Evaluation of *in vitro* Combined Antibacterial Activity of Some Commonly Used Antibiotics of Choice against Urinary Tract Infection Pathogens by Activity Index Profile.

*Ekong, U. S., Ubulom, P. M. E., and Udo, S. F.
Pharmaceutical Microbiology/Biotechnology Unit, Department of Pharmaceutics/ Pharmaceutical Technology,
Faculty of Pharmacy, University of Uyo, Uyo,
Akwa Ibom State, Nigeria.

ABSTRACT

Evaluation of the *in vitro* combined antibacterial activity of antibiotics of choice: ciprofloxacin ((C), penicillin (P), trimethoprim-sulfamethoxazole (TMP-SMX), and cefixime (CX) against authochthonous and allochthonous urinary tract infection (UTI) pathogens: *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), coagulase-negative *Staphylococcus aureus* (CONSA): *Staphylococcus saprophyticus* (SS) and *Staphylococcus aureus* (SA) was carried-out at 1xMIC, by the modified agar-well diffusion technique and the nature of the interactions assessed by activity-index profile (AIP). The preliminary antibacterial sensitivity assayed by agar-well diffusion technique showed excellent antibacterial activity of the agents against the tested UTI pathogens with inhibition zone diameters (IZD) within the range (20.0 – 50.0 mm), except cefixime, which was fairly active against EC and CONSA, with IZD of 12.0 mm; and inactive against PA and SA. The antibacterial activity of the agents was in the descending order: C > P > TMP – SMX > CX. The minimum inhibitory concentrations (MICs) of the active agents against the UTI pathogens determined by macrobroth-dilution technique were within the range: C (0.00781 – 0.0098 mg/ml), P(0.12 mg/ml); and TMP – SMX (4.80 – 0.60 mg/ml), with corresponding qualitative inhibitory potency, within the range (12.0 – 38.0 mm) for the UTI pathogens, except TMP-SMX which was inactive against SA. The interactions of the agents at 1xMIC against the UTI pathogens showed qualitative inhibitory activity within the range (9.0 – 25.0 mm), which were comparatively assessed by the AIP, indicating predominantly synergism and lessly indifferent for EC, CONSA and SA; and total antagonism for PA.

KEYWORD: Combined-Antibacterial Activity, Antibiotics of Choice, Urinary Tract Infection Pathogens, interactions, auchthocthonous and allocthonous pathogens.

INTRODUCTION

The need for a better-healthy life has lead to the development of medicaments, particularly antibiotics for the treatment of various microbial infections and diseases. This development has gone a long way to alleviate human health problems. A greater percentage of these antibiotics are isolated and developed from microorganisms and are generally used for the treatment of infections. Conversely, more than 70 % of the diseases affecting humans results from microbial infections from bacteria, fungi, viruses, etc; hence constituting the leading cause of death in human history (Fleming, 1980). Consequently, pathogenic microorganisms have various portals of entry into the human system, eliciting their virulence and pathogenicity. One of these portals of entry and pathogenesis is through the urinary tract via two major routes, namely: the urethra and by hematogenous spread (Katherine *et al.*, 2009). The entry through the urethra is the more common, and is often by self-inoculation with faecal bacteria; while the hematogenous route is much less common, and results from seeding by the kidney from a primary site of infection such as carbuncle, osteomyelitis, endocarditis, or empyema.

Urinary tract infection (UTI) is a condition where one or more parts of the urinary system comprising the kidney, ureters, bladder and urethra become infected by bacteria (Niceolle *et al.*, 2006). Thus, the UTI is basically a bacterial infection that affects parts of the urinary tract. The primary etiologic agents of UTI include the enteric bacteria such as *E. coli* and *Pseudomonas aeruginosa*, and also the staphylococci mostly the coagulase negative staphylococci: *Staphylococcus Saprophyticus* which are responsible for vast majority of UTIs as the predominant pathogens (Schlester and Kloos, 1978; Rupp *et al.*, 1992; Gruenberg, 1994; Mandell *et al.*, 2007; Scheffer, 2002; Kuroda *et al.*, 2009 and Takhar, 2011; Widerstorm, *et al.*, 2012).

* Corresponding Author: ubsa4@yahoo.com

Of all these, *Escherichia coli*, predominantly accounts for 80 – 85% of UTIs and *Staphylococcus saprophyticus*, accounts for 5 – 10% of UTIs. Some enterics including *Klebsiella*, *Proteus* and *Enterobacter* species are uncommon and typically related to abnormalities of urinary catheterization; while UTI due to *Stapylococcus aureus* typically occur secondary to blood-borne infections (Lane and Takhar, 2011). Rarely, could UTI_s be caused by fungal or viral species (Amdekur, *et.al* 2011). After gaining entry into the bladder, *E. coli* and other UTI pathogens attached to the bladder walls and form a biofilm that resists the body's primary immune response, (Sachaeffer, 2002; Scottist, 2010; Woodform and George, 2011).

