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# Comparative *in vitro* Activity of Some Macrolide (Erythromycin, Clarithromycin and Azithromycin) and Fluoroquinolone (Ciprofloxacin and Ofloxacin) Antibiotics Against *Bacillus anthracis*

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**Abstract:** *In vitro* activity of three members of the macrolide group of antibiotics (Erythromycin, Clarithromycin and Azithromycin) and two members of the fluoroquinolone group of antibiotics (Ciprofloxacin and Ofloxacin) were assessed against *Bacillus anthracis* by standard tube dilution method. All the isolates showed varied susceptibility to the 2 groups of antimicrobial agents. The isolates were susceptible to Ciprofloxacin ($MIC_{50}$-0.78 μg $mL^{-1}$) and Ofloxacin ($MIC_{50}$-0.31 μg $mL^{-1}$) but were resistant to Erythromycin (($MIC_{50}$-25 μg $mL^{-1}$), Clarithromycin (($MIC_{50}$-31.25 μg $mL^{-1}$) and Azithromycin (($MIC_{50}$-200 μg $mL^{-1}$). Comparing the results of the 2 groups of antibiotic susceptibility tests, we do not recommend the use of any of the 3 macrolide antibiotics tested for prophylaxis or therapy of *B. anthracis* in our geographical area, except with specific result of susceptibility test on the particular isolate(s). Ofloxacin is highly recommended as a good alternative to ciprofloxacin antibiotic.

**Key words:** Fluroquinolones, macrolides, *B. anthracis*, *in vitro* activity, antibiotics

## INTRODUCTION

Anthrax is an acute infectious disease caused by the bacterium *Bacillus anthracis*. Anthrax disease is highly lethal in some forms and it is a disease of some historical significance in microbiology. Anthrax is a zoonosis accidentally transmitted from herbivores to human with no onward person to person transmission. The clinical presentation and outcome depend on the route of infection. Cutaneous anthrax follows inoculation of spores into damaged skin. Eating poorly cooked meat contaminated with anthrax spores lead to oropharyngeal or gastrointestinal anthrax and inhalation of spores lead to pulmonary anthrax which is usually fatal (Hart and Beeching, 2001).

True incidence of human anthrax worldwide is difficult to know, because reporting of anthrax cases is unreliable (Turnbull, 1998). However, anthrax occurs globally and more commonly in agricultural regions where inadequate control programmes for anthrax in livestock are practiced, especially the developing countries. In Nigeria, many people are not adequately informed of this disease that can easily be acquired from animals and animal products. Therefore, incidence of anthrax disease cannot be excluded from lists of disease resulting in death in this country. Like other disease of bacterial origin, anthrax therapy and improved environmental hygiene have contributed to the global decline of anthrax disease. However, using antimicrobial prophylactic could induce side effects in users and resistance in bacteria. Antimicrobial agents need to be used according to standard guidelines after appropriate assessment of risks involved, especially when prolonged use is intended. *B. anthracis* including strains isolated from the recent cases in the United States of America are reported to be sensitive *in vitro* to a range of antimicrobial agents including pencicillin, amoxicillin, doxycycline and ciprofloxacin (Hart and Beeching, 2001). Other antimicrobial agents such as erythromycin, gentamycin, chloramphenicol, ofloxacin, levofloxacin, pefloxacin, gatifloxacin, clindamycin etc are also recommended and each has it side effects (Athamna *et al*., 2004; Cavallo *et al*., 2000; Bishai, 2001; Mechcatie, 2001). However, variations do exist in susceptibility pattern for microorganisms isolated from different areas due to previous exposure and/or mutation. Benzyl-penicillin, ciprofloxacin and doxycycline are at present the internationally recommended choice for the treatment of *B. anthracis*. The known fact is that, indiscriminate use of antibiotics exposes patients to serious risks without any

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potential benefit. This could speed up the development of drug-resistant organisms. The long-term therapy for anthrax (60 days) might induce antimicrobial resistance in *B. anthracis*. Human have a rich and varied normal bacterial flora which are also exposed even when expecting the infecting pathogen been killed. Thus, these myriad of normal bacteria could also be eliminated. Therefore, the decision about which drug to prescribe for anthrax prophylaxis or therapy should always be guided on what is best for the patient and effective. The objective of this study was to search for an alternative antimicrobial agent suitable for all ages including pregnant women in our geographical area. The 2 groups of antimicrobial agents (Fluoroquinolones and Macrolides) of which some are already recommended for both therapy and prophylaxis were selected and compared with those yet approved for use in order to determine their efficacy in view of the long-term therapy required for *B. anthracis*.

## MATERIALS AND METHODS

**Antimicrobial agents:** The antibiotics tested in this study were as follows: Erythromycin (Micro-labs Ltd Sipcot, Hosur-India); Azithromycin (Pfizer, Italia S.r.I Italy); Clarithromycin (Ranbaxy Lab Ltd. Area-3 Dewas India); Ciprofloxacin (Maxhael Pharmaceutical Mumbai-India) and Ofloxacin (Preintertial Buduraj Sidoarjo, East Java-Indonesia).

