International Journal of Biomedical and Health Sciences
Vol. 7, No. 2, June 30, 2011
Printed in Nigeria

0794-4748/2011 $5.00 + 0.00

http://www.asopah.org

IJBHS 2010195/7211

Studies on the degree of enhancement of activity of Amoxycillin by Clavulante at various ratios on some infectious bacteria

D. W. Taura

Department of Biological Sciences, (Microbiology Unit), Bayero University, Kano. P. M. B. 3011, Kano, Nigeria

(Received November 12, 2010; Accepted March 1, 2011)

ABSTRACT: The study was carried out with the aim of assessing the effect of increasing ratio of amoxicillin with Augment in against clinical isolates of *Staphylococcus aureus, E. coli, Klebsiella* spp. Disc diffusion susceptibility test results indicate that 4:1 preparations are more effective against the clinical isolates with mean zone diameter of 23mm, 17.6mm and 16.2mm for *S. aureus, E. coli* and *K. spp* respectively, followed by 2:1 with mean zone diameter of 19.4mm, 13.4mm and 11.4mm for *S. aureus, E. coli* and *K. spp* respectively, followed by the rest of the preparations i.e. 6:1, 8:1, 10:1. The susceptibility of 4:1 decreases from 80%, 60%, 40% for *S. aureus, E. coli* and *Kleb.* Spp. The result of the Statistical analysis indicates that there was a highly significant difference between 4:1 and the rest of the preparations against *S. aureus,* and *E. coli* but there was no significant difference between the preparations against *Kleb* Species.

Key words; Effects, Increasing ratio, Amoxicillin, Augmentin, Bacterial Isolate

Introduction

It is common to fear patient with medical problem to be taking many drugs combination which work synergistically, it is also becoming increasingly obvious to physicians and other members of health care team that many drugs combinations when use inappropriately have the inherent potential to interact adversely. The combination of amoxicillin and clavulanate in different ratio 2:1, 4:1, 7:1, 5:1, 16:1 in Augmentin are very effective against many clinical isolates. Augmentin in form of 16:1 are used for the treatment of community acquired pneumonia and acute bacterial sinusilis (Food and Drug Administration, Federal Register, 1972). The ratios of Clavulanic acid to amoxicillin in commercially available product are 2:1 in oral preparations and 1:5 in IV preparations (Francis *et al*., 1972). *E. coli* one of the most common pathogen community acquired and nosocomial infection is usually susceptible to amoxicillin/clavulanate (Manages *et al.,* 2001) with increasing use of this antimicrobial agents however resistance has began to emerge (Mathai *et al.,* 2001).

E-mail: tauradw@yahoo.com

Clavulanic acid was tested against a multi-drug resistance strain of *Klebsiella pneumoniae* which had been causing cross infection for several years in Nastiville hospital. The mean MIC of cephalothin for strain of these organisms was 376µg/ml, but in the presence of 1, 5 and 10µg/ml of Clavulanic this fell to 2, 1.5 and 0.7µg/ml respectively (Lawrence *et al.*, 1985). In the presence of low concentration of Clavulanic acid (0.5 – 1mg/ml) the MICs of amoxicillin for B-lactamase producing *S. aureus, B. catarrhalis, N. gonorrhea, H. ducreyi, H. influenza, Enterobacteria* and *B. fragilis* both reduce 8-64 fold (Francis *et al.*, 1992) both inhibitory and bactericidal are enhanced. A constrictive B-lactamase appear to be involved in the resistant of *M. tuberculosis* to B-lactams and in a study in which only 4/15 strain were inhibited, but not killed by 8mg/ml amoxicillin or less in the presence of 2mg/l Clavulanic acid, 14/15 were killed by 4mg or less. Similar effect are demonstrated with a wide range of B lactamase labile penicillin and cephalosporin's, but the degree of enhancement varies with agent and organisms, depending on the intrinsic activity of the agent, it penetrates and affinity relative to the inhibitor for the B lactamase (Francis *et al.*, 1992 It antimicrobial activity is almost identical with that of ampiciliin. It is hydrolysed all the clinically important B-lactamase except the class Cephasporins of *E. coli* and *Enterobacter* (Francis *et al.*, 1992). Amoxicilin/clavulanic acid is very potent against many microorganisms including *Haemophilus infuelnzae, E. coli, Klebsiella* spp, *Moraxella catarrhalis.*Minimum Inhibitory Concentration (MIC) 2mgml or less against most 90% strain of *Nesseria gonorrhea*, MICs of 4mg/ml or less against most $\geq$ 90% of *S. aureus* and aerobic bacteria and 8mcg/ml or less against most $\geq$ 90% strain of other listed organisms safety and effectiveness of amoxicillin/Clavulanic acid in treatment of clinical infection due to the microorganisms has not established in adequate and well control clinical trial. (Glaxo SmithKline, 2002). In children aged 3 – 14 years given 25mg/Kg of syrup formulation containing amoxicillin and Clavulanic acid in the ratio of 4:1, the average peak plasma concentration of Clavulanic acid was 2mg/l at 1-1.5 with a half life of 1 hour. The aims of the study was to assess the activity of different preparations of Augment in and to recommend physicians to emphasize on the prescription of more effective one. Therefore, it was also designed to assess the effect of increasing ratio of amoxicillin on the activities of augment in against clinical isolates of *Klebsiella, E. coli* and *S. aureus.*

