INTRODUCTION

In rational drug therapy, the concurrent administration of two or more antimicrobial agents is often essential and sometimes mandatory in order to achieve the desired therapeutic aim to treat co-existing diseases. However, drug interaction may have different effects on the host as well as the infecting organism (1) and can decrease potency, increase adverse effect or toxicity (2). Treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of anti-bacterial activity in the treatment of specific infections and prevention of the emergence of resistant microorganism (3) have been a problem militating against combined antimicrobial therapy. As a result, it is highly expedient that the in vitro interaction of combination of antimicrobials be evaluated using suitable test microorganisms before such combination are clinically used.

The antimicrobial actions of glycine have empirically been demonstrated (4 - 7). Gerberick and Castric demonstrated synergistic antibacterial effects between combinations of glycine and carbenicillin or EDTA against Pseudomonas aeruginosa using a microtitre modification of the checkerboard technique (8). The checkerboard technique, good as it may be in assessing antimicrobial interactions is plagued by inherent methodological limitations. Sanders et al (9) pointed out some of the limitations and went ahead to describe

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**ABSTRACT**

The decimal assay for additivity (DAA) method was used to evaluate the in vitro interaction of glycine (Gly) with penicillin G (Pen G); cloxacillin (Clox) and ampicillin (Amp) against a clinical isolate of *Staphylococcus aureus*. In the interaction between Pen G/glycine with biological equivalent factor (BEF) 5 mg/62 mg, the decimal combination of 4 parts Pen G and 6 parts glycine showed antagonism, whereas in the interaction between Clox/glycine with BEF 1.26 µg/62.5 mg only 7:3 combination gave a synergistic effect. Others showed antagonism. In Amp/glycine combination with BEF, 1.74 µg/62.5 mg, the decimal combinations of 4:6 gave additive effect; 9:1 gave synergistic effect while 7:3 combination gave indifferent effect.

**Keywords:** Glycine, interaction, penicillins, decimal assay for additivity, in vitro.
a new *in vitro* method for evaluating antimicrobial combinations devoid of these problems. This new test – the Decimal Assay for Additivity (DAA) is based on disc diffusion and was designed to have a precisely defined end point for additivity so that interactions greater or less than additivity (that is synergism or antagonism) could be accurately identified. Resistance of isolates of *Staphylococcus aureus* to conventional penicillins like ampicillin, penicillin G and cloxacillin is rapidly increasing (10, 11). Since glycine has earlier been shown to exhibit synergistic interaction with a penicillin (carbenicillin), the present study is aimed at evaluating the possible effect of combination of glycine with other conventional penicillins using a much recent method of assessing interaction.

**MATERIALS AND METHODS**

**Materials**

The culture media include nutrient agar (Oxoid, USA) and Nutrient broth (Merck, England). the test microorganism was a clinical isolate of *Staphylococcus aureus* obtained from the Pharmaceutical Microbiology laboratory of the University of Nigeria, Nsukka. The antibiotics used were crystalline penicillin G (Troge, India), ampicillin (Helm, Germany), cloxacillin and glycine.

**Methods**

**Isolation, purification and characterization of test microorganisms**

The clinical isolates of *Staphylococcus aureus* were purified by streaking on sterile nutrient agar plates and pure colonies were sub-cultured on selective media (Mannitol salt agar), characterized finally and stored in nutrient agar slants inside a refrigerator at 4°C. the stock culture was reactivated before use and the inoculum size standardized to MacFarlands 0.5 turbidity standard.

**Sensitivity and interaction studies**

**Determination of the standard dose-response curve**

A sterile 20 ml molten nutrient agar in a sterile plate was seeded with 0.1 ml suspension of the standardized microbial culture and allowed to set on a horizontal plane to ensure uniformly thick agar layer. Sterile filter paper discs impregnated with various concentrations of the test antibiotics (Amp. Pen G and clox) were aseptically placed on the set nutrient agar plates and allowed to stay at room temperature for 30 min for pre-diffusion before incubation at 37°C for 24 h. the exercise was repeated in triplicates. The inhibition zone diameter (IZD) was measured after the incubation period. A standard dose response curve was plotted from the various antibiodycics masses and their corresponding IZDs. A target size of IZD was selected from the midrange so that increases or
decreases due to any interaction could be detected. The corresponding antibiotic masses of the target IZDs were obtained by linear regression analysis for ampicillin, penicillin G, cloxacillin and glycine. From these, the biological equivalent factor (BEF) was estimated according to the method described by Sanders et al (9). The BEF was used as the stock concentration of the antibiotics for the interaction/combination studies.

**Determination of combined activity of glycine with the test penicillins**

Decimal combinations of glycine and either Amp, Pen G or Clox ranging from 0:10 to 10:0 were prepared in sterile test tubes. Sterile discs were saturated with these solutions and the discs aseptically placed on nutrient agar plates already seeded with a standardized inoculum of *S. aureus*. Thirty minutes pre-diffusion was allowed before incubation at 37°C for 24 h. IZDs were measured after 24 h and the interaction types if any were ascertained by comparing the IZDs of the decimal mixtures with those of the single antibiotics and the result analysed statistically at p < 0.05 (ANOVA).

