Synergistic Activities of Amoxicillin and Erythromycin Against Bacteria of Medical Importance

1O.O. Olajuyigbe and 2T. Animashaun

1Department of Biosciences and Biotechnology Babcock University, PMB 21244, Ikeja, Lagos, Nigeria
2Phytomedicine Research Centre, University of Fort Hare, Alice 5700, South Africa

Abstract: Background: The use of macrolides where there are allergies or non-compliance to beta-lactams is a common practice. There are reports on the diminished susceptibility to these classes of antibiotics globally with a dearth of information on the interactions between them. On this basis, the influence of combining amoxicillin and erythromycin on their respective antibacterial activities was investigated. Methods: The antibacterial activities of each antibiotic alone and their combinations were investigated using the agar diffusion and broth dilution methods. Different concentrations of each antibiotic were prepared by serial dilution while corresponding concentrations of the antibiotics were combined before being inoculate with the test organism. The inhibition zones, Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and interactions between the antibiotics were determined. Results: The obtained result indicated that the interactions between amoxicillin and erythromycin were synergistic and additive. Combining the two antibiotics resulted in significant increases in the MICs of both antibiotics and no growths were found in concentrations higher than the obtained MIC values for each antibiotic. Resistant colonies were not isolated within inhibition zones produced by the combined antibiotic. The obtained result showed that combined antibiotics produced higher bactericidal effects than the individual antibiotics. Conclusion: it is concluded that the combination of the two antibiotics is a veritable source of potential resistance modifying agents. This study, therefore, further strengthened drug-drug combination for effective antibacterial therapy.

Key words: Amoxicillin, erythromycin, drug-drug combination, antibiotics

INTRODUCTION

Multi-drug combinations are vital in modern medicine (Fitzgerald et al., 2006). Current clinical practices emphasize the use of multidrug treatments primarily to increase the spectrum of activity (Rybak, 2006), to increase efficacy (Walsh, 2003) and in some pathogens, to decrease the likelihood of the emergence of resistance (Pillai et al., 2005). While more drugs and combinations of drugs are being used than ever before, there are potential risks and benefits to patients when medication therapies are combined to cure, slow the progression, or reduce the symptoms of diseases. More side-effects of drugs and drug-drug interactions are being reported as highly effective drugs are developed and multiple-drug therapies are increasingly used. These drug combinations have resulted in perturbations categorized into additive, synergistic or antagonistic interactions (Loewe, 1953; Hartman et al., 2001). Despite numerous reports on drug interactions, physicians have failed to recognize these risks (Glassman et al., 2002) and continue to prescribe contraindicated drugs (Chen et al., 2005) that often resulted in frequent medication errors as well as constituting a public health problem (Moore et al., 2007).

While Rybak and McGrath (1996) reported that antimicrobial combinations provide broad-spectrum coverage until the causative pathogens are isolated and identified, Martinez et al. (2003) indicated that combining a macrolide with a β-lactam reduced in-hospital mortality amongst patients. In patients where allergy or non-compliance prevents the use of penicillins and other beta-lactams, macrolides are used (Strahilevitz and Hooper, 2010). However, reports of a rising Minimum Inhibitory Concentration (MIC) or diminished susceptibility to penicillin have been published (Amabile-Cuevas et al., 2001). Global increases in macrolide resistance and therapeutic failures have also been observed (Shackloth et al., 2004; Erdem et al., 2005). Use, abuse or misuse of antimicrobial agents has encouraged the evolution of bacteria towards resistance that often results in therapeutic failure (Strait et al., 1995). Prescribing practice of specific class of antibiotics to

Corresponding Author: O.O. Olajuyigbe, Department of Biosciences and Biotechnology, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria
certain organisms has been found to play a critical role in development of resistance against that antibiotic (Costelloe et al., 2010). Self-medication resulting in inadequate dosage or reduced amount of active drugs especially if they are counterfeit drugs is common. Also, the effects of patients’ requesting physicians to prescribe antimicrobials even in the absence of appropriate indications could not be underestimated.

In the clinical settings, combination therapy is mostly given empirically without the use of in vitro synergy data. Combining penicillin with other antibiotics such as erythromycin, fusidic acid, chloramphenicol and oxytetracycline has been reported to be effective against penicillinase-producing bacteria (Michel et al., 1975, 1977). Penicillin and erythromycin are combined as empirical therapy of community-acquired pneumonia and serious infections caused by Streptococcus pneumoniae (Deshpande and Jones, 2003). While the combination of beta-lactams and macrolides is often recommended for the initial empirical treatment of acute pneumonia to obtain broad spectrum antimicrobial activity against most pathogens involved, a concern about potential antagonism between the two drugs was raised by Johansen et al. (2000). This combination may be inexpedient as the bacteriocinotic agent may antagonize the effect of the bactericidal agent. However, this view needs to be substantiated. While, there is a lack of information on the interactions between amoxicillin and erythromycin and the possible outcomes against clinical isolates, this study was designed to investigate the influence of combining these antibiotics on their respective antibacterial activities.