UTIs could be uncomplicated or a simple cystitis (bladder infection) when it affects the lower urinary tract (Kuroda *et al.,* 2005; Foster, 2008; Nicolle 2008); and complicated or pyelonephritis (kidney infection), when it affects the upper urinary tract (Nicolle, 2005; Neal, 2008; Lughten-Berger and Houton 2008). It could also be a primary or recurrent depending whether the infection is occurring for the first time, or is a repeat infection (Gruenber, 1994; Schaeffer, 2002; Conway *et al.*,2001). UTIs are the most common of all bacterial infections, and can occur at anytime in the life span of an individual (Schaeffer, 2008; Conway *et al.*,2001; Nicolle, 2008; Gopal and Patel, 2009; Bhatt *et al.,* 2011). Almost 95 % cases of UTIs are caused by typically multiplying bacteria at the opening of the urethra and travel up to the bladder; and much less often by bacterial spread to the kidney from bloodstream (Conway *et al.,* 2001). Although, the female and male urinary tracts are relatively the same, except for the length of the urethra, which is shorter in women than in men. Hence, women are more prone to and in severity of UTI_s than men, and these infections tend to recur in women (Azzarone *et al,* 2007; Foster, 2008; Rossi *et al.,* 2010; Dilubaza and Schaeffer, 2011). The other predisposing factors of women vulnerability to UTI_s include frequent sexual intercourse especially the sexually active women, continuous use of contraceptive spermicides and diaphragms as well as the loss of estrogen on menopause which thins the linings of the urinary tract, hence increases the susceptibility to bacterial infections, including the UTI_s (Azzarone *et al.,* 2007; Nicolle, 2008; Salvatore *et al.,* 2013). The most common symptoms of the lower UTI (cystitis) which may vary from mild to severe are: burning sensation on urination and frequent urination, or an urge to urinate in both sexes, or the observe of vaginal discharges with significant pains above the pubic bone or in the lower back as well as dysuria without fever or chills (Nicolle, 2008). The symptoms of the upper UTI (pyelonephritis) include: flank –pains, fever or nausea and vomiting, other signs of systemic inflamatomy response, in addition to the classic symptoms of the lower UTI (Schaeffer, 2008; Lane and Takhar, 2011).

Urinary tract infections are generally treated with antibiotics of choice such as: penicillins, cephalosporins, quinolones and competitive metabolic inhibitors: trimethoprim-sulfonamides. In most cases, the UTIs clear up after days of treatment, with these antibiotics; but the more severe and complicated cases may require several weeks of antibiotics treatments and antibiotics-combinations (Nicolle *et al.,* 2008; Gopel and Patel, 2009; Scottish, 2010; Woodward and George, 2011). These combined treatments which will invariably lead to interactions become inevitable for such therapy. Antibiotics interactions have been widely reported to often result in four different outcomes or effects: synergism, additivity (potentiation), indifferent and antagonism (Rahal, 1978; Lorian, 1991; Tatro, 1992; Akunyili and Akubue, 1995; Harter, 1995; Rybak and McGrath, 1996; Sabbath and Lorian, 1997; Chait, *et al.,* 2007). Several methods including: strip-agar diffusion, killing rate kinetics, checkerboard techniques (isobologram or fractional inhibitory concentration, FIC) and disc-agar diffusion (Lorian, 1991; Akunyili and Akubue, 1995; Sabbath and Lorian, 1997; Chait, *et al.,* 2007), have been respectively reported for the evaluation of antibiotics combinations. However, there has been no reported used of activity index profile to evaluate antibiotics combination. Thus, there is a dearth of information on the efficacy of the combined antibacterial activity of the antibiotics of choice and their nature of interactions against the UTI pathogens assessed by the activity index profile with respect to the resultant effect on potency against the UTI pathogens. Consequently, in this work, the *in vitro* evaluation of the combined antibacterial activity of interactions of some antibiotics of choice against UTl pathogens, assessed by the activity index profile has been reported.

MATERIALS AND METHODS

Test Organisms

The bacterial cultures used in the study, were obtained from the stock culture collection of the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria; and the Department of Microbiology Laboratory, Faculty of Sciences, University of Uyo, Uyo Nigeria. They were: *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 27853); Coagulase negative *Staphylococcus aureus*: *Staphylococcus saprophyticus* (clinical isolate) and *Staphylococcus aureus* (NCTC 6571). They were cultured on sterile nutrient agar, NA

(Oxford, England) plates at 37 ^{o}C for 24 h and purified by repeated subculturing on NA plates. They were subsequently maintained as slant cultures, by bi-monthly subculturing on NA and stored as slant cultures at 4 ^{o}C.

Standardization of Bacterial Inocula

Standard bacterial suspensions were prepared by aseptically subculturing a loopful of the purified bacterial cultures into sterile nutrient broth, NB (Oxoid, England) and incubated at 37 ^{o}C for 24 h. The turbidity of the broth cultures were aseptically adjusted by ten-fold serial dilution with sterile NB to that of 0.5 McFarland nephelometer standard with an approximated cell density of 1 x 10^{8} cfu/ml following the methods of Tilton and Howard (1987); Baron and Finegold, (1990),with modifications (Ekong, *et al.,* 2004; 2008). Cultures of Gram-positive bacteria were diluted to 1:1000, while cultures of Gram-negative bacteria were diluted to: 1:10,000 (Ekong, *et al.,* 2004; 2008). The purity of the standardized inocula was assessed by aseptically spread-plating 0.1ml of each culture suspension onto NA plate, and incubated under standard conditions (Ekong, *et al.,* 2004).