**RESULTS AND DISCUSSION**

The dose response curve (Fig 1) showed that the activity of penicillins decreased from cloxacillin through ampicillin to penicillin G. Glycine had a high BEF of 62.5 mg compared to those of cloxacillin (1.25 μg), penicillin G (5 μg) and ampicillin (1.74 μg).

The result of the combined activity determination (Table 1) demonstrated that the different decimal combination of glycine and the penicillins gave different effects ranging from synergism to antagonism. The combination of ampicillin and glycine at 9:1 ratio respectively gave synergistic effect whereas at 4:6 ratio, additivitiy resulted. All the decimal combinations in the penicillin G/glycine gave additive effects except one whereas cloxacillin/glycine showed synergism at 9: 1 and addititity at 7:3 respectively. However, the presence of glycine potentiated the effects of the penicillins but not in a strikingly clear manner. The inhibitore effect of glycine on cell growth is more clearly seen in resistant microorganism than in susceptible ones because the strain of *S. aureus* used was not very resistant to the penicillin since the effect of the glycine was not much observed and the penicillin alone was able to produce antimicrobial effects.

The mechanism of or the synergy demonstrated with these agents is yet unknown. Penicillins are known to decrease the stability of the cell wall by inhibiting both the transpeptidase and the D-alanine carboxypeptidase enzymes and so acted synergistically with glycine. However, the effect obtained correlated with the findings of Gerberick et al (8), which showed that the synergistic effects of some drugs combined with glycine decrease as the concentration of glycine increases. As a matter of fact, infections caused by penicillin resistant isolates such as *Escherichia coli*, *S. aureus* and *Pseudomonas aeruginose* could be treated with some penicillin/glycine combination regimens at minimal glycine concentration as occurred in the 9:1 ratio of ampicillin, cloxacillin/glycine combination.
Glycine inhibits bacterial growth by replacing both D- and L-alanine residues of the peptidoglycan (5, 6). Thus, penicillins and glycine, both of which inhibit cross-linking of peptidoglycan strands by different modes of action would be expected to have synergistic mode of action. Failla et al (12) demonstrated that total parenteral nutrition solution can be rendered antibacterial by decreasing the content of the alanine and increasing that of glycine – they further proposed that high concentration of glycine was antibacterial either by inhibiting such enzyme as D-Alanine ligase, alanine racemase, or L-alanine ‘adding’ enzymes or by replacing both D- and L-alanine residues in peptidoglycan subunits, thereby impairing transpeptidization within the cell wall (12). Tomoeda et al (7) have shown that glycine is effective in eliminating drug resistance of Escherichia coli K-12 JE2100 strain harbouring the R100 – 1 factor. However, the mechanism by which glycine acts as a curing agent is still unknown.

Although synergy was demonstrated, it is felt that further research should be done with other strains of S. aureus. Also, in vivo research could be designed with laboratory animals to investigate the efficacy of conventional penicillins (ampicillin, penicillin G and cloxacillin) and glycine as topical agents for the treatment of skin diseases of bacterial origin.

Table 1: Antimicrobial activity of glycine in combination with Amp, Pen G and Clox (AG =Amp/Glyc; PG = Pen G/Glyc; CG = Clox/Glyc)

<table>
<thead>
<tr>
<th>Drug combination (AG, PG, CG)</th>
<th>Mean inhibition zone diameter (mm)</th>
<th>Confidence intervals</th>
<th>Type of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG</td>
<td>PG</td>
<td>CG</td>
</tr>
<tr>
<td>0+10</td>
<td>9.6 (0.58&quot;)</td>
<td>11.0 (0.71)</td>
<td>6.75 (0.50)</td>
</tr>
<tr>
<td>2+8</td>
<td>16.2 (0.69)</td>
<td>20.4 (1.34)</td>
<td>16.6 (1.82)</td>
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<tr>
<td>4+6</td>
<td>20.2 (0.84)</td>
<td>20.0 (1.23)</td>
<td>19.8 (1.30)</td>
</tr>
<tr>
<td>5+5</td>
<td>19.0 (0.71)</td>
<td>22.6 (1.52)</td>
<td>19.4 (0.89)</td>
</tr>
<tr>
<td>7+3</td>
<td>21.0 (0.71)</td>
<td>23.0 (1.73)</td>
<td>12.4 (0.71)</td>
</tr>
<tr>
<td>9+1</td>
<td>22.2 (0.84)</td>
<td>22.8 (0.45)</td>
<td>25.4 (0.55)</td>
</tr>
<tr>
<td>10+0</td>
<td>21.0 (0.89)</td>
<td>21.8 (1.10)</td>
<td>24.6 (0.55)</td>
</tr>
</tbody>
</table>

Fig. 1 : Dose-response relationship of a clinical isolate of Staphylococcus aureus to ampicillin, penicillin G and cloxacillin.
aetiology.

REFERENCES