MATERIALS AND METHODS

Organisms: The bacterial isolates used in this study included Escherichia coli ATCC 8739, Proteus vulgaris ATCC 6830, Acinetobacter calcoaceticus anitratus CSIR, Shigella flexneri KZN, Staphylococcus aureus OK10, Salmonella typhi TC8, Salmonella typhi TC4, Streptococcus pyogenes TD9, Streptococcus pyogenes TD10, Streptococcus pneumoniae TE3 and Streptococcus pneumoniae TE10.

Preparation of antibacterial agents: Standard laboratory powders of erythromycin and amoxicillin were used in this study. Stock solutions of each antibiotic were prepared by dissolving each antibiotic in ace tone and sterile deionized distilled water, respectively. From the stock solutions, different concentrations of erythromycin (0.012-50.0) µg mL⁻¹ and amoxicillin (0.12-500.0) µg mL⁻¹ were prepared by serial dilutions in sterile Mueller Hinton broth. For drug-drug interactions, different concentrations, from the highest to the least, of the antibiotics were combined for antimicrobial assay.

Antibacterial susceptibility testing with erythromycin and amoxicillin: The susceptibility screening of the test bacteria to each antibiotic and their combinations were done in accordance with the methods described elsewhere (Irobi et al., 1996; Akimpelu et al., 2008). The inoculums of each test strain were standardized at 5×10⁵ CFU mL⁻¹ using McFarland Nephelometer standard. Sterile Mueller Hinton agar plates were seeded with test bacterial strains and allowed to stand at 37°C for 3 h. Wells were then bored into the agar medium using a sterile 6 mm cork borer and labeled according to the concentrations of each antibiotic to be tested. From the stock solutions, 100 µL of (62.5, 125 and 250) µg mL⁻¹ of amoxicillin and (6.25, 12.5 and 25) µg mL⁻¹ of erythromycin and their combinations were dispersed into the correspondingly labeled wells taking care not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand on the laboratory bench for 30 min to allow proper diffusion of the antibiotics before being incubated at 37°C for 24 h. After 24 h incubation period, inhibition zones were measured using a calibrated transparent meter rule. The inhibition zones produced by interactions between the two antibiotics were compared with inhibition zones produced by each antibiotic. Experiments were done in duplicates under a sterile inoculating hood.

Determination of minimum inhibitory concentration by broth dilution methods: The Minimum Inhibitory Concentrations (MIC) of each drug and their combinations were determined by macrobroth dilution method using different concentrations of each antibiotic serially diluted in Mueller Hinton broth. One hundred microliter of each adjusted isolate was inoculated into tubes containing each antibiotic and their combinations. The broth cultures were incubated at 37°C for 24 h. MIC values were expressed as the lowest concentrations which inhibited growth as judged by lack of turbidity in the tube.

As a control, a tube containing antibiotic alone and a tube containing inoculums alone were incubated with other culture tubes for MIC determination.

Determination of minimum bactericidal concentration (MBC): For the determination of the MBC, one standard loopful of culture was taken from each of the first three broth tubes that showed no growth in the MIC tubes and inoculated on fresh nutrient agar plates. After 24 h incubation period, the least concentration of the antibiotics that showed no colony formation on the agar was taken as the MBC.
Checker board titration: The antibacterial effects of combining amoxicillin and erythromycin antibiotics were assessed using a checker board titration (Climo et al., 1999; Jung et al., 2005). The Fractional Inhibitory Concentration Index (FICI) was calculated as follows:

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\text{FICI} = \frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}} + \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}}
\]

The FICI was interpreted as follows: synergy, FICI = 0.5; indifference, 0.5 < FICI < 4; antagonism, FICI > 4 (Petersen et al., 2006).

