Synergistic potentials of benzylpenicillin, amoxicillin and streptomycin antibiotics against selected bacterial species

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Abstract: The development of multiple antibiotic resistances in microbial communities is gaining momentum as many chemotherapeutic agents are obsolete and ineffective to curtail the menace of pathogenic microbes. Consequently, efforts are now geared towards the development of new potent antibiotics while we explore the potentials of drug combinations to improve antibiotic effectiveness. This study investigated the effect of combining benzylpenicillin, streptomycin and amoxicillin against bacterial isolates by agar dilution and agar diffusion methods. The results showed that streptomycin was the most effective against the bacterial isolates having MICs ranging between 2 µg/ml and 200 µg/ml while amoxicillin inhibited the bacterial isolates at MICs ranging between 20 µg/ml and 400 µg/ml and benzylpenicillin inhibited the isolates at concentrations ranging between 20 µg/ml and 200 µg/ml. Amoxicillin combined with benzylpenicillin and amoxicillin combined with streptomycin were not synergistic against any isolate. Amoxicillin combined with benzylpenicillin resulted in additive interaction against *K. pneumoniae* ATCC 1538, *S. aureus* ATCC 29213 and *E. coli* ATCC 23922 but antagonistic against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Amoxicillin combined with streptomycin resulted in additive interaction against *E. coli* ATCC 23922, *K. pneumoniae* ATCC 1538 and *E. coli* ATCC 25922 but antagonistic against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. Benzylpenicillin combined with streptomycin resulted in synergistic interaction against *S. aureus* ATCC 29213, *E. coli* ATCC 23922 and *P. aeruginosa* ATCC 27853, additive interaction against *S. aureus* ATCC 29213 and antagonistic interaction against *E. coli* ATCC 25922. This study showed that synergistic drug pairs can effectively eradicate bacteria while antagonistic drug pairs would reverse the trend especially when targeting bacterial strains with a combination of drugs of unknown interactions.
[Olufunmiso O Olajuyigbe, Morenike O. Adeoye-Isijola, **Otunola Adedayo. Synergistic potentials of benzylpenicillin, amoxicillin and streptomycin antibiotics against selected bacterial species.** *Life Sci J* 2016;13(8):37-44]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 7. doi:10.7537/marslsj130816.07.

Keywords: Drug combinations, additive interaction, synergistic interaction, antagonistic interaction and resistant bacteria

Introduction

Chemotherapy has advanced a lot over the years and central to this improvement is the use of antibiotics to combat the numerous disease-causing microorganisms around the world. However, the widespread use and misuse of antibiotics both inside and outside of medicine contributed significantly to the emergence of resistant bacteria (Goossens *et al.,* 2005). Although there were low levels of pre-existing antibiotic-resistant bacteria before the widespread use of antibiotics (D'Costa *et al.,* 2011), evolutionary pressure, natural selection and molecular mechanisms arising from their use have played significant roles in the development of multidrug resistant bacteria and the spread of resistance between bacterial species (Nelson, 2009; Hawkey and Jones, 2009 and; Caldwell and Lindberg 2011). While the volume of antibiotic prescribed is a major factor in increasing rates of bacterial resistance rather than compliance with antibiotics (Pechère, 2001) and a single dose of antibiotics could lead to a greater risk of resistance development for up to a year in its recipient (Costelloe *et al.,* 2010), inappropriate prescription of antibiotics has been attributed to a number of causes (Arnold and Straus, 2005). Suboptimum antibiotic concentrations in critically ill people has increased the frequency of antibiotic resistant organisms (Thomas *et al.,* 1998) and shortening the course of antibiotics may actually decrease rates of resistance development (Pechère, 2001; Li *et al.,* 2007).

There are several molecular mechanisms of acquiring antibiotic resistance to antimicrobial agents. This may include inactivation of drugs, alteration of the target sites, reduction in cellular uptake and increased efflux (Smith, 2004). While Witte (2004) reported that the spread of antibacterial resistance often occurs through vertical transmission of mutations during growth and genetic recombination of DNA by horizontal genetic exchange, Alekshun and Levy (2007) indicated that intrinsic antibacterial resistance may be part of the genetic makeup of bacterial strains and that acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. Witte (2004)

and Baker-Austin *et al*., (2006) indicated that the genes for antibiotic resistance can be exchanged between different bacterial strains or species through plasmids carrying these resistance genes. The plasmids with different resistance genes can confer resistance to multiple antibiotics and cause cross-resistance to several antibiotics (Baker-Austin *et al*., 2006) or result in preferential growth of resistant bacteria while growth of susceptible bacteria is inhibited by the antibacterial agents (Levy, 1994). Thus, the misuse of antibiotics and the immense contribution of antibiotic resistance to clinical therapy had resulted in human's inability to curtail or treat infections.