Antibiotics

The antibiotic of choice used in the study were; penicillin VK, 250 mg (Penicillin®); ciprofloxacin 500mg USP (Cipro®); trimethoprim-sulfamethoxazole 960mg (Primepex ®); and cefixime, 400mg (Cefixime®).

Determination of Antibacterial Sensitivity

The antibacterial activity of the antibiotics was evaluated by the agar-well diffusion technique (Collins and Lyne, 1979). Stock concentrations of the antibiotics were aseptically prepared by ten-fold serial dilutions with distilled water to yield. 0.25 mg/ml (P); 0.5 mg/ml (C), 9.6 mg/ml (TMP – SMX) and 4.0 mg/ml (CX). Thereafter, 0.1 ml standardized inocula of the cultures were aseptically spread-plated by sterile glass-spreader, on sterile NA plates. Wells 4mm x 4mm were aseptically bored on the respective assay plates, previously seeded with the different bacterial cultures, and 0.2 ml of stock concentrations of the antibiotics was aseptically introduced. The plates were held at 4 ^{o}C for 1 h, followed by incubation at 37 ^{o}C for 24 h (Ekong, *et al.*, 2004). The inhibition zone diameters (IZD), denoting the potency of the antibiotics against the test bacterial cultures were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the antibiotics against the bacterial cultures was determined using the macrobroth-dilution technique (Ekong, *et al.,* 2004), a modification of the microbroth-dilution method of Tilton and Howard, (1987); Baron and Finegold, (1990). Two fold serial dilutions of the respective antibiotics stock concentrations were made in sterile test-tubes to yields graded concentrations of the antibiotics. Thereafter, 0.1ml inocula of the bacterial cultures were inoculated and incubated at 37 ^{o}C for 24 h. Uninoculated NB served as positive controls. Following incubation, the MIC of the antibiotics against the bacterial cultures were taken as the least concentrations of the agents that inhibited the growth of the bacterial cultures, as measured by turbidity compared with the control.

Evaluation of Antibacterial Activity

Evaluation of the antibacterial activity of the antibiotics was carried out at inhibitory concentrations by the agar-well diffusion techniques (Ekong *et al.,* 2008). In the assay, 0.1 ml aliquot of the 24 h old standardised broth bacterial cultures were spread-plated on sterile NA in duplicate. Wells were aseptically bored with sterile cork borer and aliquots of the antibiotics at 1xMIC against the respective bacterial cultures were introduced and kept at 4 ^{o}C for 1 h, and thereafter incubated at 37 ^{o}C for 24 h. After incubation, the IZD denoting potencies were measured and recorded for the respective antibiotics –bacterial culture combinations

Evaluation of Combined Antibacterial Activity of Antibiotics of Choice against UTI Pathogens.

Evaluation of the combination –antibacterial activity of the antibiotics of choice against the UTI Pathogens was carried out at 1xMIC, using the modified agar-well diffusion technique (Ekong *et al*., 2008; 2010; Ekong, 2013), a modification of the agar-disc diffustion combination test (Sabbath and Lorian, 1997), and Strip –agar diffusion technique (Okore, 2009). In the assay, the antibiotics solutions at 1xMIC were mixed in the equimolar ratio (1:1 $^{v}/_{v}$); and 0.2 ml of the antibiotics mixtures was aseptically introduced into holes aseptically bored on assay plates previously seeded with 0.1 ml inocula of 24 h old standardized bacterial broth cultures. The plates were held at 4 ^{o}C for 1 h (Ekong, *et al*., 2004), and incubated at 37 ^{o}C for 24 h. After incubation, the IZD for the respective antibiotics- combinations, against the bacterial cultures were measured and recorded.

Evaluation of Nature of Interaction between Antibiotics of Choice and UTI Pathogens

The nature of the interactions of the antibiotics of choice against the UTI pathogens was determined by the activity index profile, AIP (Ekong, 2013): a modification of fractional inhibitory concentration, concept of the checkerboard method (Ekong *et al.,* 2010). The AIP compares the IZD of the combined antibiotics with that of the more potent uncombined antibiotics against the bacterial cultures at sub-inhibitory

concentrations. The differences obtained were expressed as the percentage change in the IZD of the antibiotics in combination with respect to the IZD of the combined antibiotics. The AIP values obtained were compared to and interpreted from the strip-agar diffusion interaction ranges specified by Okore (2009), to determine the nature of the interactions of the combined antibiotics of choice against the UTI pathogens.

RESULTS AND DISCUSSION

Table 1: Antibacterial activity and MIC of of antibiotics of choice against urinary tract infection (UTI) Pathogens

Test organisms	Antibacterial Activity (mm)				MIC (µg/ml)		
	C	P	TMP – SMX	CX	C	P	TMP – SMX
Escherichia coli	45.0	40.0	20.0	12.0	0.00195	0.125	4.8
Pseudomonas aeruginosa	*40.0*	40.0	40.0	-	0.00195	0.125	4.8
Staphylococcus saprophyticus (CONSA)	30.0	39.0	20.0	12.0	0.0078	0.126	4.8
Staphylococcus aureus	50.0	50.0	-	-	0.00195	0.125	1.2

- = No activity.

