. The MIC values obtained using the CBD are similar to those obtained using the National Committee for Clinical Laboratory Standards (NCCLS) procedure (28).

RESULTS

Isolated organisms
Out of the 200 specimens, 52 S epidermidis, 28 (40 %) coagulase negative staphylococci other than S epidermidis , 43(20%) Staphaureus, 22 (10%) streptcoccus spp, 20 Gram negative bacilli,15 enterococci, and 7 candida spp were isolated (table: 2).

Table (2) shows isolated organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S epidermidis</td>
<td>52 (28)</td>
</tr>
<tr>
<td>Coagulase negative staphylococci</td>
<td>28 (15)</td>
</tr>
<tr>
<td>Staphaureus</td>
<td>43 (23)</td>
</tr>
<tr>
<td>Streptcoccus spp</td>
<td>22 (12)</td>
</tr>
<tr>
<td>Gram negative bacilli</td>
<td>20 (11)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>15 (8)</td>
</tr>
<tr>
<td>Candida spp</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>187 (100)</td>
</tr>
</tbody>
</table>

Detection of Slime (biofilm)-producing phenotype of S.epidermidis by CRP plate test:
36/52 (69 %) of S. epidermidis isolates were proved to be biofilm producing as assessed by culture on CRP while 16 (31%) strains were non biofilm producers.
PCR detection of icaA and icaD:
The PCR technique was applied to the 52 S. epidermidis clinical isolates. 39/52 (75%) were PCR positive for both icaA & icaD gens. Out of these 39 strains, 36 strains were CRP +ve and 3 strains were CRP –ve. All the positive strains for icaA (giving a 103 bp. band) were also positive for icaD (giving a 198 bp. band). The non biofilm producing S. epidermides were negative for both gens. Typical results are shown in the figure.

![Figure showing PCR detection of icaA & icaB](image)

PCR detection of icaA & icaB in the same amplification process
Lane 1: Molecular weight marker
Lanes 2, 5: no bands means non slime producing strains
Lanes 3, 4 & 6 : 2 bands one 103 bp of icaA and the other198 bp of icaB

The concentrations of antibiotic required to inhibit planktonic bacteria (MIC50 &MIC90) and those required to kill biofilm bacteria (MBEC50 & MBEC90) are summarized in Table (3). In this study, a clear difference in antibiotic susceptibility was seen between planktonic populations of S.epidermidis and that of the biofilm populations of the same strain. Vancomycin, oxacillin, ciprofloxacin and cephalothin antibiotics had MBEC some what higher than their MIC. Gentamycin and erythromycin had MBEC much higher than their MIC.

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC50</th>
<th>MBEC50</th>
<th>MIC90</th>
<th>MBEC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>1</td>
<td>32</td>
<td>16</td>
<td>128</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>4</td>
<td>16</td>
<td>8</td>
<td>128</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8</td>
<td>64</td>
<td>16</td>
<td>128</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.50</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>4</td>
<td>16</td>
<td>32</td>
<td>128</td>
</tr>
</tbody>
</table>

Table(3) shows MIC50, MBEC50, MIC90 and MBEC90 of Staphylococcus epidermidis strains.

MIC 50 versus MBEC 50 was as follow: for vancomycin, 2 µg/ml versus 8 µg/ml; gentamycin, 1 µg/ml versus 32 µg/ml; oxacillin, 4 µg/ml versus 16 µg/ml; erythromycin, 8 µg/ml versus 64 µg/ml; ciprofloxacin, 0.5 µg/ml versus 2 µg/ml; and for cephalothin 4 µg/ml versus 16 µg/ml.

MIC90 versus MBEC90 was as follow: for vancomycin were 4 µg/ml versus16 µg/ml; gentamycin, 16 µg/ml versus 128 µg/ml; oxacillin, 8 µg/ml versus 128 µg/ml; erythromycin, 16 µg/ml versus 128 µg/ml; ciprofloxacin, 4 µg/ml versus 8 µg/ml; and cephalothin 32 µg/ml and 128 µg/ml.
DISCUSSION

Staphylococcus epidermidis is an important pathogen in orthopaedic implant infections (3,38). It can colonize artificial surfaces in a self-generated viscous biofilm composed of polysaccharides called slime (24). In this study Staphylococcus epidermidis was the most common organism isolated from infected cases. This was in agreement with von Eiff et al (40) who explained this phenomenon by the easy access of this skin inhabitant to wounds and implants.

