The Kinetics Of Ampicillin, Gentamycin, Ampicillin And Gentamycin Combined, Vancomycin On Urinary Tract β-Lactamase Positive And Negative Staphylococci Infections In Benin City, Nigeria

O ONI, F ESUMEH, P OTUAGA

Citation

Abstract
β-Lactamase production by some bacteria is a cause of decreased clearance of bacteria in infected tissues by β-lactam antibiotics like Ampicillin and the Cephalosporins. A total of two hundred and fifty clinical isolates of staphylococcus aureus (180) and coagulase negative Staphylococci (70) had their time killed kinetics to Ampicillin, Gentamycin, Ampicillin combined with Gentamycin, and Vancomycin tested using different concentrations of the drugs. This study revealed that the Minimum Inhibitory Concentration (MIC) of Ampicillin was 10µg/ml for β-Lactamase negative staphylococci. However, there was a reduction of 58% from the initial cfu/ml of 4.8x10^6 to 2.05x10^6 in 10 hours at a concentration of 25µg/ml of Ampicillin for β-lactamase producing strains. Gentamycin had a minimum inhibitory concentration of 5µg/ml 10 hours after incubation for β-Lactamase negative and β-Lactamase producing Staphylococci respectively. Furthermore, synergism was observed in the Ampicillin and Gentamycin combination which had a minimum inhibitory concentration of 5µg/ml in this study. Vancomycin was found to have an MIC of 4µg/ml in this study. In conclusion, it was observed that the β-Lactamase negative (non producing) staphylococci were more sensitive to ampicillin than the β-Lactamase producing Staphylococci. However, the grand difference between the sensitivity of β-lactamase positive and negative staphylococci to the antibiotics was significant when P-value was set at 0.05.

INTRODUCTION
The genus staphylococcus was identified in a purulent material by Robert Koch in 1878 and later cultivated by Louis Pasteur. They form the natural floral of human skin and mucous membrane. Staphylococcus is a member of the family micrococcacea in the order Eubacteriales. They are gram positive and coccospherical in shape. The most widely encountered staphylococci are staphylococcus aureus, epidermidis and saprophyticus. Staphylococcus aureus is the most important medically because of its association with human diseases. Staphylococcus aureus is one of the most versatile nosocomial and dangerous human pathogen since publication of its role in sepsis by Ogston in 1880 and 1882. Hence, the treatment of staphylococcus can be challenging and the associated mortality ranges from 20-30% despite the availability of highly active antimicrobial agents.

Treatment of staphylococci infections is still based on the penicillins in many rural areas in Nigeria, despite the availability of more expensive highly active drugs. However, clinical failures with delayed sterilization of the affected tissue and environment have been reported with the penicillins. The first case of penicillin resistance was identified among the β-lactamase producing staphylococcus aureus in the 1960s. Based on this understanding, the idea of prescribing the penicillins against β-lactamase producing organisms like staphylococci isolates may be reconsidered, since staphylococcus strains resistant to the β-lactam antibiotics is becoming a significant pathogen especially in developing countries like Nigeria. Kalsoom et al investigated the resistance pattern of staphylococcus aureus to cephalosporins, quinolones, aminoglycoside, penicillins and vancomycin. She reported that gentamycin was the second most effective drug against staphylococcus aureus after vancomycin. Mohsen et al reported a similar work and he concluded that the susceptibility of staphylococci to gentamycin was 100%. However, Moellering et al, observed an enhanced bactericidal activity offered by β-lactam-aminoglycoside antibiotic combination therapy.
emphasized that immunocompromised patients are most likely to gain from such enhanced combination. The work of Leibovici et al corresponds with that of Moellering et al as he reported a better killing activity by synergism of combined drug than monotherapy. This study sought to determine the individual effectiveness of ampicillin, gentamycin, ampicillin and gentamycin combined, vancomycin. We also determined the minimum therapeutic dose of the antibiotics through their minimum inhibitory concentrations.