Antibacterial Sensitivity Test

The preliminary antibacterial sensitivity test of the antibiotics of choice on the UTI pathogens is presented in Table 1. The result showed that ciprofloxacin and penicillin were the most active agents against the UTI pathogens, followed by trimethoprim-sulfamethoxazole, and lessly active by cefixime in the descending antibacterial activity sequence: C > P > TMX-SMX > CX. This antibacterial activity sequence indicated that ciprofloxacin and the other agents, except cefixime are active to both the Gram-positive and Gram-negative UTI pathogens tested. The preliminary sensitivity study showed excellent antibacterial activity of the agents against the UTI pathogens, which was highest for *Staphylococcus aureus*, than the other UTI organisms in the descending antibiogram sequence: SA > EC > PA > SS.

The excellent antibacterial activity of the agents against the UTI pathogens is encouraging, as the UTI pathogens were sensitive to the agents, despite the intrinsic and biochemical resistance mechanisms of bacteria, including the UTI pathogens to antibiotics. These include the elaboration of bacterial inactivation enzymes, particularly β-lactamases and mostly with an extended spectrum of activity to many antibiotics classes. These enzymes which often inactivated antibiotics, as the most frequent biochemical resistant mechanism by both Gram-positive and Gram-negative bacteria has been widely reported (Davies, 1979; Neu, 1983; Neu, 1984; Davies, 1984; Roy *et al.*, 1985; Philipion *et.al 1989*; Williams, 2000). Furthermore, the wider activity of the agents against the Gram-negative UTI pathogens under study is laudable in view of the intrinsic physiological and biochemical resistance of these strains to antibiotics. This could be explained on the basis of the presence of the recalcitrant outer-membrane (OM) in the Gram-negative organisms. The role of the OM as an intrinsic and biochemical resistance mechanisms, serving as a transport diffusion barrier (porins), preventing the passage of hydrophobic and large molecular weight hydrophilic substances including antibiotics into the intracellular compartments of Gram-negative bacteria has been widely reported (Nikaida *et al.*, 1983; Vara and Vaara, 1983; Nikaido and Normark, 1987; Nikaido, 1989). Minimum Inhibitory Concentration (MIC)

The summary of the MICs of the antibiotics of choice against the UTI pathogens is presented in Table 1. The result indicated and established ciprofloxacin as the most potent of the antibiotics of choice tested against the UTI pathogens, given the least MIC value of 0.0098 mg/ml for the UTI pathogens. This may confirmed the potency of ciprofloxacin with respect to the other antibiotics of choice in the therapy of UTI, in the sequence: C > P > TMP – SMX. The MIC has been reported to be the standard for determination of susceptibility of humans and animals pathogens to antimicrobial agent (Gruenberg, 1984). It is recognized that antibiotics which are ineffective in vitro in preventing the growth of a particular organism, using the MIC assay, will also be clinically or in vivo ineffective and vice-versa (Gould et al., 2010).

Table 2: Antibacterial activity of antibiotics at sub-inhibitory concentrations (IXMIC) against the UTI pathogens

Test organisms	Antibiotics Activity at I x MIC					
	Uncombined activity/IZD(mm)			Combined Activity /IZD (mm)		
	C	P	TMP – SMX	C/P	C/TMP-SMX	P/TMP-SMX
Escherichia coli	19.0	15.0	15.0	19.0	20.0	25.0
Pseudomonas aeruginosa	23.0	11.0	13.0	12.0	13.0	9.0
Staphylococcus saprophyticus (CONSA)	15.0	12.0	11.0	24.0	23.0	15.0
Staphylococcus aureus	23.0	15.0	-	11.0	17.0	15.0

– = *no activity*

Table 3: Evaluation of nature of combined activity of antibiotics of choice against UTI pathogens

Test Organization	Combined activity/IZD(mm)			Activity Index (%)[a]			Inference		
	C/P	C/TMP-SMX	P/TMP-SMX	C/P	C/TMP-SMX	P/TMP-SMX	C/P	C/TMP-SMX	P/TMP-SMX
Escherichia coli	19.0	20.0	25.0	0.0	5.0	40.0	IND	ADT	SYN
Pseudomonas aeruginosa	12.0	13.0	9.0	-91.67	-76.92	-33.33	ANT	ANT	ANT
Staphylococcus saprophyticus (CONSA)	24.0	23.0	15.0	37.50	34.78	30.00	SYN	SYN	SYN
Staphylococcus aureus	11.0	17.0	15.0	-18.18	23.53	0.0	ANT	SYN	IND

a=Acticity Index: ≥20.0% (synergism, SYN); < 20.0% (Additivity, ADT); 0.0% (Indiferent, IND); - 0.0% (Antagonism, ANT).

However, in the light of this assertion, there are many arguments for and against this submission, as many active agents *in vitro* are inactive *in vivo*. This has been reported to be mostly due to challenges of extrapolation, toxicity and delivery to target sites, etc. But, despite these shortcomings, the MIC assay remains the best approach to select potentially active and effective antimicrobial agents for effective therapy (Andrews, 2001). Consequently, in this study, the MIC_s of the antibiotics of choice against the Gram-negative UTI pathogens were compared to those of the Gram-positive counterpart, indicating high activity or potency. This excludes any possibility of bacterial enzymatic inactivation, as well as an enhanced transportation of the antibiotics across the highly undulating porins repleted OM into the intracellular compartments. These factors could have resulted in the higher intracellular concentrations of the antibiotics particularly ciprofloxacin and penicillin; hence, the lower MICs, recorded. Thus, the lowest MIC values recorded especially for ciprofloxacin, and partly penicillin against the UTI pathogens in view of antibiotics inactivation enzymes such as β-lactamases and possession of the OM by the Gram-negatives is clinically interesting. This laudable activity further adds credence to the potency of ciprofloxacin and the other agents in the therapy of UTI.