RESULTS

In this study, the influence of combining amoxicillin and erythromycin on their respective antibacterial activities was investigated. While amoxicillin inhibited more organisms than erythromycin, *Escherichia coli* ATCC 8739, *Proteus vulgaris* ATCC 6830, *Proteus vulgaris* CSIR 0030 and *Salmonella typhi* TC4 were not susceptible at the concentration of erythromycin used for the agar assay (Table 1). The combined antibiotics produced varied inhibition zones when tested against each bacterial isolate. The inhibition zones obtained from the combined antibiotics were similar or slightly bigger than those obtained from either of the two antibiotics. The macrobroth dilution assay justified the differences between the antibacterial activities of each of these antibiotics and their combinations (Table 2). The susceptibility of the test organisms varied from one organism to another as shown by the differences in the minimum inhibitory concentrations (MICs) obtained from both antibiotics. The results obtained from both agar diffusion and macrobroth assays are complementary. Organisms with high MIC values had smaller inhibition zones. While agar diffusion assay suggested that the interactions between amoxicillin and erythromycin were both synergistic and additive, macrobroth dilution assay indicated that the interactions between the two antibiotics combined were synergistic resulting in significant increases in the MICs of both antibiotics. In the combined antibiotics, no growths were found in concentrations higher than the obtained MIC values for each antibiotic. The MIC values of the combined antibiotics were mostly a concentration lower than the MIC values obtained from erythromycin alone while, in comparison to those of amoxicillin, they were 2-4 folds lower resulting in increased antibacterial activities. The Fractional Inhibitory Concentration Index (FICI) obtained from the checker board titration, however, indicated that the interactions between amoxicillin and erythromycin were synergistic and additive or indifference but not antagonistic. Generally, the Minimum Bactericidal Concentrations (MBC) values were similar or one-fold higher than the MIC values. The antibacterial activities of the combined antibiotics were additive or indifference against five bacterial isolates and synergistic against four isolates. No resistant colonies were isolated or fuzzy inhibition zones were observed within and around the edges of each inhibition zone produced by each antibiotic and their combinations.

Table 1: Susceptibility of different bacteria to Amoxicillin (A), Erythromycin (E) and their combinations (A/E)

| ATCC 8739                          | 250 | 28 | 0.25 | 25 | 20 | 0.25 | 22 | 17 | 0.22 | 22 | 12 | 2.00 | 19 | 17 | 0.26 | 22 | 12 | 2.00 | 18 | 17 | 0.26 | 22 | 12 | 2.00 | 19 | 17 | 0.26 | 22 | 12 | 2.00 | 19 | 17 | 0.26 | 22 | 12 | 2.00 | 19 | 17 | 0.26 | 22 | 12 | 2.00 |
| ATCC 6830                          | 125 | 24 | 0.26 | 22 | 20 | 0.22 | 22 | 18 | 0.26 | 22 | 17 | 2.00 | 20 | 18 | 1.00 | 22 | 17 | 2.00 | 24 | 18 | 2.00 | 22 | 17 | 2.00 | 20 | 18 | 1.00 | 22 | 17 | 2.00 | 20 | 18 | 1.00 | 22 | 17 | 2.00 | 20 | 18 | 1.00 | 22 | 17 | 2.00 |
| CSIR 0030                          | 62.5 | 22 | 0.22 | 20 | 14 | 0.17 | 22 | 19 | 0.22 | 25 | 20 | 2.00 | 24 | 22 | 1.00 | 25 | 20 | 2.00 | 24 | 22 | 1.00 | 25 | 20 | 2.00 | 24 | 22 | 1.00 | 25 | 20 | 2.00 | 24 | 22 | 1.00 | 25 | 20 | 2.00 | 24 | 22 | 1.00 | 25 | 20 | 2.00 |

Key: A/E = Average inhibition zones

Table 2: Antibacterial activities of amoxicillin (A), erythromycin (E) and their combinations (A/E) as determined by macrobroth dilution method

<table>
<thead>
<tr>
<th>Name of the strains used</th>
<th>A (MIC µg ml⁻¹)</th>
<th>E (MIC µg ml⁻¹)</th>
<th>A (MBC µg ml⁻¹)</th>
<th>E (MBC µg ml⁻¹)</th>
<th>A/E (MIC µg ml⁻¹)</th>
<th>E (MBC µg ml⁻¹)</th>
<th>FICI (µg ml⁻¹)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td>7.813</td>
<td>3.125</td>
<td>7.813</td>
<td>7.813</td>
<td>3.906/0.390</td>
<td>0.625</td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> ATCC 6830</td>
<td>62.5</td>
<td>3.125</td>
<td>62.5</td>
<td>3.125</td>
<td>15.625/1.563</td>
<td>0.75</td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> CSIR 0030</td>
<td>0.12</td>
<td>6.25</td>
<td>0.625</td>
<td>6.25</td>
<td>0.03/0.006</td>
<td>0.25</td>
<td>Synergistic</td>
<td></td>
</tr>
<tr>
<td><em>Actinobacter calcoaceticus</em></td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.971/0.098</td>
<td>0.504</td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> OK2a</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.971/0.098</td>
<td>0.504</td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> TC4</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.971/0.098</td>
<td>0.504</td>
<td>Additive</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumonia</em></td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.195</td>
<td>0.971/0.098</td>
<td>0.504</td>
<td>Additive</td>
<td></td>
</tr>
</tbody>
</table>