**Bacterial strains and growth condition:** The bacteria isolates used were stored in sterile 30% glycerol in normal saline and were cultured on meat infusion blood agar. The plates were incubated overnight at 37°C to obtain discrete colonies (vegetative form). A single colony was picked and inoculated into 10 mL iso-sensitest broth and incubated at 37°C over night and then titrated to determine the Colony Forming Unit per milliliter (CFU/mL).

**Determination of Minimum Inhibition Concentration (MIC):** The antimicrobial agents to be tested were prepared as stock concentrations in sterile de-ionise water. Two fold dilutions in iso-sensitest broth were used in concentration range from 0.098-50 μg $mL^{-1}$ for erythromycin and ciprofloxacin; 0.39-200 μg $mL^{-1}$ for azithromycin; 0.039-20 μg $mL^{-1}$ for ofloxacin and 0.98-500 μg $mL^{-1}$ for clarithromycin. A 10 μL volume of bacteria culture which contained $10^5$CFU/mL was then added into each dilution. Following incubation of the tubes for 18-24 h at 37°C in ambient air, MIC was determined. The MIC was recorded as the lowest concentration of antibiotic that completely inhibited visible growth of the bacteria (NCCLS, 2004). The $MIC_{50}$ was the concentration that inhibited the growth of 50% of the isolates.

## RESULTS AND DISCUSSION

The average concentrations that inhibited the growth of 50 % of the isolates ($MIC_{50}$) are shown in Table 1. The categorical interpretations (susceptible, intermediate and resistant) for *B. anthracis* are yet to be established by the National Committee for Clinical Laboratory Standards (NCCLS), therefore, we based our results on the breakpoint for staphylococci for the selected antibiotics and the general breakpoints for nonfastidious organisms (NCCLS, 2001) as shown in Table 2.

Considerable numbers of previous studies have examined the susceptibility of *B. anthracis* to various antimicrobial agents (Lightfoot *et al*., 1990; Doganay and Aydin, 1991; Athamna *et al.,* 2005) without any standardized methods given for testing and interpretive criteria established for these organisms. In our study, two fluoroquinolones (ciprofloxacin and ofloxacin) and three macrolides (erythromycin, clarithromycin and azithromycin) were selected to determine the best choice for both prophylactic and therapeutic measures against anthrax disease in our geographical area. Hart and Beeching (2001) reported that anthrax disease can present 50 days or more after exposure. Therefore, prophylaxis should continue for 60 days unless exposure has been excluded. The $MIC_{50}$ for ciprofloxacin from out results was 0.781 μg $mL^{-1}$ which would be interpreted as susceptible by use of the NCCLS breakpoints for staphylococci and other non-fastidious organisms (NCCLS, 2001) (Table 1). Our results fall within the recommended range given by Inglesby *et al.* (1999) that ciprofloxacin could be used as one of the primary agents for post exposure prophylaxis of adults including pregnant women and children. However, there are normally contraindications tothe use of fluoroquinolones by children and pregnant women (Knudson, 1986; Dixon *et al*., 1999). Other fluoroquinolones tested was ofloxacin. Choe *et al*. (2000) reported that the ofloxacin MIC for *B. anthracis* (Sterne Strain) could be increased from 0.2-0.8 μg $mL^{-1}$ by continuous passage *in vitro*. This recommended MIC range for ofloxacin still remains within the susceptible range defined for non-fastidious organisms. ofloxacin is also recommended as a possible secondary therapeutic agent and prophylaxis for inhalation anthracis (CDC, 2001). In our study, the $MIC_{50}$ for this antibiotic was 0.31 μg $mL^{-1}$, which falls within the recommended susceptibility range for this antimicrobial agent. Therefore, our results show that this antibiotic is useful in therapy and prophylaxis against anthrax disease. Fluoroquinolones are useful drugs with broad spectrum bactericidal activity especially ciprofloxacin which is often used for empiric treatment. However, ciprofloxacin was reported to be associated with rupture of tendons and neuropsychiatric disorder in elderly people (Harrel, 1999;

Table 1: Summary of $MIC_{50}$ titration

| Antimicrobial agent | Stock Soln. Conc. | $^1/_{10}$ dilution | Two fold dilution concentration μg $mL^{-1}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Erythromycin | 05 mg $mL^{-1}$ | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | 0.391 | 0.195 | 0.0976 |
| 500 mg (Tab) | | - | - | + | + | + | + | + | + | + | + |
| Ciprofloxacin | 0.5 mg $mL^{-1}$ | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | 0.390 | 0.195 | 0.0976 |
| 500 mg (Tab) | | - | - | + | + | + | + | + | + | + | + |
| Azithromycin | 0.2 mg $mL^{-1}$ | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | 0.390 |
| 200 mg (powder) | | - | + | + | + | + | + | + | + | + | + |
| Ofloxacin | 0.02 mg $mL^{-1}$ | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.312 | 0.156 | 0.078 | 0.039 |
| 200 mg (Tab) | | - | - | - | - | - | - | - | + | + | + |
| Clarithromycin | 5 mg $mL^{-1}$ | 500 | 250 | 125 | 62.5 | 31.25 | 15.62 | 7.81 | 3.90 | 1.95 | 0.976 |
| 500 mg (Tab) | | - | - | - | - | - | + | + | + | + | + |