S. epidermidis is now well established as major nosocomial pathogens associated with infections of indwelling medical devices. Biofilm formation is one of major virulence factors of this organism, often leading to persistent infections (6). Our study revealed that most S.epidermidis isolated strains (75%) were biofilm producer. This result agreed with Arciola et al who indicated that out of 123 Staphylococcus epidermidis strains isolated from orthopedic implant infections, 69(56%) were biofilm producers(3). These finding also agreed with other studies which showed high incidence of biofilm producing staphylococci isolation from clinically significant infections of different sites especially sepsis including intravenous catheter related bacteremia and other prosthetic device infections (22,42).

Our results found that the sensitivity of PCR in detecting Slime (biofilm) producing strains (75%) was better than that of CRA (65%). PCR could detect ica operon in strains that appeared to be non biofilm producers with CRA. These reasults were in agreement with those of other authors (10,17,11), who explained this by occurrence of mutation in the icaA gene of biofilm negative strains or due to 1,332 bp sequence element known as Is 256 that cause inactivation of icaA gene and lead to biofilm negative phenotype. Also Handke et al.(17) stated that the presence of the ica locus in some biofilm negative CRA strains might be explained by low level of expresion of ica locus in those strains.

Our study revealed that both icaA and icaD genes were either present in a certain strains or absent and no single strain had shown the presence of one genes. This result confirmed the fact that both genes were part of one operon and so either the entire operon was present or absent. This was in agreement with other literatures (2,10). In addition our results showed that both genes (icaA & icaD) were present in all biofilm producing strains, this indicated that the presence of both genes was essential for biofilm formation. This was supported by Mack et al. (25) who had inactivated each gene separately by insertion of different transposon and by co-insertion of both genes that had lead to complete inactivation of PIA synthesis.

Production of biofilm by coagulase negative staphylococci is widely considered an important determinant of prosthetic device related infections thus ica operon plays an important role in disease pathogeneses (10). In some studies of human disease (2,43), ica operon was found to be more common in S.epidermidis strains associated with disease than in carriage strains. Inactivation of the icaA was reported to be associated with a decrease in the pathogenicity of a strain of S. epidermidis in two animal models of foreign body infection (35).

The MIC has been used as a gold standard for determination of anti-microbial sensitivities for animal and human pathogenic bacteria (6). It is recognized that an antibiotic that is ineffective in preventing growth of a particular organism using the MIC assay will also be clinically ineffective (23). However, an organism that is sensitive in vitro may not be effective in vivo (34). It is clear that the antibiotic susceptibilities of planktonic populations, as determined by MIC methodologies, are not necessarily applicable to effective treatment of the same organism once a biofilm has been established. So in our study we aimed to measure the antibiotic susceptibility of a biofilm (MBEC) in device-related infections caused by organisms for which MIC has not provided clinically relevant information. This was done by using CBD which considered as easy, reliable and reproducible assay.

Antibiotic resistance is a serious problem encountered with human pathogens (18) particularly notable with S.epidermidis, since many clinical isolates of this organism are resistant to many different antibiotics (11,32). A number of assays have been used to quantify antibiotic susceptibility of cells in biofilms, but such assays do not provide a fair
comparison with the standard MIC assay for planktonic bacteria (19). This is because NCCLS standards stipulate the use of low inocula of the planktonic bacteria whereas bacteria are at a high cell density in established biofilm. This difference affects antibiotic susceptibility assays in some antibiotics as vancomycin which exhibits a cell density-dependent effect(4,31).

In our study, we compared the conventional MIC of planktonic bacteria with MBEC of the biofilm, the studied antibiotics were chosen as they are frequently used to treat patients with staphylococcal infections. MBEC50 & MBEC90 for the antibiotics used in the study (vancomycin, gentamycin, oxacillin, erythromycin, ciprofloxacin and cephalothin) were much higher than those of conventional MIC50 & MIC90. This indicated that the biofilm producing bacteria were resistant to the antibiotics compared to the planktonic forms. This result agreed with the results obtained in other studies (20,33,37). Strong reduction in antibiotic activity of biofilm producing strains can be explained by the hypothesis that glycocalyx acts as penetration barrier by providing resistance to diffusion and delaying the equilibration of antibiotic concentration between external medium and the depth of the biofilm (20,39). The exopolysaccharide matrix of medically important bacteria is negatively charged or less commonly neutral leading to an interaction with positively charged antibiotic molecules like vancomycin and aminoglycosides (13,20). Reduced growth rates in established bacterial biofilms may be an other cause of reduced susceptibility to antibiotics. Reduced growth rates in bacterial biofilms may be due to limited diffusion of nutrients and oxygen and accumulation of waste products (20). Quinolones and aminoglycosides activity may be affected by low pH and reduced oxygen levels in deeper parts of the biofilm (21). Beta lactame antibiotics can be affected by changes in the cell envelop of biofilm producing bacteria affecting the expression of different penicillin binding proteins in response to environmental changes (20).