Materials and Methods

Sample Collection

Five clinical isolates each of *Staphylococcus aureus, E. coli* and *Klebsiella* spp were collected from Clinical Microbiology Laboratory of Aminu Kano Teaching Hospital (AKTH). The clinical isolates were identified base on the method described in Cheesbrough (2004).

Drug Samples

Augmentin capsules 370mg, 625mg and 875mg manufactured by Glaxosmithkline in form of 2:1, 4:1 and 6:1 ratio of amoxicillin/Clavulanic acid respectively were purchased from pharmaceutical chemist at Sabon Gari market Kano. Amoxicillin capsule 250mg (Glaxosmithkline) was purchased from Academic Staff Union of University (ASUU) pharmaceutical chemist BUK old site, whilst 8:1, 10:1 was prepared in the laboratory by adding 250mg and 500mg of amoxicillin on 875mg Augmentin capsule (powder) respectively.

Susceptibility Test

Disc diffusion susceptibility test was carried out using Kirby – Bauer (1966) as recommended by National Committee for Clinical Laboratory Standard (NCCLS, 1997). Kirby – Bauer method: This is now the official method of food and drug administration in the USA (Federal Register, 1972). The method defines three degree of sensitivity according to zone diameter and makes no exception for organisms in the urine. Zone diameter is interpreted by reference to published table, and the performance of the test, therefore be strictly standard. Mueller – Hinton agar is specified with single usually high content discs for each drug. Plates are inoculated with a swab dipped into bacterial suspension adjusted to the same density as a barium sulphate standard. The result in just confluent growth. The Kirby – Bauer discs diffusion

susceptibility test performed in accordance to NCCLS method (NCCLS, 1997) give reliable results and hence predict clinical efficacy of the antibiotics tested.

Medium

Mueller-Hinton agar (Bio Science, Spain, MD) was used for discs diffusion test as recommended by National Committee for Clinical Laboratory Standard (NCCLS, 1997).

Preparation of Sensitivity Tests

Discs were prepared base on the method described by Chessbrough (2004) as follows:

Punch disc of 5-6mm diameter from a sheet of what man N0. I Filter paper.
Place disc in a Petri dish at a distance of 2-4mm between each and sterilized in hot air oven at 160^{o}C for 1 hour. Allow to cool and add correct concentration of sterile antimicrobial solution. A standard disc absorbs 0.1ml.

Method of Inoculation

Using a sterile wire loop, touch 3-5 well isolated colonies of similar appearance of the test organism and emulsified in 3-4ml of sterile physiological saline.
In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use) hen comparing turbidities it was easier to view against a printed card or sheet of paper. Using a sterile swab, a plate of Mueller Hinton agar was inoculated. Excess fluid was removed by pressing and rotating the swab against the side of the tubes above the level of the surface of the medium in three directions rotating. The approximately 60% to ensure even distribution. With the Petri dish lid in place allow 3-5 minutes or longer then 15 minutes) for the surfaces of the agar to dry. Using a sterile forceps, appropriate antimicrobial discs were evenly distributed on the inoculated plates.
Within 30 minutes of applying the disc, the plates were inverted and incubated aerobically for 18 – 24 hours at 35^{o}C.