To combat this menace, it is expected that keeping away from antibiotics would be an effective measure. However, lives cannot be left at the risk of mortality while long-term measures, including the use of drug combinations with the hope that they act synergistically and able to combat drug-resistant microbes are put in the pipelines. Antimicrobial combinations are used for expansion of antimicrobial spectrum, minimization of drug toxicity, minimization of antimicrobial resistance and antimicrobial synergism (King *et al*., 1981; Eliopoulos and Eliopoulos, 1988 and Eliopoulos and Moellering, 1996) and to achieve synergistic inhibitory or bactericidal activity (Eliopoulos, 1989). While broad-spectrum combination therapy will increase or improve clinical outcomes and ensure effective therapy as compared with monotherapy (Kumar *et al.,* 2010; Micet *et al.,* 2010; Martinez *et al.,* 2010 and Traugott *et al.,* 2011), delayed administration of appropriate antibiotic therapy will increased mortality in patients with septic shock (Kumar *et al.,* 2010). As a result, scientific studies on drug combinations ensure that effective therapeutic combinations are attained since antagonism or indifference may result from the use of incompatible drugs with serious consequences. This study was, therefore, designed to investigate the potency of selected antibiotics, and qualitatively determine the synergistic action or otherwise between the antibiotics and subsequent provision of information that would prove important in the fight against antibiotic resistant organisms.

Materials and Methods

Source of samples

The antibiotics, namely; benzylpenicillin (Pemapen by Shijiazhuang pharma; and Crysgen, by Shijiazhuang pharma, used for zone of inhibition), streptomycin (Septocin, manufactured by CSPC Zhongnuo pharmaceutical) and Amoxicillin (Amoxicillin, produced by Reyoung pharmaceutical Co., Ltd. and Amoxil, produced by Beecham Pharmaceuticals U.K, used for zone of inhibition), were purchased from chemists in Lagos.

The microorganisms, namely *Escherichia coli* 25922, *Klebsiella pneumoniae* ATCC CT1538, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 23922 and *Pseudomonas aeruginosa* ATCC 27853, against which the antibiotics were tested were obtained from the Microbiology Section of the department of Biosciences and Biotechnology, Babcock University, Ilisan Remo, Ogun State, Nigeria. The microorganisms were cultured and maintained on double strength nutrient agar plates incubated at 37^{o}C for 24 h before being kept in the refrigerator at 4^{o}C.

Preparation of turbidity standard

A 0.5 McFarland standard was prepared. Briefly, 0.5 ml aliquot of 0.048 mol/l $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 mol/l H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1 cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm was about 0.10 for the 0.5 McFarland standards. The barium sulphate suspension was transferred in 4 to 6 ml aliquots into tubes of the same size as those used in growing or diluting the bacterial inoculum. These tubes were tightly sealed and stored in the dark at room temperature. The barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniformly turbid appearance.

Preparation of inoculums

To prepare the inoculums for the bacterial cultures, the organisms were grown in 9 ml full strength nutrient broth overnight for about 15 h. At 15 h, the broth cultures were centrifuged at 1,500 rpm for 10 min and the supernatant discarded. Then, 9 ml of 0.85% normal saline was added to each of the bacterial pellets before being centrifuged at 1,500 rpm for 10 min again. The supernatant was discarded and the residues were resuspended in about 5 ml of normal saline. This is usually kept in the refrigerator for use within 24 - 48 h. To prepare the inoculum, the bacterial suspensions were serially diluted three times each (10^{-3}), by adding 0.5 ml into 4.5 ml of normal saline each time. This gave turbidity comparable to the 0.5 McFarland turbidity standards and an approximate bacterial population of 10^6 cfu/ml.

