Journal of Pharmacy and Allied Health Sciences

ISSN 2224-2503

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Journal of Pharmacy and Allied Health Sciences 2 (1): 12-20, 2012
ISSN 2224-2503 / DOI: 10.3923/jpahs.2012.12.20

Synergistic Influence of Tetracycline on the Antibacterial Activities of Amoxicillin Against Resistant Bacteria

O.O. Olajuyigbe
Phytomedicine Research Centre, Department of Botany, University of Fort Hare, Alice, 5700, South Africa

Corresponding Author: O.O. Olajuyigbe, Department of Biosciences and Biotechnology, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria Tel: +27730562219

ABSTRACT

In this study, the *in vitro* influence of antibacterial activity of amoxicillin and tetracycline on each other was investigated by macrobroth dilution and checkerboard assay methods. Each of the antibiotics as well as their combinations proved inhibitory against all the tested organisms with the exception of *Acinetobacter calcoaceticus* showing high level of resistance. The inhibition zones produced by the combination of tetracycline and amoxicillin with those obtained from each antibiotic showed synergistic interaction which is concentration dependent. The checkerboard assay results showed 87.5 synergy and 12.5% antagonism with a high reduction in the Minimum Inhibitory Concentrations (MICs) and a strong bactericidal activity against the test organisms. Results of macrobroth dilution and checkerboard assays are complementary and indicated a strong synergistic interaction between amoxicillin and tetracycline.

Key words: Antibiotics, checkerboard assay, macrobroth dilution, synergy, antagonism

INTRODUCTION

The changing antimicrobial sensitivity pattern of causative organisms has posed a therapeutic challenge in treating patients in the recent time. Unregulated provision of antibiotics, dispensing of insufficient doses, reduced adherence to complete dose regimens and the poor quality of the drug supply (Goossens *et al*., 2005; Blomberg *et al*., 2007; Cars *et al*., 2008), decreasing effectiveness of conventional antimicrobial drugs (Park *et al*., 2011) coupled with the overuse or misuse of antibiotics accelerating antibiotic resistance (Collignon *et al*., 2009; Love *et al*., 2011) and Extended Spectrum Beta Lactamases (ESBL) (Gururajan *et al*., 2011) has caused serious problems due to the rapid emergence of multidrug-resistant pathogens as well as creating a major challenge for the public health in the world (Zhang *et al*., 2011). Resulting from these challenges, antibiotic resistance threatens human health worldwide (Karou *et al*., 2009; Hogberg *et al*., 2010), affect treatment decisions, patient outcome, health care expenditure and public perceptions of health care delivery (Okeke *et al*., 2005; Sosa *et al*., 2010). Since there is a growing awareness of public health concerns associated with the emergence of drug-resistant strains of bacteria (Raghunath, 2008) and antibiotic resistance is currently involving most of the known classes of natural and synthetic antibiotics (Pednekar *et al*., 2011), there is an urgent need for antibiotics with novel scaffolds and mechanism of actions. In response to identifying alternative means to combat these situations, combination therapy or polypharmacy has been acknowledged.

Combination therapy or polypharmacy (Alam *et al.*, 2011) which is the concurrent use of two or more medications in a single patient has increased progressively with increasing global population, progression of the existing infections, onset of new microbial infections and development of resistance in infectious agents. It is known to be superior to single modality therapy (Masoudzadeh and Khalilian, 2007), able to minimize side effects (Haidari *et al.*, 2008) and may be more effective in the treatment of resistant infections. The mitigating factors may include proliferation of new antimicrobial agents thought to be better tolerated than previously available drugs, the increasing tendency to treat partial response by introducing additional agents and increased use of drugs to alleviate adverse effects accompanying use of existing anti-infective agents. However good it is by simplifying treatment and improve drug compliance by reducing the burden of taking multiple drugs (Chobanian *et al.*, 2003; Mancia *et al.*, 2007), the mechanistic basis of many drug-drug interactions is well established (Stockley, 2002). The drug-drug interactions can occur as a result of changes in pharmacodynamics and/or pharmacokinetics. A pharmacodynamic interaction occurs when drugs act on the same receptor, site of action, or physiologic system. A drug or substance that interferes with or accentuates the absorption, distribution, or elimination of a second drug produces a pharmacokinetic interaction. While some drug interactions are beneficial, others are clinically harmful and may eventually result in therapeutic failures.