The results of the present study confirms that ica genes can be considered a virulence marker in the pathogenesis of implant associated orthopedic infection by S epidermidis. This study also demonstrates marked differences between the results of susceptibility testing performed according to standard NCCLS guidelines and testing based on biofilm susceptibility testing. This study recommends that we can use MBEC assay to predict clinical failure and clinical success of certain antibiotics used to treat diseases due to device-associated infections in humans. Also this study recommends conducting a clinical trials to compare the efficacy of antibiotic therapy based on biofilm susceptibility testing with that based on standard testing thus we can know whether these in vitro differences will translate to improved bacterial killing in vivo and better clinical response to therapy.

REFERENCES


سبيد الأسخدام المتزايد للأجهزة الطبية الداخلية زيادة هامة في العدوى المتعلقة بتلك الأجهزة. و تعدد المكروبات العنقودية البشريّة سبباً هاماً لتلك العدوى حيث إن لها القدرة على تكوين مادة الرداغ الذي يمكّنها من الإمساك بالموزعات البكترية.

وكان الغرض من هذه الدراسة تحديد أهمية تكوين الرداغ في عدوى البذال التجbstractيّة والمكروبات العنقودية البشريّة وبحث ما إذا كان تكوين الرداغ له تأثير على حساسيتها للمضادات الحيوية.

وقد أجري هذا البحث بأخذ 200 مسحة من مرضى بقسم العظام مركب للذين يعانون من الباختيار البكترية السببية للعدوى باستخدام الطرق البكترية التقليدية. تم اختبار تفاعل Congo red test and PCR لتقريب المكروبات العنقودية البشريّة المكرونة للرداغ ثم تم عمل اختباري للكزم الأولي للمضادات الحيوية المزيلة للعدوى و التركزي الأولي للمضادات الحيوية المزيلة للعدوى (MIC) لهذه المكروبات و كانت النتائج

1- تم عزل 80% من المكروبات العنقودية السالبة من السكان البكترية.
2- تم عزل 50% من المكروبات العنقودية البشريّة.
3- كان 31% من المكروبات العنقودية البشريّة مكرونة للرداغ في حين كان 31% سلبياً لتكوين الرداغ باستخدام الأجاز المكرونة البكترية السببية للعدوى.
4- كان 25% من المكروبات العنقودية البشريّة مكرونة للرداغ في حين كان 25% سلبياً لتكوين الرداغ باستخدام التفاعل المتسلسل لإنزيم البيلموري.
5- كان التركزي الأولي للمضادات الحيويّة إلى المكروبات السالبة من السكان البكترية (MBEC) أكبر من التركزي الأولي للمضادات الحيويّة إلى المكروبات السالبة من السكان البكترية (MIC).

وخلص البحث إلى أن جين إيكيا يمكن أن يعتبر عامل فعال للمكروبات العنقودية البشريّة أثناء التسبب في العدوى المصاحبة للدلايل التجbstractيّة. و أثبت البحث فرق واضح بين نتائج الحساسية للمكروبات الحيوية باستخدام المكرونة البكترية البشريّة المكرونة للرداغ (MIC) والتركيز الأولي للمضادات الحيوية إلى المكروبات السالبة من السكان البكترية (MBEC) والتركيز الأولي للمضادات الحيويّة إلى المكروبات السالبة من السكان البكترية (MIC).

وأوصى البحث بإضافة دراسة كلينيّة لمقارنة مدى نجاح العلاج بالمضادات الحيويّة المعتمدة على كل من الطرفيتين المعرفة ما إذا كان هناك فائدة كلينيّة من استخدام الطريقة الجديدة لحساسية المكروبات الحيوية طريقة التركزي الأولي للمضادات الحيويّة المزيلة للعدوى (MIC). (MBEC).