MATERIALS AND METHODS

A total of two hundred and fifty clinical isolates of staphylococcus species from different urine samples were obtained from the microbiology laboratory of the university of Benin Teaching Hospital.

The isolates were identified using catalase test and the tube coagulase test in accordance with procedure described by Cowan and Steal in 1985. The criteria included colony morphology, size, shape, surface elevation, edge, colour and capacity. Gram stain was done on suspected colonies as described by Stokes in 1980. Gram stain slide was examined under the microscope using oil immersion of (100x objective). Those that appeared as blue black cocci in the cluster were suspected to be staphylococcus species. Generally, when gram stain is applied on a slide, gram positive bacteria appears blue white and gram negative are pink on microscopy.

Control experiments were set up using known catalase positive staphylococcus aureus and catalase negative staphylococci species. β-lactamase production by staphylococcus species was detected using the iodometric method.

The minimum inhibitory concentration is defined as the least concentration of anti-bacterial agent that inhibits the visible growth of bacterial after incubation for 24hrs. MIC was carried out on the β-lactamase negative and positive strains of staphylococci using ampicillin, gentamycin, ampicillin and gentamycin combination, vancomycin. Appropriate dilution of the stock solution of the antibiotics were made in nutrient broth to give different concentrations. A range of dilutions of ampicillin 1mg/ml was made in nutrient broth to form concentrations of 5, 10, 15, 20 and 25µg/ml. The same dilution were obtained for gentamycin 1mg/ml but at concentrations of 2.5, 5, 10, 15 and 20µg/ml. Thereafter a volume of 2mls was taken from each antibiotic to form concentrations of ampicillin-gentamycin combination of 2.5, 5, 7.5, 10 and 15µg/ml. In addition, 1gm of vancomycin was also diluted from 1mg/ml to provide different concentration of 2, 4, 6, 8 and 10µg/ml.

Initial inoculum count of 4.8 x 10⁶ cfu/ml was introduced into bottles containing the different antibiotic(s) concentrations. The nutrient agar plates were labeled with different concentrations of the antibiotic(s). The nutrient broth in bottles containing antibiotics and organisms were inoculated on nutrient agar plates at different time intervals of 2, 4, 6, 8 and 10 hours respectively after incubation for 24 hours. The colonies were later counted. The MIC was taken as the lowest drug concentration that showed no visible growth for each antibiotic(s).

RESULTS

Table 1 showed the prevalence of Staphylococcus aureus and coagulase negative Staphylococci in different urine samples. Out of a total of 250 isolates obtained from different urine samples, S. aureus and coagulase negative staphylococci were 180 (72%) and 70 (28%) respectively. In Table 2, the incidence of β-Lactamase producing strain of S. aureus and Coagulase negative Staphylococci were 176 (98%) and 12 (17%) respectively. The results of Table 3 showed the susceptibility of β-lactamase negative Staphylococci isolates to Ampicillin. At 0 hour, the inoculum of 4.8 x 10⁶ cfu/ml gradually reduced until there was no visible count 10 hours after incubation. The minimum inhibitory concentration was found to be 10µg/ml 10 hours after incubation.