To avoid unpleasant pharmacological and therapeutic actions related to drug-drug interactions (Rashidul Bari *et al.*, 2000) and forestall therapeutic failures, development of bacterial resistance and increased cost of treatment often associated with the inept polypharmaceutical practices, researches in synergistic mechanisms of action for controlling microbial infections with reduced side effects becomes indispensable. Hence, since there is a lack of information on the interactions between amoxicillin and tetracycline and the effects of their combination against bacteria of clinical importance, this study was designed to investigate the influence these antibiotics could have on the antibacterial activity of one another.

MATERIALS AND METHODS

Bacterial strain: The bacteria used in this study included *Bacillus cereus* (ATCC 10702), *Staphylococcus aureus* (ATCC 6538), *Salmonella typhi* (ATCC 13311), *Acinetobacter calcoaceticus* UP, *Klebsiella pneumoniae* KZN, *Proteus vulgaris* KZN, *Enterococcus faecalis* KZN and *Staphylococcus aureus* OK_{2a}. These organisms were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. The antibacterial assays were carried out using Mueller Hinton II Agar (Biolab) and broth.

Antibiotics used in this study: Antibiotic powders of Amoxicillin (Duchefa) and Tetracycline hydrochloride (Duchefa) were used. Stock antibiotic solutions were prepared and dilutions made according to the CLSI (Clinical Laboratory Standardization Institute) method or manufacturer's recommendations (NCCLS, 1997; Richard *et al.*, 2007).

Antibiotic susceptibility testing: Agar diffusion method: Each of the isolates was standardized using colony suspension method. Each strain's suspension was matched with 0.5 McFarland standards to give a resultant concentration of 1.5×10^{8} CFU mL^{-1}. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique (Cheesbrough, 2006) by swabbing the Mueller-Hinton Agar (MHA) (Oxoids UK) plates with the resultant saline suspension of each strain. Wells were then bored into the agar medium with heat

sterilized 6 mm cork borer. The wells were filled with 100 µL of different concentrations (31.25, 62.5 and 125 µg mL^{-1}) of each of the antibiotics taking care not to allow spillage of the solutions onto the surface of the agar. To determine the combinatorial effect of the antibiotics, different solutions containing combined concentrations (31.25, 62.5 and 125 µg mL^{-1}) of tetracycline and amoxicillin were prepared and used. The plates were allowed to stand for at least 30 min before being incubated at 37°C for 24 h (BSAC, 2002). The determinations were done in duplicates. After 24 h of incubation, the plates were examined if there is any zone of incubation (Bauer *et al.*, 1966). The diameter of the zone of inhibition produced by the antibiotic alone and its combination were measured and interpreted using the CLSI zone diameter interpretative standards (CLSI, 2008). Synergism was considered when combinations exhibited inhibition zones increment of 0.5 mm above those produced by the individual antibiotics (Ahmad and Aqil, 2007).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC): The Minimum Inhibitory Concentrations (MICs) for the two antibiotics under study were determined in duplicate by the macrobroth dilution method in Meuller Hinton broth according to CLSI (Clinical Laboratory Standardization Institute) (Richard *et al.*, 2007). To determine the MICs of each antibiotic, the concentrations used for each of the antibiotics (0.0019-500) µg mL^{-1} were prepared by serial dilution in Mueller Hinton broth. To determine their combinatorial effects, combinations of different concentrations used in the determination of the MICs of each of the antibiotics were used. The tubes were inoculated with 100 µL of each of the bacterial strains. Blank Mueller Hinton broth was used as negative control. The bacterial containing tubes were incubated aerobically at 37°C for 24 h. Each combination assay was performed two times. The MIC was defined as the lowest dilution that showed no growth in the Mueller Hinton broth. The MBC was defined as the lowest dilution that did not show growth on Mueller Hinton agar after subculturing from the lowest concentrations that showed no bacterial growth (Lorian, 1980).