Results

Sensitivity test

The zone diameter produce by each preparation against the clinical isolates were recorded as sensitive, intermediate and resistant by comparing with standard, published by NCCLS (1997). The standards are as follows; for *Staphylococcus aureus* $\geq$ 20mm are said to sensitive and $\leq$ 19 resistant. And for all other organisms except *Streptococcus pneumoniae* and *N. meningitis*, zone diameter $\leq$ 18 are said to be sensitive while 14 – 17mm intermediate and $\leq$ 13 are resistant.

Table 1: Sensitivity Test for *S. aureus* given five level of Augmentin treatment

Species		2:1	4:1	6:1	8:1	10:1	Inference
S. aureus	A	24	27	16	20	21	Base NCCLS standard isolate A was sensitive to 2:1, 4:1, 8:1 and 10:1 and resistant to 6:1
S. aureus	B	15	19	10	14	10	Base NCCLS standard isolate B was are resistant to all preparations
S. aureus	C	18	22	16	16	16	Base NCCLS standard isolate C was sensitive to 4:1 and resistant to all others.
S. aureus	D	19	21	16	17	17	Base NCCLS standard isolate D was sensitive to 4:1 and resistant to all others
S. aureus	E	21	26	17	16	19	Base NCCLS standard isolate E was sensitive to 2:1, 4:1 and resistant to all other preparations

Table 2: Sensitivity Test for *E. coli* given five level of Augmentin treatment

Species		2:1	4:1	6:1	8:1	10:1	Inference
E. coli	A	18	17	13	13	15	Base NCCLS standard isolate A was sensitive to 2:1 and intermediate sensitive to 4:1 and 10:1 and resistant to 6:1 and 8:1
E. coli	B	00	11	00	00	00	Base NCCLS standard isolate B was resistant to all preparations
E. coli	C	17	20	17	15	15	Base NCCLS standard isolate C was highly sensitive to 4:1, intermediate sensitive to 2:1,6:1, 8:1 and 10:1.
E. coli	D	21	27	19	20	18	Base NCCLS standard isolate D was highly sensitive to 2:1, 4:1, 6:1, 8:1 and 10:1.
E. coli	E	11	13	00	00	00	Base NCCLS standard isolate E was resistant to all other preparations

Table 3: Sensitivity Test for *K.* spp given five level of Augmentin treatment

Species		2:1	4:1	6:1	8:1	10:1	Inference
K. spp	A	16	16	12	14	15	Base NCCLS standard isolate A was intermediate sensitive to 2:1, 4:1, 8:1 and 10:1 and resistant to 6:1
K. spp	B	17	00	00	00	00	Base NCCLS standard isolate B was intermediate sensitive to 4:1 and resistant to all preparations
K. spp	C	10	11	00	00	10	Base NCCLS standard isolate C was resistance to all preparations
K. spp	D	17	20	20	20	19	Base NCCLS standard isolate D was highly sensitive to 4:1, 6:1, 8:1 and 10:1 but intermediate to 2:1
K. spp	E	14	17	10	15	13	Base NCCLS standard isolate E was intermediate sensitive to 2:1, 4:1 and 8:1 and resistant to 6:1 and 10:1

Discussion

Staphylococcus aureus, E. coli, Klebsiella spp are important nosocomial and community acquired pathogens. A major cause of concern is their increasing multi-drug resistance which now encompasses what consider the drug of choice. *E. coli, S. aureus, Klebsiella* spp were usually susceptible to amoxicillin – clavulanate, however with increasing use of antimicrobial, resistance begun to emerge; the resistance often conferred by plasmid encoded TEM – type B lactamase – production (Nicolas, 1997 and Reguera *et al.*, 1997). Other mechanisms that have been described include acquired plasmid encoded cephalosporinase (AMPC – types, hyper production of chromosomal AMPC intrinsic to *E. coli*. The alternative of porin channels production of OXA B lactamase and inhibitor resistance mutant of TEM and SHV enzyme (Livermore, 1995, M 'zali *et al.*, 1997; Oliver *et al.*, 1999 and Regeura *et al.*, 1997). From Table one, two, three amoxicillin clavulanate were found to be effective against *E. coli*, because majority of *S. aureus E. coli, a*nd all of species of *Klebsiella* spp are B lactamase producer and clavulanic acid are potent inhibitor of B lactamase, which include class I, II, III and IV, R – Factor mediated enzyme including TEM – 1 and 2, SHV, OXA, 1, 2, 3 and PSE, 1, 2, 3, and 4, and it is also very effective against K1 and Chromosomal enzyme (Harold and Francis, 1992). 4:1 preparation is more effective against the clinical isolates than the