Determination of the minimum inhibitory concentrations (MIC) by agar dilution assay

To determine the MIC of each antibiotic, the agar dilution method was used. Mueller-Hinton agar (19 ml) was dispensed into McCartney bottles before being sterilized at 121^{o}C for 15 min. This was allowed to cool in a water bath maintained at 50^{o}C before 1 ml of each of the antibiotic solutions was added. Stock solutions of benzylpenicillin (8000 µg/ml), amoxicillin (8000 µg/ml) and streptomycin (4000 µg/ml) were

prepared with sterile deionized distilled water. Different concentrations of each of the antibiotics ranging between 0.002 µg/ml and 400 µg/ml were prepared in the molten Mueller Hinton agar, mixed thoroughly before being poured into sterile Petri dishes. One plate without antibiotics served as the control plate. The agar plates were allowed to set before being partitioned into five portions and labelled for each organism. Each test organism was then streaked on the appropriate portion of each experimental and the control plates. The plates were incubated at 37°C for 24 h. After the incubation period, the plates were observed for bacterial growth and the MIC of each antibiotic for each organism was recorded as the lowest concentration that prevented any visible growth of the streaked bacterial isolates.

Determination of bactericidal activity of antibiotics

The appropriate amount of inoculum of each organism was prepared in 3 tubes of sterile normal saline to a total of 9 ml. One millilitre (1 ml) of 2 x MIC of each of the antibiotics prepared in sterile broth was inoculated with 100 µl of each of the adjusted bacterial isolates. A loopful of each bacterial culture was streaked on sterile Mueller Hinton agar after 15 and 30 h. The plates were labelled, inverted and incubated for 24 h at 37°C. After 24 h of incubation, the plates were examined for bacterial growth. The above process was repeated for 10 x MIC of each of the antibiotics. After incubating for 15 and 30 h, 100 µl of each adjusted broth culture was inoculated on sterile nutrient agar plates and spread with a sterile glass spreader. The plates were labelled, inverted and incubated for 24 h at 37°C. After 24 h incubation period, the plates were examined for bacterial growth.

Qualitative assessment of drug interaction by agar diffusion assay

The qualitative assessment of drug interaction was carried out using the agar diffusion method. A suspension of each of the bacterial isolates comparable to a 0.5 McFarland turbidity standard was prepared for use as the inoculum. The susceptibility screening of the test bacteria to each of the antibiotics and their various combinations was determined by using the modified Kirby-Bauer diffusion technique (Cheesbrough, 2002) involving swabbing Mueller-Hinton agar (MHA) (Oxoids Ltd, Basingstoke, Hampshire, UK) plates with the resultant saline suspension of each adjusted bacterial strain. Wells, appropriately labelled, were then bored into the agar medium using a heat sterilized 6 mm cork borer. A stock solution of 1000 µg/ml of each of benzylpenicillin, amoxicillin and streptomycin were prepared. From these stock solutions, equal volumes of each of the antibiotics were combined while 100 µl of each of the antibiotics and their combinations was dispensed into each labelled agar well taking care not to allow spillage of the solutions onto the surface of the agar. The culture plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of each of these solutions before being incubated at 37°C for 24 h. The determinations were done in duplicates. After 24 h of incubation, the plates were examined for inhibition zones. The diameters of the inhibition zones produced by the concentration of each of the antibiotics (benzylpenicillin, amoxicillin and streptomycin) and their combinations (amoxicillin + benzylpenicillin, amoxicillin + streptomycin, and benzylpenicillin + streptomycin) were measured in millimeters (Wikler, 2007) and interpreted using the CLSI zone diameter interpretative standards (Wikler, 2008). The inhibition zones were interpreted using the pattern of interpreting zone diameters in Kirby-Bauer test (Bauer *et al.,* 1966). A diameter less than 12 mm indicates a resistant organism, 12-17 mm indicates intermediate resistant and greater than 17 mm, a sensitive organism.