Checkerboard assay: The range of drug concentration used in the checkerboard assay was such that the dilution range encompassed the MIC for each drug used in the analysis. The Fractional Inhibitory Concentration (FIC) was derived from the lowest concentrations of the two antibiotics in combination permitting no visible growth of the test organisms on the Mueller Hinton agar plates after an incubation for 24 h at 37°C (Mandal *et al.*, 2004). FIC indices were calculated using the formula FIC index = (MIC of tetracycline in combination/MIC of tetracycline alone) + (MIC of amoxicillin in combination/MIC of amoxicillin alone). Synergism by the checkerboard method was defined as an FIC index of ≤1, additive effect was defined as an FIC index = 1, Indifference effect was defined as an FIC index of >1 and ≤4 and antagonism effect was defined as an FIC index of > 4 (Kamatou *et al.*, 2006; Petersen *et al.*, 2006). Concentrations within the FIC panel were such that the MIC of each antibiotic was in the middle of the range of concentrations tested but lower than the MICs of the respective antibiotics.

RESULTS

In this study, each of the antibiotics as well as their combinations proved inhibitory against all the tested organisms with the exception of *Acinetobacter calcoaceticus* that showed high level of resistance to these antibiotics at the concentrations used singly or in combinations *in vitro* (Table 1). The resistance exhibited by this isolate at the low concentration was corroborated by the result obtained with the minimum inhibitory and bactericidal concentrations (Table 2). A

Table 1: Zones of inhibition resulting from each antibiotic alone and their combinations

	Tetracycline alone (± 1.0 mm)			Amoxicillin alone (± 1.0 mm)			Amx + Tet combined (± 1.0 mm)		
	125	62.5	31.25	125	62.5	31.25	125/125	62.5/62.5	31.25/31.25
Bacteria	(μg mL^{-1})								
Bacillus cereus (ATCC 10702)	30	28	25	23	21	18	35	33	31
Staphylococcus aureus (ATCC 6538)	23	20	18	27	24	21	33	30	28
Salmonella typhi (ATCC 13311)	32	30	26	33	31	28	38	35	32
Acinetobacter calcoaceticus UP	0	0	0	0	0	0	0	0	0
Klebsiella pneumoniae KZN	17	15	0	22	20	17	27	24	20
Proteus vulgaris KZN	17	13	0	30	28	25	32	29	26
Enterococcus faecalis KZN	16	13	0	32	30	27	42	37	30
Staphylococcus aureus OK_{2a}	22	19	17	25	23	20	30	28	25

Table 2: Minimum inhibitory and bactericidal concentration values for amoxicillin and tetracycline antibiotics

	Tetracycline (μg mL^{-1})		Amoxicillin (μg mL^{-1})		Tet/Amx combined (μg mL^{-1})
Bacteria	MIC	MBC	MIC	MBC	MIC
Bacillus cereus (ATCC 10702)	0.0305	0.122	7.8125	15.625	0.007625/0.007625
Staphylococcus aureus (ATCC 6538)	3.906	7.8125	125	125	1.953/1.953
Salmonella typhi (ATCC 13311)	0.0305	0.061	0.4883	0.488	0.0038/0.0038
Acinetobacter calcoaceticus UP	125	250	250	250	62.5/62.5
Klebsiella pneumoniae KZN	7.8125	7.8125	31.25	31.25	3.9063/3.9063
Proteus vulgaris KZN	7.8125	15.625	0.488	0.488	1.953/1.953
Enterococcus faecalis KZN	15.625	15.625	0.9766	0.976	0.488/0.488
Staphylococcus aureus OK_{2a}	0.9765	1.953	62.5	125	0.488/0.488