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DISCUSSION

Drug-drug interactions occur when one drug influences the effects of another drug subsequently causing a change in the pharmacodynamic or pharmacokinetic parameters which may lead to lack of efficacy or increased efficacy, or to an increase or a decrease in the number of reported adverse drug reactions. For busy health professionals, the search for and evaluation of drug interaction information at the time it is needed is a great problem (McNamara et al., 1992; Ely et al., 1992). While the chances that patients will have a clinically significant drug interaction increase with the number of medications (Nies, 2001), studies looking at the frequency of interactions have reported incidences ranging from 4 to 20% (Laine et al., 2000) occurring in 9 to 70% of patients in the outpatient setting (Jankel and Speedie, 1990). In vivo studies have favoured adverse drug reactions without enough due considerations for the in vitro influences that combining these drugs could have on infectious agents. 

β-lactams and erythromycin combinations have been widely studied and many different interaction results have been described (How et al., 1958; Penn et al., 1982). Darras-Joly et al. (1996) reported synergy between β-lactam antibiotics and gentamicin against Streptococcus pneumonia. Chan et al. (2007) also reported that phenothiazines interact synergistically with erythromycin and amoxicillin against Burkholderia pseudomallei. Over two decades earlier, antagonism between ampicillin and chloramphenicol against Haemophilus influenzae (Lapointe et al., 1986), penicillin and tetracycline against pneumococci (Olsson et al., 1961), penicillin and erythromycin against group A streptococci (Strom, 1961; Johansen et al., 2000) was also reported. In this study, synergy and additive interactions of amoxicillin and erythromycin against some bacteria of medical importance are indicated.

The impact of antibiotics on a pathogen is specific and differs from pathogen to pathogen. Different antibiotics exercise their inhibitory activity on various pathogenic organisms by killing them outright or by arresting their growth. While combined antibiotic therapy has been shown to delay the emergence of bacteria resistance, it also produced desirable synergistic effects in the treatment of bacterial infection. The synergistic effect often surpasses their individual inhibitory activity. The double attack of both agents on different target sites of the bacteria could theoretically lead to either an additive or a synergistic effect as obtained in this study. Here, significant reduction in the MICs of the antibiotics resulting from their combinations indicated that the combination of the two antibiotics is a veritable source of potential resistance modifying agents (Dickson et al., 2006; Sibanda and Okoh, 2007).

Since potential drug-drug and drug-disease interactions are frequent when patients receive multiple prescriptions, clinical consequences include increased incidence of adverse events, hospitalizations, changes in therapeutic efficacies of the combined medicines with resultant poor control of the diseases under treatment and deaths (Kniff-Dutmer et al., 2003; Ray et al., 2004). In most cases, they are erroneously interpreted as patient deterioration because of illness, poor adherence to prescribed treatment, or infection (Seymour and Routledge, 1998). This study showed that combining amoxicillin and erythromycin could give beneficial chemotherapeutic outcomes because the resultant synergistic effect surpasses their individual performances. This synergistic effect may easily help in understanding if there are therapeutic efficacies or failure in treatment situation. While combinations of drugs may inhibit bacterial growth in complex ways, deviating from the neutral situation expected when the drugs do not interact (Greco et al., 1995), the inhibitory effect of the combined antibiotics may be due to certain complex formed between them which become more effective either by inhibiting the cell wall or protein synthesis or by causing its lyses or death. The absence of resistant colonies within inhibition zones or fuzzy zones around the edges of the inhibition zones emphasized the definite bactericidal activity of the combined antibiotics.

In conclusion, in order to effectively control a particular disease, in vitro experiments should be carried out with various antibiotics and their combinations so that a right drug combination may be administered to the patient for early and safe recovery from a specific ailment. Since all combinations do not produce synergistic effects, synergistic antibacterial activities in other chemotherapeutic agents could only be identified through investigations. While a further pharmacological study is recommended, the relevance of these combinations may not be underestimated. The synergistic effect of combining amoxicillin and erythromycin may be particularly useful in the outpatient setting in areas with high rates of penicillin- and/or macrolide-resistant pathogens. Combining the two antibiotics in drug formulation and chemotherapy may be an alternative means of treating patients who are allergic or failed to respond to beta-lactams or macrolides and/or live in areas with a high prevalence of multidrug-resistant strains of any of these bacteria.

REFERENCES