Results

In the determination of the minimum inhibitory concentrations (MIC), streptomycin showed the highest potency with the MIC 2 µg/ml against *S. aureus* ATCC 29213, *Escherichia coli* ATCC 23922 and *P. aeruginosa* ATCC 27853, while *K. pneumoniae* ATCC 1538 and *E. coli* ATCC 25922 were inhibited at a minimum concentrations of 20 µg/ml and 200 µg/ml respectively. Although streptomycin was the most effective against the bacterial isolates having MICs ranging between 2 µg/ml and 200 µg/ml, amoxicillin inhibited the bacterial isolates with MICs ranging between 20 µg/ml and >400 µg/ml and benzylpenicillin inhibited the isolates at concentrations ranging between 20 µg/ml and 200 µg/ml. While benzylpenicillin also had an MIC of 200 µg/ml against *E. coli* ATCC 25922, amoxicillin exhibited the lowest potency (MIC >400 µg/ml) against *E. coli* ATCC 25922 and these two antibiotics inhibited *Ps. aeruginosa* ATCC 27853 at the highest MIC of 20 µg/ml (Table 1).

After exposure to a concentration twice the MIC (2 x MIC) of each antibiotic for 15 h, all the isolates showed growth after incubating the subculture for 24 h with the exception of *E. coli* ATCC 25922 treated with streptomycin and *Escherichia coli* ATCC 23922 and *P. aeruginosa* ATCC 27853 treated with amoxicillin. After 30 h incubating subcultured samples from 2 x MIC, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 exposed to streptomycin and benzylpenicillin respectively did not grow (Table 2).

When the bacteria were exposed to tenfold MIC (10 x MIC) for 15 h, all the cultured plates showed growth for all organisms except *S. aureus* ATCC

29213 and *E. coli* ATCC 23922 exposed to benzylpenicillin. After incubating for 30 h, *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. coli* ATCC 23922 did not grow after being exposed to benzylpenicillin. Also, *P. aeruginosa* ATCC 27853 did not grow after being exposed to streptomycin (Table 3).

In Table 4 showing the inhibition zones produced by each antibiotics alone and those of their combinations, the isolates were resistant to amoxicillin but highly susceptible to streptomycin. While *K. pneumoniae* ATCC 1538 was resistant to benzylpenicillin, other bacterial isolates were susceptible with the exception of *P. aeruginosa* ATCC 27853 that showed intermediate resistance. The combination of amoxicillin and benzylpenicillin resulted in *E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. coli* ATCC 23922 being more susceptible than when amoxicillin was used alone while *K. pneumoniae* ATCC 1538 was resistant and *P. aeruginosa* ATCC 27853 showed intermediate resistance. However, the combination of amoxicillin and streptomycin and those of benzylpenicillin and streptomycin produced inhibition zones wider than when each of the antibiotics was used alone.

From Table 5, the interactions between the different combinations of antibiotics are divided into synergistic, antagonistic, and additive based on whether the inhibitions zones are respectively larger, smaller or similar to the largest individual inhibition zones produced by each antibiotic against each bacterial isolate. The varied combination showed that amoxicillin combined with benzylpenicillin and amoxicillin combined with streptomycin were not synergistic against any isolate. However, amoxicillin combined with benzylpenicillin resulted in additive interaction against *K. pneumoniae* ATCC 1538, *S. aureus* ATCC 29213 and *E. coli* ATCC 23922 but antagonistic against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Amoxicillin combined with streptomycin resulted in additive interaction against *E. coli* ATCC 23922, *K. pneumoniae* ATCC 1538 and *E. coli* ATCC 25922 but antagonistic against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. On the other hand, benzylpenicillin combined with streptomycin resulted in synergistic interaction against *S. aureus* ATCC 29213, *E. coli* ATCC 23922 and *P. aeruginosa* ATCC 27853, additive interaction against *S. aureus* ATCC 29213 and antagonistic interaction against *E. coli* ATCC 25922.

Table 1: The minimal inhibitory concentrations of the antibiotics against each organism

	Minimum inhibitory concentrations of the antibiotics		
	Benzylpenicillin	**Streptomycin**	**Amoxicillin**
	----------µg/ml----------		
E. coli ATCC 25922	200	200	>400
K. pneumoniae ATCC 1538	>400	20	>400
S. aureus ATCC 29213	200	2	200
E. coli ATCC 23922	400	2	200
P. aeruginosa ATCC 27853	20	2	20

Table 2: Effects of 2 x MIC of antibiotics on bacterial isolates after exposure to the antibiotics for 15 and 30 h

		2 x MIC (15 h)			**2xMIC (30 h)**	
	Pen	**Str**	**Amx**	**Pen**	**Str**	**Amx**
E. coli ATCC 25922	+	-	+	+	-	+
K. pneumoniae ATCC 1538	+	+	+	+	+	+
S. aureus ATCC 29213	+	+	+	+	+	+
E. coli ATCC 23922	+	+	-	+	+	+
P. aeruginosa ATCC 27853	+	+	-	-	+	+