comparison of the inhibition zones produced by the combination of tetracycline and amoxicillin with those obtained from each antibiotic showed there is synergistic interaction which is concentration dependent between the two antibiotics. With the exception of *Acinetobacter calcoaceticus,* inhibitory effects of the combined antibiotics were consistently higher than the inhibitory effects of the individual antibiotic. *Staphylococcus aureus* (ATCC 6538), *Acinetobacter calcoaceticus* UP and *Staphylococcus aureus* OK_{2a} considered resistant to amoxicillin by having low MIC values were drastically inhibited by the combined antibiotics. While the MIC values for tetracycline ranged between 0.0305 and 125 μg mL^{-1}, the MIC values of amoxicillin ranged between 0.061 and 250 μg mL^{-1}. Although most of these bacterial isolates had higher MIC values in amoxicillin than in tetracycline, all the bacteria exhibited higher inhibition zones when tested against amoxicillin as compared to tetracycline.

The results of checkerboard assay combinations showed 87.5% synergy and 12.5% antagonism (Table 3). Synergistic interactions between tetracycline and amoxicillin were observed against *Bacillus cereus* (ATCC 10702), *Staphylococcus aureus* (ATCC 6538), *Salmonella typhi* (ATCC 13311), *Acinetobacter calcoaceticus* UP, *Klebsiella pneumoniae* KZN, *Enterococcus faecalis* KZN and *Staphylococcus aureus* OK_{2a} (FIC indices of 0.132-0.625) while combinations against *Proteus vulgaris* KZN exhibited antagonistic interaction (FIC index of 4.25). Although synergy did not depend on the bacterial susceptibilities to each of the antibiotics when used separately but was observed for most of the strains, a high decrease in MIC and a strong bactericidal activity against these strains is of significant importance.

Table 3: Fractional inhibitory concentration values for the combination of amoxicillin and tetracycline antibiotics

Bacteria	FIC TET	FIC AMX	FICI	REMARK
Bacillus cereus (ATCC 10702)	0.25	0.0009	0.251	Synergy
Staphylococcus aureus (ATCC 6538)	0.5	0.156	0.516	Synergy
Salmonella typhi (ATCC 13311)	0.125	0.0078	0.132	Synergy
Acinetobacter calcoaceticus UP	0.5	0.125	0.625	Synergy
Klebsiella pneumoniae KZN	0.5	0.125	0.625	Synergy
Proteus vulgaris KZN	0.25	4	4.25	Antagonistic
Enterococcus faecalis KZN	0.031	0.5	0.531	Synergy
Staphylococcus aureus OK_{2a}	0.5	0.0078	0.508	synergy

FIC TET: Fractional inhibitory concentration of tetracycline alone, FIC AMX: Fractional inhibitory concentration of amoxicillin alone, FICI: Fractional inhibitory concentration indices

DISCUSSION

Amoxicillin is a broad-spectrum bacteriolytic antibiotic while tetracycline is a broad-spectrum bacteriostatic antibiotic. They are widely used to treat community and healthcare-associated infections. The emergence and dissemination of antimicrobial resistance to these antibiotics represents a significant threat to human health (Woodford and Livermore, 2009). For these antibiotics, numerous resistance mechanisms including expression of drug-destroying enzymes such as β-lactamases (Paterson and Bonomo, 2005), altered drug targets such as conformational changes in penicillin-binding proteins (PBPs), decreased bacterial permeability, increased drug efflux (Wright, 2005; Piddock, 2006; Chen *et al.*, 2011), ribosomal protection and tetracycline modification (Roberts, 2003, 2005) have been described. Discovering a combination therapy capable of preventing or inhibiting the expression of these different mechanisms of resistance will be a giant stride towards effective chemotherapy using these antibacterial agents.