Table 4 showed the susceptibility of β-lactamase negative Staphylococci to gentamycin. The cfu/ml at 0 hour was 4.8x10⁶. This gradually reduced until 10 hours after incubation, when there was no visible growth. The minimum inhibitory concentration was found to be 5µg/ml. Table 5 revealed that ampicillin-gentamycin combination totally inhibited the growth of the staphylococci isolates 8 hours after incubation. Furthermore, as the concentration of the drugs increased, the duration of inhibition of the staphylococci isolates reduced to 2 hours at a concentration of 15 µg/ml. The MIC of the combined drug was also found to be 5µg/ml. The results from table 6 revealed that the staphylococci isolates were consistently killed by vancomycin at a concentration of 10µg/ml, 4 hours after incubation. However, the minimum inhibitory concentration of vancomycin was found to be 4µg/ml 10 hours after incubation.
Tables 7-10 give the results of the kinetics of β-lactamase producing Staphylococci by the various antibiotics. Table 7 showed that there was no complete inhibition of β-lactamase producing Staphylococci isolates by ampicillin, 10 hours after incubation. At 5µg/ml, the viable bacteria count remained the same. However, at 10µg/ml and 10 hours after incubation, 75% of the total count were still found to be viable. Furthermore, at 25µg/ml the total count reduced to 42% 10 hours after incubation. The results of Table 8 showed that gentamycin’s minimum inhibitory concentration was 5µg/ml 10 hours after incubation and that there was no visible count 6 hours after incubation at a concentration of 15µg/ml. Table 9 revealed that the initial inoculum of $4.8 \times 10^6$ cfu/ml was eliminated at a concentration of 15µg/ml of both ampicillin and gentamycin after 6 hours after incubation. The minimum inhibitory concentration was 5 µg/ml 10 hours after incubation. Finally, Table 10 showed an initial inoculum of $4.8 \times 10^6$ cfu/ml at 0 hours. 10 hours after incubation, there was a complete inhibition of the Staphylococci cells at a vancomycin concentration of 4µg/ml, which was taken as the minimum inhibitory concentration. However, there was no visible growth on the nutrient agar plate 4 hours after incubation at a concentration of 10µg/ml.

Table 1: the incidence of Staphylococcus aureus and coagulase negative staphylococci

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>CNS</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180(72%)</td>
<td>70(28%)</td>
<td>250(100%)</td>
</tr>
</tbody>
</table>

*CNS = Coagulase negative staphylococci

Table 2: Incidence of β-Lactamase production amongst Staphylococcus aureus and Coagulase negative staphylococci isolates.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus (n=180)</th>
<th>CNS (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>176(98%)</td>
<td>12(17%)</td>
</tr>
</tbody>
</table>

*CNS = Coagulase negative staphylococci

n = number tested
Table 6: The kinetics of Vancomycin on β-lactamase negative staphylococci

**Figure 6**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci count (CFU/ml)</td>
<td>4.8 x 10^6</td>
<td>1.1 x 10^6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin at µg/ml conc.</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
</tr>
</tbody>
</table>

* N = Numerous
µg/ml = Microgram per milliliter

Table 7: The kinetics of Ampicillin on β-lactamase producing staphylococci

**Figure 7**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
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</tbody>
</table>

* N = Numerous
µg/ml = Microgram per milliliter

Table 8: The kinetics of Gentamycin on β-lactamase producing staphylococci

**Figure 8**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci count (CFU/ml)</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
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<tr>
<td>Gentamycin at µg/ml conc.</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
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</table>

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Table 9: The kinetics of Ampicillin-Gentamycin combination on β-lactamase producing staphylococci

**Figure 9**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci count (CFU/ml)</td>
<td>4.8 x 10^6</td>
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<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
</tr>
<tr>
<td>Ampicillin-Gentamycin at µg/ml Conc.</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
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</table>

* N = Numerous
µg/ml = Microgram per milliliter

Table 10: The kinetics of Vancomycin on β-lactamase producing staphylococci

**Figure 10**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>0</th>
<th>2</th>
<th>4</th>
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<tbody>
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<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
</tr>
<tr>
<td>Vancomycin at µg/ml conc.</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
<td>4.8 x 10^6</td>
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**DISCUSSION**