Key: Pen = Benzylpenicillin; Str = Streptomycin; Amx = Amoxicillin; + = growth; - = no growth

Table 3: Effects of 10 x MIC of antibiotics on bacterial isolates after exposure to the antibiotics for 15 and 30 h

	10 x MIC (15 h)			**10 x MIC (30 h)**		
	Pen	**Str**	**Amx**	**Pen**	**Str**	**Amx**
E. coli ATCC 25922	+	+	+	-	+	+
K. pneumoniae ATCC 1538	+	+	+	+	+	+
S. aureus ATCC 29213	-	+	+	-	+	+
E. coli ATCC 23922	-	+	-	-	+	+
P. aeruginosa ATCC 27853	+	+	-	+	-	+

Key: Pen = Benzylpenicillin; Str = Streptomycin; Amx = Amoxicillin; + = growth; - = no growth

Table 4: Mean inhibition zone (mm ± 1.00) produced by each antibiotics used alone and in combinations against each bacterial isolate

	Mean zones of inhibition					
	Amx	**Pen**	**Str**	**Amx + Pen**	**Amx + Str**	**Pen + Str**
	----------(mm ± 1.00)----------					
E. coli ATCC 25922	0.0 (R)	23.0 (S)	32.0 (S)	20.0 (S)	28.0 (S)	29.0 (S)
K. pneumoniae ATCC 1538	0.0 (R)	0.0 (R)	18.0 (S)	0.0 (R)	18.0 (S)	18.0 (S)
S. aureus ATCC 29213	0.0 (R)	25.0 (S)	22.0 (S)	24.0 (S)	21.0 (S)	27.0 (S)
E. coli ATCC 23922	0.0 (R)	20.0 (S)	23.0 (S)	19.0 (S)	20.0 (S)	31.0 (S)
P. aeruginosa ATCC 27853	0.0 (R)	17.0 (I)	28.0 (S)	13.0 (I)	28.0 (S)	29.0 (S)

Key: Pen = Benzylpenicillin; Str = Streptomycin; Amx = Amoxicillin; R: resistant (<12mm); I: intermediate (12-17mm); S: sensitive (>17mm).

Table 5: Interactions of the different antibiotic combinations against the bacterial isolates

	Synergistic	**Additive**	**Antagonistic**
Amx + Pen		*K. pneumoniae* ATCC 1538	*E. coli* ATCC 25922
		S. aureus ATCC 29213	*P. aeruginosa* ATCC 27853
		E. coli ATCC 23922	
Amx + Str		*E. coli* ATCC 23922	
		K. pneumoniae ATCC 1538	*S. aureus* ATCC 29213
		E. coli ATCC 25922	*P. aeruginosa* ATCC 27853
Pen + Str	*S. aureus* ATCC 29213	*S. aureus* ATCC 29213	*E. coli* ATCC 25922
	E. coli ATCC 23922		
	P. aeruginosa ATCC 27853		

Key: Pen = Benzylpenicillin; Str = Streptomycin; Amx = Amoxicillin

Discussion

A favourable clinical response to the treatment attainable using antimicrobial agents, alone or in combination, to produce bactericidal effects is the ultimate criterion for successful therapy. To achieve this goal, drug combinations are increasingly being used in the treatment of many diseases and infections (Caminero *et al.,* 2010 and Keith *et al.,* 2005). Theoretically, antibiotics are combined for the treatment of serious infections to achieve bactericidal synergistic effect (Raad *et al.,* 2007), broaden coverage (O'Neill *et al.,* 2001), prevent the development of resistance (Firsov *et al.,* 2006; Chait *et al.,* 2007 and Hegreness *et al.,* 2008; Kim *et al.,* 2014), enhance activity against isolates with a minimum inhibitory concentration (MIC) at/or approaching the breakpoint for susceptibility, provide activity against organisms growing in biofilm and stationary-phase organisms (Saginur *et al.,* 2006 and Rose and Poppens, 2008), reduce toxicity and augment cells and tissues penetration (Yamaoka, 2007).