In this study, the impact of each antibiotic on each organism is specific and differs from organism to organism. Amoxicillin and tetracycline showed a powerful bactericidal activity to most of the test organisms and their combinations have apparent synergistic effects. The synergistic effect may be due to the formation of certain complexes which becomes effective in inhibiting the affected microorganisms either by inhibiting the bacterial protein and cell wall synthesis, by causing its lyses or death or by attacking different target sites apart from their known target sites of actions. Bliss (1939) and Greco *et al.* (1995) indicated that combinations of drugs may inhibit bacterial growth in complex ways, deviating from the neutral situation expected when they do not interact. Hence, the double attack of both agents on different target sites of the bacteria could have theoretically lead to either an additive or a synergistic effect (Esimone *et al.*, 2006) as obtained in this study.

The synergistic interactions resulting in drastic increase in the MICs of the test antibiotics are significant. The ability of these combinations to inhibit Gram-negative and Gram-positive as well as those considered resistant to amoxicillin to a great extent showed that combined therapy of tetracycline and amoxicillin against infectious agents could be more effective than their monotherapy and amoxicillin-clavulanic acid as well as killing pathogens before resistance development. While combined antibiotic therapy has been shown to delay the emergence of bacteria resistance, the synergistic effects are often known to surpass their individual inhibitory activity. In agreement with the report of Sato *et al.* (2004), the combination of the two agents showed significant synergism because the test organisms had a high MIC to at least one of the agents. The significant increase in the MICs of the antibiotics in the combined state showed that the

combination of the two antibiotics is a veritable source of potential resistance modifying agents (Dickson *et al.*, 2006). Hence, while new therapy can be developed by combining synthetic, semi synthetic and natural drugs in pharmaceutical formulations (Kadam *et al.*, 2010), combining the two antibiotics while reducing their doses and the risk of dose-related toxicity will result in enhanced efficiency (Salam *et al.*, 2009).

Although, there is a dearth of information on the interaction between amoxicillin and tetracycline, interactions between these antibiotics and other chemotherapeutic agents have been reported. Drug-drug interactions between amoxicillin (Wood and Deeble, 1993; Soto *et al.*, 1993; Bandrowsky *et al.*, 1996) as well as amoxicillin-clavulanic acid (Penning-van Beest *et al.*, 2001) with warfarin have been reported *in vivo*. Most drug interactions of tetracycline are pharmacokinetic and reflect changes in their absorption and elimination or the absorption and elimination of other agents. The plasma concentrations of tetracycline are markedly reduced (30 to 90%) with the concurrent administration of products containing divalent, trivalent and multivalent cations. Tetracycline lowers plasma concentrations of atovaquone and increases that of quinine when concomitantly administered against *Plasmodium falciparum* (Pai *et al.*, 2006). Vancomycin combined with cephalosporins and penicillins has been shown to synergistically inhibit a number of gram-negative bacilli (Donabedian and Andriole, 1977). Darras-Joly *et al.* (1996) indicated synergy between ß-lactam antibiotics and gentamicin against *Streptococcus pneumonia*. Olsson *et al.* (1961) reported antagonistic interactions for penicillin combined with tetracycline against pneumococci, Chan *et al.* (2007) reported that phenothiazines interact synergistically with amoxicillin against *Burkholderia pseudomallei*.

In conclusion, these *in vitro* data still need to be validated by assessing the clinical performances of these combinations. These findings could also prove to be a promising alternative in the treatment of patients for whom existing antimicrobial treatment fails. Despite the lack of knowledge for the underlying mechanism of the synergistic effect of amoxicillin and tetracycline combinations, there is a great potential for use of such combinations against infectious pathogens since it may be able to make some untreatable resistant infections treatable at the recommended dosages that are often marginally effective against resistant strains when used alone. A wider study with increase in the number of drugs and clinical isolates are necessary to establish the mode of action and the mechanism of synergy which is fundamental to the development of a more effective pharmacological agent.