The worldwide emergence of antibiotics resistance of the versatile nosocomial and dangerous human pathogen, staphylococci has changed the approach to its chemotherapy. The increasing resistance of β-lactamase producing staphylococci isolates to penicillins has rejuvenated the need to investigate other antibiotics like gentamycin, ampicillin combined with gentamycin, vancomycin as alternatives to treat infections caused by staphylococci isolates. The result of this present study shows that gentamycin had an excellent antibacterial activity against staphylococci isolates irrespective of β-lactamase production. This occurred because gentamycin is not a β-lactam antibiotics and its mode of action is by
inhibition of protein synthesis of bacteria. The result of this study corresponds with the work of Scott et al and Nobert et al.\textsuperscript{16,17} Contrarily, Bint et al reported an outbreak of gentamycin resistant staphylococcus aureus.\textsuperscript{18} However, other researchers like Linhua et al have reported good results with the use of gentamycin and vancomycin.\textsuperscript{19} He revealed that staphylococcus aureus and epidermidis were susceptible to gentamycin and other antibiotics like vancomycin, ciprofloxacin, amikacin etc. This present study also agrees with the work of Lelievre et al who reported that the emergence and spread in French hospital of meticillin resistant staphylococcus aureus had an increasing susceptibility to gentamycin and vancomycin.\textsuperscript{20} Synergism was observed between ampicillin and gentamycin in this present study. This is in agreement with the work of Moellering et al and Leibovici et al who demonstrated a better killing activity by synergism of ampicillin and gentamycin combination.\textsuperscript{10,11} In conclusion, Observation of the results obtained from this work showed that β-lactamase producing Staphylococcus species were resistant to ampicillin but susceptible to gentamycin, vancomycin and the synergistic action of ampicillin and Gentamycin combination. Furthermore, vancomycin is the drug of choice in its action against β-lactamase negative and positive Staphylococci infections due to its lower MIC as demonstrated in this study.

**RECOMMENDATION**

The results of this study made it imperative to recommend that gentamycin should be prescribed alone or in combination with ampicillin by physicians due to their synergistic effect in the treatment of β-lactamase producing Staphylococcus. In addition, vancomycin has been shown to be the most effective and a first line drug in the management of β-lactamase negative and positive staphylococcal infections. However, it should be used with care due to its serious side effects. We recommend that more work should be done in this field using other common and less expensive antibiotics for the absolute benefit of patients who cannot afford the expensive drugs.

**ACKNOWLEDGMENT**

We remain grateful to the staff of microbiology department, University of Benin Teaching Hospital (UBTH), Benin City and Ambrose Alli University (AAU), Ekpoma, for their unparalleled assistance, especially Dr F. Esumeh who graciously supervised this work.

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**APPENDIX 1**

Student T-test was used to determine the probability that the difference between the MICs is a real difference and not a chance difference.

\[ X_1 = \text{MIC of the antibiotics against β-lactamase negative staphylococci.} \]

\[ X_2 = \text{MIC of the antibiotics against β-lactamase positive staphylococci.} \]

**Figure 11**

Because there was no 100% MIC in Ampicillin against β-lactamase positive staphylococcus, 25µg/ml of ampicillin which reduced the bacteria load to < 50% was used.

The following formular was used in the student t-test.

\[ X_1 - X_2 = \text{mean values of the two groups.} \]

\[ \Sigma x_1^2 \text{ and } \Sigma x_2^2 = \text{sum of the squared values.} \]

\[ x_1^2 \text{ and } x_2^2 = \text{square of the mean of the two groups.} \]

\[ n=\text{size of the observations.} \]

\[ \Sigma x_1=24 \text{, } \Sigma x_2=39 \text{, } \Sigma x_1^2=166 \text{, } \Sigma x_2^2=691 \]

\[ X_1=24/4=6 \text{, } X_2=39/4=9.75 \]

Substituting these values in the above t-test formulae, we thus have the following:
The Kinetics Of Ampicillin, Gentamycin, Ampicillin And Gentamycin Combined, Vancomycin On Urinary Tract β-Lactamase Positive And Negative Staphylococci Infections In Benin City, Nigeria

Figure 13

The degree of freedom in t-test is calculated as

\[ Df = N1+N2 - 2 \]

\[ Df = 4+4-2 = 6 \]

A two tailed test at 0.05 level of significance would be 2.45. This is the critical level at which conclusion is made. Since the calculated t-value of 0.71 is less than the critical or table value, the null hypothesis is accepted. On this basis, we can conclude that the difference in the kinetics of the antibiotics on the β-lactamase negative and β-lactamase positive isolates of staphylococci species was significant.

References

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