Antibacterial Activity at Sub-inhibitory Concentration

The antibacterial activity of the antibiotics of choice in single and in combinations against the UTI pathogens at sub-inhibitory concentrations is presented in Table 2. The result indicated excellent qualitative sensitivity assessment of the UTI pathogens to the antibiotics of choice, notably ciprofloxacin, while penicillin and the trimethoprim-sulfamethoxazole was highly active against the Gram-negative UTI pathogens, but moderately active against the CONSA (*Staphylococcus saprophyticus)* and inactive against *Staphylococcus aureus.*

The excellent antibacterial activities of the agents at the sub-inhibitory concentrations against the UTI pathogens further support the earlier assertion of the potency of the agents to circumvent the permeability barrier of the OM of the Gram-negative UTI pathogens. This promotes and enhanced adequate uptake and intracellular concentration of the agents; and also counters any possibility of enzymatic inactivation of the antibiotics by the UTI pathogens. However, the absence of activity by TMP-SMX against *Staphylococcus aureus* could possibly be linked to

the propensity of elaboration of inactivation enzymes, probably β-lactamases with extended spectrum of activity. This may invariably inactivated the antibiotic, resulting in its inactivity against the particular UTI pathogen.

Equally, the result qualitatively compared the combined antibacterial activity of the antibiotics to the uncombined agents. Hence, the result indicated that all the antibiotics –combinations showed varied levels of antibacterial activity against the UTI pathogens tested. This is promptly noticed in the potentiated activity of TMP-SMX by ciprofloxacin and penicillin, especially against *Staphylococcus aureus,* which hitherto was inactive against the organism when uncombined. This result further corroborated the merits of antibiotics combinations, which include the enhancement of antimicrobial activity of one agent by another; effective treatment of both specific and mixed microbial infections, especially those of unknown etiologic agents; prevention of emergence of resistant strains; as well as reduction of dose-related toxicity to the host (Lorian, 1991; Tatro, 1992; Akunyili and Akubue, 1995; Harter, 1995; Rybak and McGrath, 1996). In this study, the potentiation of the activity of TMP-SMX by both ciprofloxacin and penicillin against *Staphylococcus aureus* confirmed the merit of enhancing the antimicrobial activity of one antibiotic by another. The elevated activity could invariably resulted in effective treatment, thereby preventing the probable emergence of resistant strains of *Stphylococcus aureus* to TMP-SMX in the study. Thus, the problem of antimicrobial resistance has considerably reduced since the advent of combined antimicrobial chemotherapy. Hence, the combination of two antimicrobics has for many years been recognized as an important method for at least delaying bacterial resistance to antibiotics (Harter, 1995). Nevertheless, according to Zinner *et.al.*, (1981), antimicrobial combination may also produce desirable synergistic effects in the treatment of diverse specific and mixed bacterial infections. Consequently, combined antibiotics therapy has been indicated for the treatment of different types of UTIs (Azzarone *et al.*, 2007; Nicolle, 2005).

Nature of Interaction of Antibiotics of Choice against UTI Pathogens

The nature of interactions of the combined antibiotics of choice against the UTI pathogens is presented in Table 3. The result generally indicated the following interactions predominantly synergism and lessly additivity or indifferent and antagonism. Specifically, the three different antibiotics of choice combinations (C/P; C/TMP-SMX; P/TMP – SMX) against the aucthocthonous UTI pathogens indicated synergism for *Staphylococcus saprophyticus,* one of indifferent, additivity or synergism for *Escherichia coli;* and predominantly antagonism for *Pseudomonas aeruginosa*. Equally, the combined antibiotics of choice against the allocthonous UTI pathogens *Staphylococcus aureus* indicated antagonism, synergism and indifferent respectively.

In the study, the nature of the interactions obtained against the UTI pathogens by the AIP are in line with those reported for other antibiotics combinations against other organisms by several techniques (Ekong et al., 2008; Okore, 2009; Ekong *et al.*, 2010; Ekong, 2013). Accordingly, synergism occurred when the activity index is $\geq$ 20.0 %; additivity resulted when activity index is $<$ 20.0 %; indifferent interaction occurred at activity index of 0.0 %; and antagonism when activity index is - 0.0 % (Ekong, 2013). Furthermore, besides, the concept of activity index, the nature of the interactions could be inferred from the mode of action of the antibiotics. Following, it has been widely reported that interactions between bactericidal agents in combinations are enhanced by another resulting in synergism; while interactions between a bacteriostatic and bactericidal; or two bacteriostatic agents often resulted in antagonism, as the action of the bactericidal agent is inhibited by the bacteriostatic agent (Rahal 1978, Lorian, 1991; Tatro, 1992; Harter 1995; Rybak and McGrath, 1996; Okore, 2009). Accordingly, synergism occurred by enhancement of action of an agent by another through the blockade of sequential steps in a metabolic pathway; inhibition of enzymatic inactivation of one agent by another; enhanced microbial uptake of one antibiotic by another. Thus, in this study, the synergistic interactions which may be additivity or potentiation noticeable in C/TMP-SMX and P/TMP-SMX could be attributed to any of these mechanisms of synergism. This resulted in the enhanced combined antibacterial activity of the antibiotics especially TMP-SMX against CONSA (*Staphylococcus saprophyticus)* and *Escherichia coli* the authocthonous UTI pathogens; as well as *Staphylococcus aureus,* the allocthonous UTI pathogen. Similarly, the authors also reported that antagonism resulted by the inhibition of two static agents, from the induction of enzymatic inactivation of one antibiotic by another in the combination. Hence, in this report, the antagonistic interactions obtained by the combination of ciprofloxacin, a-cidal agent with the -static agents C/P and C/TMP-SMX against *Pseudomonas aeruginosa*; and the only antagonism recorded by C/P combination against *Staphylococcus aureus,* may be attributed to and confirmed these mechanisms of antagonism (Ekong, 2013). Nevertheless, in view of the mechanisms of action of the antibiotics under study, ciprofloxacin acts by