REFERENCES

Ahmad, I. and F. Aqil, 2007. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESßL-producing multidrug-resistant enteric bacteria. Microbiol. Res., 162: 264-275.

Alam, K.D., M.K. Hosain, S. Kabir, R.M.A.A. Chowdhury and S. Mahjabeen *et al.*, 2011. *In vitro* binding chemistry of amlodipine besylate (calcium channel blocker) and atorvastatin calcium (HMG-CoA reductase inhibitor) to serum albumin and their mutual effect to displace each other from the binding site. Am. J. Drug Discovery Dev., 1: 220-230.

BSAC, 2002. Disc diffusion methods for antimicrobial susceptibility testing. Br. Soc. Antimicrob. Chemother., 2: 1-46.

Bandrowsky, T., A.A. Vorono, T.J. Borris and H.W. Marcantoni, 1996. Amoxicillin-related postextraction bleeding in an anticoagulated patient with tranexamic acid rinses. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol., 82: 610-612.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bliss, C.I., 1939. The toxicity of poisons applied jointly. Ann. Applied Biol., 26: 585-615.

Blomberg, B., K.P. Manji, W.K. Urassa, B.S. Tamim and D.S.M. Mwakagile *et al.*, 2007. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: A prospective cohort study. BMC Infect. Dis., Vol. 7. 10.1186/1471-2334-7-43.

CLSI, 2008. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement. 18th Edn., Clinical and Laboratory Standard Institute, USA, ISBN-13: 9781562386535, Pages: 181.

Cars, O., L.D. Hogberg, M. Murray, O. Nordberg and S. Sivaraman *et al.*, 2008. Meeting the challenge of antibiotic resistance. Br. Med. J., 337: 726-728.

Chan, Y.Y., Y.M. Ong and K.L. Chua, 2007. Synergistic Interaction between Phenothiazines and Antimicrobial Agents against Burkholderia pseudomallei. Antimicrobial Agents Chemotherapy, 51: 623-630.

Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries, Volume 2. 2nd Edn., Cambridge University Press, UK., ISBN-13: 9780521676311, pp: 64-68, 136-142.

Chen, C.M., M. Huang, H.F. Chen, S.C. Ke, C.R. Li, J.H. Wang and L.T. Wu, 2011. Fusidic acid resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a Taiwanese hospital. BMC Microbiol., Vol. 11. 10.1186/1471-2180-11-98.

Chobanian, A.V., G.L. Bakris, H.R. Black, W.C. Cushman and L.A. Green *et al.*, 2003. The seventh report of the joint national committee on prevention, detection, evaluation and treatment of high blood pressure: The JNC 7 report. J. Am. Med. Assoc., 289: 2560-2572.

Collignon, P., P.J. Howers, T.M. Chiller, A. Aidara-Kane and F.M. Aarestrup, 2009. World health organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. Clin. Infect. Dis., 49: 132-141.

Darras-Joly, C., J.P. Bedos, C. Sauve, P. Moine, E. Vallee, C. Carbon and E. Azoulay-Dupuis, 1996. Synergy between amoxicillin and gentamycin in combination against a highly penicillin-resistant and -tolearant strain of *Streptococcus pneumonia* in a mouse pneumonia model. Antimicrob. Agents Chemother., 40: 2147-2151.

Dickson, R.A., P.J. Houghton, P.J. Hylands and S. Gibbons, 2006. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* Baill, *Securinega virosa* Roxb and willd and *Microglossa pyrifolia* Lam. Phytother. Res., 20: 41-45.

Donabedian, H. and V.T. Andriole, 1977. Synergy of vancomycin with penicillins and cephalosporins against pseudomonas, klebsiella and serratia. Yale J. Biol. Med., 50: 165-176.

Esimone, C.O., I.R. Iroha, E.C. Ibezim, C.O. Okeh and E.M. Okpana, 2006. *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. Afr. J. Biotech., 5: 1082-1086.