In this study, the potency of amoxicillin, benzylpenicillin and streptomycin as well as the possibility of synergistic, antagonistic or additive interaction between them in combinations were investigated. The minimum inhibitory concentrations (µg/ml) results indicated that streptomycin was the most potent of the antibiotics in inhibiting all bacterial isolates at concentrations ranging between 2 µg/ml and 200 µg/ml while the isolates were, however, highly resistant to other antibiotics based on their minimum inhibitory concentrations recorded. Although additivity, antagonism, synergy and autonomy or indifference have been defined (Rahal, 1978; King *et al.,* 1981; Hamilton-Miller, 1985 and Keith *et al.,* 2005), the inhibition zones and the MICs produced by the antibiotics and their combinations gave qualitative results on drug interactions vis-à-vis synergism, additive effects and antagonism. Streptomycin showed highest potency against all the bacterial isolates except *Staphylococcus aureus* ATCC 29213 against which benzylpenicillin was most active. Amoxicillin showed no zone around all the bacterial isolates except *P. aeruginosa* ATCC 27853. For synergy testing, inhibition zones from combined antibiotics wider than those produced by individual antibiotics were interpreted as indicating synergism, a lesser zone as antagonism and those with little or no change in zone diameter were regarded as additive. Thus, benzylpenicillin combined with streptomycin showed better antibacterial activity against *S. aureus* ATCC 29213, *E. coli* ATCC 23922 and *P. aeruginosa* ATCC 27853 than all the combined antibiotic solutions as this combination produced wider

inhibition zones than those of the individual antibiotics. On the other hand, amoxicillin combined with streptomycin and its combination with benzylpenicillin resulted in both additive and antagonistic interactions.

That benzylpenicillin and streptomycin showed the highest occurrences of synergism is not surprising. While drug synergy has been shown to be more efficient in clearing infections as well as achieving clearance at lower concentrations (Cottarel and Wierzbowski, 2007), synergy between beta-lactam antibiotics and aminoglycosides had been reported (Wolfe and Johnson, 1974 and Kohanski, 2010). The synergy between aminoglycosides and β-lactams has been attributed to β-lactam-mediated membrane damage leading to increased uptake of aminoglycosides (Kohanski *et al*., 2010). Such uptake effect is probably the cause of the synergy recorded between aminoglycoside and beta-lactam antibiotics (Plotz and Davis, 1962). Although Plotz and Davis (1962) indicated that penicillin enhances the intracellular entry of subinhibitory concentrations of streptomycin in *Escherichia coli*, the antagonism observed between benzylpenicillin and streptomycin may be due to the degree of resistance of *Escherichia coli* 25922 to benzylpenicillin and streptomycin combined while the roles of cellular functions such as lipopolysaccharide synthesis and adenosine triphosphate synthesis on diverse drug interactions (Chevereau and Bollenbach, 2015) may not be underestimated.

In the combination of amoxicillin and streptomycin, the additive and antagonistic interactions recorded in their combination may be alluded to the fact that bacteriostatic-bactericidal antibiotics combined often result in antagonistic interactions (Ocampo *et al.,* 2014) as killing by bactericidal antibiotics often requires cell growth which is prevented by bacteriostatic drugs (Bollenbach, 2015). It is a possibility that the low potency of amoxicillin reduced the effectiveness of streptomycin in the combination making the use of streptomycin singly a better option. Though antagonism is a warning against indeterminate treatment (Johansen *et al.,* 2000), antagonistic drug combinations require more investigation as clinical options (Yeh *et al.,* 2009) since hyperantagonistic drug combinations had been reported to select against bacterial population resistant to one of the drugs and instead favoured the completely sensitive wild type (Chait *et al.,* 2007).

In conclusion, resistance to antibiotics is becoming a difficult problem in the management of bacterial infections. Combining drugs could result in synergistic or antagonistic interactions which can either prevent the development of multidrug resistant strains, slow or accelerate antibiotic resistance evolution. This study showed that proportionate synergistic drug pairs can effectively subdue bacterial growth while antagonistic drug pairs would reverse the trend especially when targeting both sensitive and resistant strains with a combination of drugs of unknown interactions. Since there is a reduction in the production and the flow of new antibacterial drugs into the market coupled with the increasing prevalence of multidrug resistant bacterial infections, assessing the effect of combining old generations of antibiotics may be helpful in the war against antimicrobial resistance.

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8/24/2016