binding to the a-subunit of topoisomerase, preventing DNA super-coiling thereby inhibiting DNA synthesis; penicillin inhibits cell wall synthesis by blocking transpeptidization of the growing cell wall by binding to the terminal D-alanyl-D-alanine; while sulfamethoxazole-trimethoprim are competitive metabolic analogues-inhibitors, sequentially inhibiting the synthesis of folic acid by blocking the conversion of PABA to dihydrofilate, and later reduction to tetrahydrofolate. Furthermore, the quinolones have been reported to synergized with the β-lactams (Finch, 2011). In this study, this assertion has been confirmed by the interaction of C/P against the two aucthocthonous UTI pathogens: *Escherichia coli* and CONSA. The result was synergistic activity as penicillin inhibited cell wall synthesis, thereby increasing permeability of the bacterial cell envelop to the inhibitory action of ciprofloxacin. Likewise the synergism between C/TMP-SMX and P/TMP-SMX against the UTI pathogens could be explained on the basis of the concerted effects of the mechanisms of actions of the antibiotics, jointly elicited against the UTI pathogens. Equally, based on mechanisms of action of the antibiotics, the antagonistic interactions recorded in the various antibiotics combinations could be due to the inhibition of the effect or action of one agent by another, and induction of enzymatic inactivation of the agents hence resulting in the antagonism against the UTI pathogens.

Consequently, the synergistic interaction exhibited by the three antibiotics combinations against the UTI pathogens, indicated the possibility of adoption of such laudable combinations in empirical UTI therapy of any specific or mixed pathogens. The persistent antagonism demonstrated by the agents against *Pseudomonas aeruginosa*, is an indication that any of the three antibiotics combinations should not be used in the treatment of UTI of which the organism is the principal etiologic agent. Also, the only antagonism recorded against *Staphylococcus aureus* by the interaction of C/P, is laudable as these antibiotics of choice should not be employed in combinations for the treatment of a UTI caused by the organism.

CONCLUSION

In this paper, we have evaluated the potency of the antibacterial activities of antibiotics of choice; ciprofloxacin (C), penicillin (P), and trimethoprim-sulfamethoxazole (TMP-SMX), when uncombined and in combinations against both aucthocthonous and allocthonous urinary tract infection pathogens tested. The interactions from the antibiotics combinations were predominantly synergism with few indifferent and additivity against the UTI pathogens. However, all the antibiotics interactions were predominantly antagonistic against *Pseudomonas aeruginosa*. This is an indication that ciprofloxacin being so potent could be used alone or in combinations to potentiate the other antibiotics against the UTI pathogens in empirical clinical conditions. Conversely, penicillin and trimethoprim-sulfamethoxazole should not be used alone, but in combination with each other and ciprofloxacin to enhance their antibacterial activity and to counter the induction of resistance by the UTI pathogens. The synergism of the antibiotics combinations against coagulase-negative *Staphylococcus aureus, Escherichia coli* and *Staphylococcus aureus* could be exploited in therapy for containment of these problematic UTI pathogens. Conversely, the combinations of the antibiotics may be avoided for *Pseudomanas aeruginosa*, owing to the predominant antagonism, to counter the induction of resistance of this ubiquitous and problematic UTI pathogens, and hence treatment failure. It is recommended that further animals studies on the pharmacokinetics and pharmacodynamics, as well as the toxicological profiles of the combined antibiotics should be conducted and even to the level of clinical trials. These recommended combination studies would help to ascertain the efficacy or otherwise of their clinical relevance for use in UTI chemotherapy.

REFERENCES

Akunyili DN, Akinbue IP (1995). *Handbook of Drug Interactions (Led).* Kelu-printing and publishing company Enugu , Nigeria. pp 1 – 37.

Amdekar S, Singh V, Singh DD (2011). Probiotic therapy: immunomodulting approach towards urinary tract infection. *Curr. Microbiol. Biol.*, 63(5): 484 – 490.

Andrew JM (2001). Determination of minimum inhibitory concentration. *J. Antimicrob. Chemother.* , 48 (Suppl. 1): 15 – 16.

Azzarone G, Liewehrs O'Connork (2007). Cystitis. *Paediat. Rev.,* 28(12): 474 – 476.