rest of the preparations followed by 2:1 with susceptibility increase from *K.* spp to *E. coli* and then *S. aureus* i.e. 40%, 60% and 80%. This is because as the concentration of amoxicillin double from 2:1 to 4:1 the activity of Augmentin increases but as the concentration continues to double the activity remains approximately constant. *S. aureus* are more susceptible to *E. coli* and *E. coli* are more than *K.* spp, because amoxicillin/clavulanate are potent inhibitor of most enzymes produce by *S. aureus* while some of *E. coli* are extended spectrum B-lactamase producer which are often resistant to B-lactam. B-lactamase inhibitor and also many species of *E. coli* are resistant to amoxicillin/clavulanate due to production of TEM – type and CMY – 2 enzymes (Chaibi *et al*., 1999). From table one, it is indicated that there was no significant difference between 2:1 and 4:1 and there is no significant difference between pairs of two above 4:1 are more effective with mean zone of diameter of 23mm. This is because as the ratio of amoxicillin/clavulanate increase from 2 parts to 4 part, the activity of Augmentin against *S. aureus* increase but as the ratio continue to double or increase the activity remain approximately constant.

From table 2 there is significant difference between 2:1 and 4:1 but there is no significant difference between 6:1, 8:1 and 10:1. 4:1 are more effective than the rest of the preparations. 4 part amoxicillin are more likely to increase the activity of clavulanate against most B-lactamase enzyme produced by *E. coli* but with increase of amoxicillin from 4 part activity decrease. From table 3, there is no significant difference between the level of Co-amoxiclav treatment given to five clinical isolates of *K.* spp, because all known strains of *K.* spp are B-lactamase producers, therefore increasing the ratio of amoxicillin at constant ratio of clavulanate, does not have any effect due to the fact that the activity of amoxicillin against B-lactamase producing strain depend entirely on the concentration present. In general, the activity of B-lactamase (amoxicillin) are greatly enhanced by the presence of B-lactamase inhibitor (clavulanate) against B-lactamase producers, but the degree of enhancement varies with agents and organisms, depending on the intrinsic activity of agent, its penetration and its affinity relative to the inhibitor for the B-lactamase.

Conclusion

Conclusively 4:1 preparations of Augmentin are more effective than the rest of the preparations against clinical isolates of *E. coli* and *S. aureus.* Therefore, it can be use for the treatment of urinary tract and wound infection caused by these organisms, even though the activity of antimicrobial *in-vitro* does not normally reflect its activity *in-vivo* and it can also be used in the treatment of respiratory, pneumonia and bacteremia caused by susceptible organisms of *Klebsiella* spps.

Recommendations

From the research carried out 4:1 preparation are more effective *in-vitro,* therefore I recommend the physicians to subject different preparations of Augmentin currently available into clinical trials for comparative study on their activity *in-vitro.* From these, preparations which are more potent *in-vivo* are emphasized for the treatment of infection caused by susceptible organisms.

References

American Thoracic Society (2000): Guideline for the Treatment of Bacterial Sinusitis. *Otalarangal Head Surgeon*. PP 103.

Chessbrough, M. (1984): *District Laboratory manual for tropical countries.* Vol. 2 PP 132 – 137, 200 – 205.

Croydon, E.A. P., and Sutherland, R. (1971): *Antimicrobial chemotherapy*. 1970. pp 427.

Chaibi, E.B., D. Sirot, G., Poul and R. Labia (1999): Inhibitor resistance TEM – beta lactamase phenotypic genetic, biochemical characteristics. *J. Antimicrob. and Chemother*. 43:447 – 458.

Ericsson, H.M.N., Sheris, J.C. (1971): *Acta Pathogenic Microbiology.* Scand. Section B. Suppl. 271.