Goossens, H., M. Ferech, R.V. Stichele and M. Elseviers, 2005. Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. Lancet, 365: 579-587.

Greco, W.R., G. Bravo and J.C. Parsons, 1995. The search for synergy: A critical review from a response surface perspective. Pharmacol. Rev., 47: 331-385.

Gururajan, G., K.A. Kaliyaperumal and B. Ramasamy, 2011. Prevalence of extended spectrum beta lactamases in uropathogenic *Escherichia coli* and *Klebsiella* species in a Chennai suburban tertiary care hospital and its antibiogram pattern. Res. J. Microbiol., 6: 796-804.

Haidari, F., M.R. Rashidi, S.A. Keshavarz, S.A. Mahboob, M.R. Eshraghian and M.M. Shahi, 2008. Effects of onion on serum uric acid levels and hepatic xanthine dehydrogenase/xanthine oxidase activities in hyperuricemic rats. Pak. J. Biol. Sci., 11: 1779-1784.

Hogberg, L.D., A. Heddini and O. Cars, 2010. The global need for effective antibiotics: Challenges and recent advances. Trends Pharmacol. Sci., 31: 509-515.

Kadam, N.M., H.S. Chaudhari, J.K. Parikh, V.S. Modi, S.U. Kokil and V.M. Balaramnavar, 2010. De novo combination therapy in retroviral infection. Int. J. Virol., 6: 219-223.

Kamatou, G.P.P., A.M. Viljoen, S.F. van Vuuren and R.L. van Zyl, 2006. *In vitro* evidence of antimicrobial synergy between *Salvia chamelaeagnea* and *Leonotis leonurus*. South Afr. J. Bot., 72: 634-636.

Karou, S.D., D.P. Ilboudo, W.M.C. Nadembega, Y. Ameyapoh and D. Ouermi *et al*., 2009. Antibiotic resistance in urinary tract bacteria in Ouagadougou. Pak. J. Biol. Sci., 12: 712-716.

Lorian, V., 1980. Antibiotics in Laboratory Medicine. Williams and Wilkins, Baltimore, MD., USA., pp: 95-98.

Love, D.C., M.F. Davis, A. Bassett, A. Gunther and K.E. Nachman, 2011. Dose imprecision and resistance: Free-choice medicated feeds in industrial food animal production in the United States. Environ. Health Perspect., 119: 279-283.

Mancia, G., G. de Backer, A. Dominiczak, R. Cifkova and R. Fagard *et al*., 2007. ESH-ESC practice guidelines for the management of arterial hypertension: ESH-ESC task force on the management of arterial hypertension. J. Hypertens., 25: 1751-1762.

Mandal, S., M.D. Mandal and N.K. Pal, 2004. Evaluation of combination effect of ciprofloxacin and cefazolin against *Salmonella enteric* serovar *typhi* isolates by *in vitro* methods. Calicut Med. J., 2: e2-e2.

Masoudzadeh, A. and A.R. Khalilian, 2007. Comparative study of clozapine, electroshock and the combination of ECT with clozapine in treatment-resistant schizophrenic patients. Pak. J. Biol. Sci., 10: 4287-4290.

NCCLS, 1997. Methods for Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically. 3rd Edn., National Committee for Clinical Laboratory Standards, Villanova, PA., USA.

Okeke, I.N., R. Laxmaninarayan, Z.A. Bhutta, A.G. Duse and P. Jenkins *et al*., 2005. Antimicrobial resistance in developing countries. Part 1: Recent trends and current status. Lancet Infect. Dis., 5: 481-493.

Olsson, R.A., J.C. Kirby and M.J. Romansky, 1961. Pneumococcal meningitis in the adult. Clinical, therapeutic and prognostic aspects in forty-three patients. Ann. Int. Med., 55: 545-549.

Pai, M.P., K.M. Momary and K.A. Rodvold, 2006. Antibiotic drug interactions. Med. Clin. N. Am., 90: 1223-1225.