Baron JE, Finegold SM (1990). Methods for testing antimicrobial effectiveness. In: C. V. Mosdy(ed). *Bailey Scotts Diagnostic Microbiology (8ed).* Missouri, USA.

Bhatt RG, Kary TA, Place FC (2011). Pediatric urinary tract infections. *Med. Clin. North America,* 29(3): 637 – 643.,

Chait BT, Williams K, Scmidt O (2007). Antibiotics interaction. *J. Antimicrob. Chemother.*, 38 (Suppl. A): 12 – 15.

Colgan R, Williams M, Johnson JR (2011). Discuss an treatment of acute pyelinephritis in women. *America Fam. Physician,* 84(5): 519 – 526.

Collins CH, Lyne A (1979). *Microbiological Methods (4ed) . Butterworth,* London.

Conway PH, Cnaan A, Zaoutis T, Henry BV, Grundmeiur RW, Keren R (2007). Recurrent urinary tract infections in children: risk factors and association with prophylactic antimicrobials. *J. Antimicrob. Chemother*. 298(2): 179 – 186.

Davies J (1979).General mechanism of antimicrobial resistance. *Rev. Infect. Dis.*, 1**:**23 – 28.

Davies J (1984). Microbial resistance to antimicrobial agents. *In:* Rsituccia, A. M. and B. A. Cunda (ed). *Antimicrobial Therapy.* Raven Press-New York.

Dielubanza EJ, Schaeffer AJ (2011). Urinary tract infections in women. *Med. Clin. North America.*,95 (1): 27 – 11.

Ekong US, Mgbor NM, Moneke AN, Obi SKC (2004). Evaluation of the antimicrobial and some pharmacokinetic properties of an antibiotic – substance produced by an environmental *Aspergillus* species SK2, isolated from Nigerian soil. *Nig. J. Microbiol.,* 18 (1-2), 199 – 206.

Ekong US, Mgbii AI, Adikwu MU (2008). Evaluation of the *in vitro* combination effect of colloidal silver concentrate on the antifungal activity of ethanolic extract of the lichen *USnea subfloridans. Nig. Ann. Nat. Sci.,* 8(1): *1 – 5.*

Ekong US, Odenigbo NS, Adikwu MU (2010). Effect of colloidal silver concentrate on the antibacterial activity of ethanolic extract of lichen: *Parmelia perlata. J. Pharm. Allied Sci.,* 7(3): 227 – 331.

Ekong US (2013). Production, characterization and antimicrobial spectra of antibiotic substances from *Streptomyces* species isolated from different soil samples in Uyo, Nigeria. Ph.D dissertation, University of Nigeria, Nsukka, Nigeria.

Finch RG (2011). Antimicrobial therapy: principles of use of antibiotics combinations: quinolones and *β-lactams. Medicine,* 39**:** 35 – 40.

Fleming A (1980). Classes in Infectious Diseases: On the antibacterial action of cultures of Penicillin, with special reference to their use in the isolation of *B. influence Brit. J. Exptal. Pathol.*, 10:226 – 236.

Foster RT (2008). Uncomplicated tract infections in women. *Obstet. Gynecol. North America,* 35(2): 235 – 248.

Gopal RG, Patel M (2009). Urinary tract infection in hospitalized elderly patients in the united Kingdom,: the importance of making accurate diagnosis in the post broad-spectrum antibiotics era. *J. Antimicrob. Chemother.,* 63:5 – 6.

Gould CU, Umscheid CA, Agarwal RK, Kuntzapergues DA (2010). Guidelines for prevention of catheter-asociated urinary tract infections. *Infect. Control Hosp. Epidemiol.,* 31(4): 319 – 326.

Gruenberg RN (1994). Changes in urinary tract pathogens and their antibiotic sensitivity 1971 – 1972. *J. Antimicrob. Chemoth.,* 33: *(Suppl. A)1.*

Harter DP (1995). Important Drug Interactions: In: G.B katzung (ed). *Basic And Clinical Pharmacology (6ed).* Appleton-Lange, Connecticutt, USA. P. 986 – 994.

Katherine MK, Susan E (2007). Routes of transmission of infectious diseases agents from modes of introduction of exotic animal disease agents. *J. Vet. Med.* 54**:** *221 – 235.*

Kuroda M, Yamashita A, Hirakawa H (2003). Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc. Natl. Acad. Sci*, USA. 102 (37): 113272 – 113277.

Lane DR, Takhar SS (2011). Diagnosis and Management of urinary tract infection and pyelonephritis *Emergency Med. Clinic North-America,* 29(3): 539 – 552.

LichtenBerger P, Hooton TM (2008). Complicated urinary tract infections. *Curr. Infections Urol. Clin. North America,* 35:13 – 22

Lorian V (1991). *Antibiotics in Laboratory Medicine: Methods Used to Assess the Activity of Antimicrobial Combinations (3ed). Williams and Wilkins, Baltimore, Maryland,* USA. P. 434 – 444.

Mandell GL, Douglass RG, Bernett JE (2000). *Principles and Practices of Infectious Diseases.* Churchill, Livingstone, Phladelphia, USA. Pp 222 – 225.

Neal DE Jr (2008). Complicated urinary tract infections. *Urol. Clin. North America,* 35: 13 – 22.