Key: + = Concentrations that showed visible growth of the bacteria; - = Concentrations that showed no visible growth of the bacteria

Table 2: Fifty percent Minimum Inhibition Concentration ($MIC_{50}$) of antimicrobial agents against *B. anthrais* strains

| Antimicrobial agents | $MIC_{50}$ (μ $mL^{-1}$) | Staphylococcal breakpoints | $MIC_{50}$ categorical interpretation |
|---|---|---|---|
| Erythromycin | 25 | ≤0.5≥8 | Resistant |
| Ciprofloxacin | 0.78 | ≤1-≥4 | Sensitive |
| Azithromycin | 200 | ≤0.5≥2 | Resistant |
| Ofloxacin | 0.31 | ≤1-≥8 | Sensitive |
| Clarithromycin | 31.25 | ≤0.5≥8 | Resistant |

Rayer, 1996). In most countries it is not licensed for use in pregnancy or children. In children, the concern is damage to the cartilage in weight bearing joints (Hart and Beeching, 2001). Prolonged administration of fluoroquinolones may lead to elimination and/or emergence of resistance in these normal bacteria flora in the body. However, in the case of anthrax infection, some individual believed that the benefits of these drugs outweigh the risks.

Macrolides is another group of antimicrobial agents tested in this study. Erythromycin was recommended as an alternative treatment of *B. anthracis* for children and pregnant women (Lightfoot *et al*., 1990; Dixon *et al*., 1999; CDC, 2001), but Mohammed *et al.* (2000) reported that 97% of their isolates showed reduced susceptibility to erythromycin. Their results were in the intermediate range when the breakpoint for staphylococci of = 0.5 μg $mL^{-1}$ was used. In our study, the isolate $MIC_{50}$ to this antimicrobial agent was 25 μg $mL^{-1}$ indicating a resistant result. Two other macrolides selected were clarithromycin and azithromycin. The two new macrolides were reported to have good activity against Gram positive and negative pathogens as well as typical organisms involved in the aetiology of upper and lower respiratory tract infections (McCracken, 1997). They were also reported to be chemically stable, better tolerated by children, have a broader antimicrobial spectrum than erythromycin against a variety of respiratory pathogens (Alvarez-Elcoro and Enzier, 1999). The bioavailability of clarithromycin is in general more than twice that of erythromycin and the bioavailability of azithromycin is 1.5 times that of erythromycin (Eisenbery and Barza, 1994). This improved absorption is related to increase in acid stability (Bishai, 2001). Also, the elimination half lives of azithromycin and clarithromycin are greater than that of erythromycin, with azithromycin having the longest half-life (Eisenbery and Barza, 1994). The improved pharmacokinetic profile of the two new macrolide antibiotics exhibit time dependent bacterial killing activity (Hardy *et al.*, 1990). The 2 macrolide antibiotics were also reported to accumulate to a greater extent in intrapulmonary tissue than erythromycin (Patel *et al*., 1996) thus could have been of better choice over erythromycin which is now one of the recommended antibiotics for the treatment of pulmonary anthracis. Our results for clarithromycin and azithromycin show high resistance with $MIC_{50}$ 31.25 μg $mL^{-1}$ and 200 μg $mL^{-1}$, respectively when the staphylococci breakpoints for these drugs were used. Comparing our $MIC_{50}$ results with that of Mohammed *et al.* (2002) for carithromycin and azthromycin (0.12-0.25 and 1-2 μg $mL^{-1}$, respectively), there was a great parity in the 2 susceptibility results indicating that in our geographical area, these drugs are of no value in either prophylaxis or therapy of *B. anthracis*, although clarithromycin was among the secondary agents suggested for use in combination with either ciprofloxacin or doxycycline (CDC, 2001).

## CONCLUSION

In conclusion, *B. anthracis* remain susceptible to many antimicrobial agents especially the fluoroquinolone antibiotics. The good activity of fluoroquinolones against our isolates demonstrated that this group of antimicrobial agents is the best choice for prophylaxis and therapy of *B. anthracis* in our area. The macrolide group of antibiotics are generally considered to be safe in pregnant women and children if tolerated, since they are not known to be human teratogen and should have been favoured. Our isolates show no susceptibility to any of the macrolides, therefore cannot be recommended for use in prophylaxis or therapy of *B. anthracis* in our area without a susceptibility result on the isolates. From our results ofloxacin is highly recommended as a good alternative to ciprofloxacin antibiotic.

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