Park, S.C., Y. Park and K.S. Hahm, 2011. The role of antimicrobial peptides in preventing multidrug resistant bacterial infections and biofilm formation. Int. J. Mol. Sci., 12: 5971-5992.

Paterson, D.L. and R.A. Bonomo, 2005. Extended-spectrum beta-lactamases: A Clinical Update. Clin. Microbiol. Rev., 18: 657-686.

Pednekar, P., R. Jain and G. Mahajan, 2011. Anti-infective potential of hot-spring bacteria. J. Glob. Infect. Dis., 3: 241-245.

Penning-van Beest, F.J., E. van Meegen, F.R. Rosendaal and B.H. Stricker, 2001. Drug interactions as a cause of overanticoagulation on phenprocoumon or acenocoumarol predominantly concern antibacterial drugs. Clin. Pharmacol. Ther., 69: 451-457.

Petersen, P.J., P. Labthavikul, C.H. Jones and P.A. Bradford, 2006. *In vitro* antibacterial activities of tigecycline in combination with other antimicrobial agents determined by chequerboard and time-kill kinetic analysis. J. Antimicrob. Chemother., 57: 573-576.

Piddock, L.J., 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin. Microbiol. Rev., 19: 382-402.

Raghunath, D., 2008. Emerging antibiotic resistance in bacteria with special reference to India. J. Biosci., 33: 593-603.

Rashidul Bari, A.H.M., A.T.M. Zafrul Azam, M.S. Amran and M.A. Hossain, 2000. *In vivo* effects of ibuprofen and naproxen on the plasma concentration of diltiazem in rabbits. Pak. J. Biol. Sci., 3: 555-557.

Richard, S., S.M. Lynn and C.G. Avery, 2007. Antimicrobial Susceptibility Testing Protocols. CRC Press, New York.

Roberts, M.C., 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol. Lett., 245: 195-203.

Roberts, M.C., 2003. Acquired tetracycline and macrolide-lincosamides-streptogramin resistance in anaerobes. Anaerobe, 9: 63-69.

Salam, M.A., M.A. Baki, A.T.M. Zafrul Azam, M.S. Amran, F.M. Amjad, B. Rokaya and M.A. Hossain, 2009. *In vitro* and *in vivo* effects of glipizide and gliclazide on the protein binding, plasma concentration and serum glucose, cholesterol and creatinine levels of ibuprofen. J. Pharmacol. Toxicol., 4: 307-313.

Sato, Y., H. Shibata, T. Arai, A. Yamamoto, Y. Okimura, N. Arakaki and Higuti, 2004. Variation in synergistic activity by flavonone and its related compounds on the increased susceptibility of various strains of methicillin-resistant *Staphylococcus aureus* to ßlactam antibiotics. Int. J. Antimicrob. Agents., 24: 226-233.

Sosa, A.J., D.K. Byarugaba, C.F. Amabile-Cuevas, P.R. Hsueh, S. Kariuki and I.N. Okeke, 2010. Antimicrobial Resistance in Developing Countries. Springer, New York .

Soto, J., J.A. Sacristan, M.J. Alsar, C. Fernandez-Viadero, R. Verduga, 1993. Probable acenocoumarol-amoxycillin interaction. Acta Haematol., 90: 195-197.

Stockley, I H., 2002. Drug Interactions. The Pharmaceutical Press, London.

Wood, G.D. and T. Deeble, 1993. Warfarin: Dangers with Antibiotics. Dent Update, 20: 352-353.

Woodford, N. and D.M. Livermore, 2009. Infections caused by Gram-positive bacteria: A review of the global challenge. J. Infect., 59: S4-S16.

Wright, G.D., 2005. Bacterial resistance to antibiotics: Enzymatic degradation and modification. Adv. Drug. Deliv. Rev., 57: 1451-1470.

Zhang, T., X.X. Zhang and L. Ye, 2011. Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. PLoS One, Vol. 6, No. 10. 10.1371/journal.pone.0026041.