Fatima, B.M. (2003): *Introduction to Biostatistics.* 1st ed. Samarib Publishers, Kano. Pp230 -234.

Federal Register (1972): 37:20525.

Francis, W. Or Grady, and Harold, P.L. (1992): *Antibiotic and Chemotherapy*. 6th ed. By Longman group, Limited UK. Pp 84 – 85.

Glaxosmithkline (2002): Augmentin insert leaflet. Beecham Pharmaceutical Factory, England.

Harold, P.L., Francis, W., Or Grady, and Lambert, (1992): *Antibiotic and Chemotherapy*. 6th ed. By Longman group, Limited UK. Pp 83 – 85.

Jackson, R.T., Harris, L.F. and Alfred, R.H. (1978): *Antimicrobial and chemotherapy.* 6th ed. UK. Pp 84 – 85.

Jones, R.N. and M.N. Dudley (1997): Microbiology and pharmacodynamic principles applied to antimicrobial susceptibility testing analysis of correlation between the *in-vitro* test result and clinical response. *Diagnostic microbio. Infect. Disease*. 28:5 – 18.

Kaleida, P.H., C.D. Bluestone, H.E. Rokette, L.W., Basses, J.H., Woltson, J.M., Braek, and E.B. Ubinger (1987): Amoxicillin – clavulanate potassium compare with cefavelo for acute otitis media in infant and children. *Peaditric Inf. J. Dis. J.* 6:265 – 267.

Kirby, M. and Bauer, A.W. (1966): Antibiotics Susceptibility test by Standard Single Disc Method. *Am. J. Clin. Path.* 45:493 – 496.

Lawrence, P.G., Arnold, P. Lambert, Francis, W. O' Grady, (1992): *Antibiotic and Chemotherapy*. 6th ed. By Longman group, Limited UK. Pp 86 – 87.

Livermore, R.R., I.R. Johnson, B. Foxman, T.T. O' Bryan, K.E., Fullerton and L. W. Riley (2001): Wide spread distribution of Urinary tract infection. Cosn by Multidrug resistance *E. coli* clonal group. *N. Eng. J. Med.* 345:1007 – 1013.

Manges, A.R., I.R. Johnson, B. Foxman, T.T.O' Bryan, K.E., Fullerton and L.W. Riley (2001): Wide spread distribution of Urinary tract infection caused by multi-drug *E. coli.* Clonal group. *N. Eng. J. of Med.* 345:1007 – 1013.

Mathai, D., R.N., Jone and M.A. Pfaller (2001): Epidemiology and Frequency of resistance among pathogen causing urinary tract infection in 1510 hospitalized patients. A report from the Sentry antimicrobial surveillance programme (North America). *Diagn. Microbiology of Infection Dis.* 405:129 – 136.

M' zali, F.H., J. Herritage, D.M, Gascoigne – Binzi, M. Denton, N.J. Todd and P.M. Hawkey (1997): Transcontinental importation into the UK of *E. coli* expressing a plasma mediated AMPC – type B-lactamase, exposed during an out break of SHV – 5 extended spectrum B-lactamase in a Leed Hospital. *J. Antimicrobial Chemother.* 40:823 – 831.

National Committee for Clinical Laboratory Standards (1997): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard. NCCLS Document M 7-A4

National Committee for Clinical Laboratory Standards (1998): NCCLS document. M100 -158. 8th ed.

Oliver, A., M. Perez- Vazquez, M. Martinez – Ferre, F. Baquero, J. De Rafael and R. Canton (1999): Ampicilin – Sulbactan and amoxicillin – clavulanate susceptible test of *E. coli* isolate with different B-lactam resistance phenotypes. *Antimicrobial Chemother.* 43:862 – 867.

Reading, L., and Core, M.N. (1977): *Antimicrob and Chemother*. 11:852.

Reguera, J. F. Baquero, J.C., Perez-Diaz and J.L. Martinez (1991): Factors determining resistance to beta lactam combined with beta lactamase inhibitor in *Escherichia coli. J. Antimicrob. Chemother*. 27:569 – 575.

Sutherland, R., and Rolinson, G.N.S. (1970): *Microbial and Chemother*. P411

WHO (1977): Expert Committee on Biological Standardization. *Technical Report Series*. 610. p 98.