Neu AC (1983). The emergence of bacterial resistance and its empirical therapy. *Rev. Infect. Dis.*, **5:** 59 – 63.

Neu HC (1984). Changing mechanisms of bacterial resistance *Amer. J. Med.*, 776: 11 – 13.

Nicolle LE (2005). Complicated urinary tract infection in adults. *Can J. Infect. Dis. Med. Microbiol.,* 16**:** 349 – 360.

Nicolle LE (2008). Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. *Urolog. Clin. North American,* 35(1): 1 – 2.

Nicolle LE, Bradley S, Colgan R (2006). Infectious diseases society of America: Guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin. Infect. Dis*., 40:634 – 654.

Nikaido A (1989). Outer-membrane barrier as a mechanism of antimicrobial resistance. *Antimicrob. Agents Chemother.*, 33: 1831 – 1836.

Nikaido H, Normark S (1987). Sensitivity of *Escherichia coli to various β- lactams* is determined by the interplay of outer-membrane permeability and *β- lactamases: a qualitative predictive treatment. J. Mol. Biol***.,** 1: 29 – 36.

Nikaido H, Rosenberg EX, Foulds J (1983). Porin channels in *Escherichia coil.:* studies with β-lactamase in intact cells. *J. Bacteriol,* 153: 232 – 240.

Okore VC (2009).*Principles of Pharmaceutical Microbiology* (2ed). Ephrata Publishers, Nsukka, Nigeria. P. 196 – 260.

Phillipion A, Lehra R, Jacoby G. (1989). Extended spectrum of β- lactamses. *Antimicrob. Agents Chemother*., 3:131 – 1136.

Rahal JJr. (1978). Antibiotics combinations: the clinical relevance of synergy and antagonism. *Medicine,* **57:** 179 – 195.

Rossi R, Porta S, Canovi B (2010). Overview on cranberry and urinary tract infections in females. *J. Clin. Gastrointerol. (*44Suppl. 1). 86 – 92.

Roy CC, Segura C, Tirado M, Reig R, Hermida M, Teruses D, Fo A (1985). Frequency of plasmid determined β –lactmases in 680 consecutively isolated strains of enterobacteriaceae. *Euro. J. Clin. Microbiol*., 4: 146 – 147.

Rupp ME, Soper DE, Archer GL (1992). Colonization of female genital tract with *Staphylococcus saprophyticus. J. Clin. Microbiol.,* 30 (11): 2975 – 2979.

Rybak JM, McGrath JB (1996). Combined antimicrobial therapy for bacterial infections: guidelines for clinicians. *Drugs*, 52(3):309 – 402.

Sabbath D, Lorian V (1997). *In vitro tests for antibacterial activity of antibiotics in combinations. In: A Bodi, J. T. Bartola, and J. E. Prier (eds).The Clinical laboratory As An Aid in Chemotherapy of Infectious Diseases.* University Park press, Baltimore, Maryland, USA. P 196 – 227.

Salvatore S, Cattori E, Eiesto G, Serati M, Soruce P, Tonella M (2011). Urinary tract infections in women. *Euro J. Obste. Gynecol. Repro. Biol.,* 156 (3): 131 – 136.

Schaeffer AJ (2002). Infections of the urinary tract. *In Walsh, P. C.,* Refik, A B., Vangham, E. O., and Weri, A. J. (eds). *Campbell's Theology (ed).* Saunders, Philadelphia, USA. P 515 – 602.

Schleifer KH, Kloos WE (1978). Isolation and characterization of *Staphylococcus saprophyticus* and description of three new species. *Int'l J. Syst. Bacteriol.,* 25(1): 50 – 61.

Scottish MU (2010). Intercollegiate guidelines network: Management of suspected bacterial urinary tract infection in adults. *NHS Qual. Improv. Scotland.* 27**:**145 – 175.

Tatro DS (1992). Drug Interactions. *In:* E. T. Herfindal, D. R. Gourley and L. L. Hert. *Clinical Pharmacy and Therapeutics* (5ed). Williams and Wilikings , Baltimore, Maryland, USA. P. 37 – 44.

Tilton RC, Howard BJ (1987). Antimicrobial susceptibility testing. *In:* B. J. Howard (ed). *Clinical and Pathogenic Microbiology.* C. V. Mosby, Missouri, USA.

Vaara M, Barra T (1983). Polycations sensitized enteric bacteria to antibiotics. *Antimicrobs. Agent Chemother*., 24: 107 – 113.

Widerstorm M, Wistrom J, Sjostedt A, Monsen T (2012). Coafulase – negative staphylococcus; update on molecular epidemidogy and clinical presentation, with focus on *Staphylococcus*

saprophyticus. Euro. J. Clin. Microbiol. Infect. Dis., 31 (7): 7 – 20.

Williams R (2000). Antimicrobial resistance: The facts. *Essential Drug Monitor*, 28/29:7 – 8.

Woodford HJ, George J (2011). Diagnosis and management of urinary tract infections in older people. *Clin. Med.*, 11 (7): 80 – 83.

Zinner SN, Lasterky J, Grays H, Bernard C, Ryff IC (1981). *In vitro and in vivo* studies of three antibiotic combinations against Gram-negative bacteria and *Staphylococcus aureus. Antimicrob. Agents Chemother.*, 20: 463 – 469.