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Original Article

SYNERGISTIC COMBINATIONS OF BROAD SPECTRUM ANTIBIOTICS AGAINST *ACINETOBACTER SPP*

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ABSTRACT

Objective: The present study was aimed to investigate the resistant pattern of *Acinetobacter spp*. against 4 broad spectrum antibiotics and the synergistic activity between 3 different combinations of antimicrobials.

Methods: For conducting research we obtained 52 sample of bacterial spp. from different infection sites of patients admitted to tertiary care hospital. The procedure was carried out in two parts. At first the Kirby Bauer Disc Diffusion Method was adopted to evaluate resistance pattern according to CLSI standards. 08 broad spectrum antibiotics were used. In second part of the study the synergistic activity between different combinations of selected antibiotics were estimated by Double Disc Synergy Method.

Results: It was evident that male patients were highly infected by *Acinetobacter spp.* The organism was found responsible for infecting the respiratory tract of elderly patients at high rate. Colistin and Polymixin effectively inhibited *Acinetobacter spp.* 98% each. On contrary, it was found to be highly resistant against β-lactam (98%). Cephalosporins, Quinolones, Aminoglycosides, Carbapenem were also proven inactive when used alone. All the isolates when subjected to DDS test. Highest synergy was observed between combinations of Fosfomycin-Polymixin (90.38%), Almost 52% isolates were successfully inhibited when the combination of Colistin and Fosfomycin was checked. However, more than 61% isolates showed the highest resistance when the Polymixin-Imipenemwere used together. However, all the tested combinations were highly effective against *Acinetobacter*isolates obtained from tracheal aspirate.

Keywords: Double Disc Synergy, Resistance, Broad spectrum.

INTRODUCTION

The resistant strains today are emerging as a leading cause of morbidity and mortality. Organisms that were indigenous flora of human being are now causing life threatening infections. The species of Acinetobacter have been found to be the leading cause of resistant infections today [1]. Especially in those patients who are admitted to the hospital in MICU [2], It is the environment that had been stringently controlled to ensure inhibition of communicable diseases from patient to other patient as well as to avoid transfer of infectious agents from staff personnel [3]. There are several factors, identified by the previous relevant studies that have played key role as a contributing factor towards the development of resistant strains of indigenous bacterial strains. By at large the mutation in the genetic configuration of common pathogens has resulted in serious and life threatening incidence [4-8]. Other factors include improper intake of antibiotic dosage which initiated the activation or synthesis of certain inhibitory enzymes by bacteria rendering them to be untreatable by traditionally used broad spectrum antibiotics. [9, 10] Acinetobacter sp. is one amongst those opportunistic and multidrug resistant pathogens like *S. aureus, P. aeruginosa* [11], that are not inhibited effectively by the use of the single antibiotic. Several double and multiple combinations of broad spectrum antibiotics have been tested by different methods including Double Disc Diffusion method and some of them gave promising results [1, 12-19] giving hope and a new direction to the researchers and physicians. It also increased the options to avoid treatment failure.

Tatman-Otkun studied the trend of resistance of *Acinetobacter spp.* from 1996 to 2000. They observed a huge rate of increase in resistance against all the tested antibiotics especially the highest rate of resistance was against Ceftazidime. At the same time they also investigated the synergy between different combinations and observed that there was no detection of antagonism when used together and the most successful combination proved to be Ceftazidime-Amikacin and Ampicillin/Sulbactam-Tobramycin inhibiting 50% of bacterial isolates each [20]. The ability of genetic modification and production of enzymes by *Acinetobacter spp.* and *Pseudomonas aeruginosa* was also reported through researchers. Multiple reasons have been identified including mutation in genetic coding [21]. *Acinetobacterbaumannii* is the most prevalent bacterial isolate obtained from different hospitals today. One of related studies also ascertained the production of enzymes by *A. baummannii* and its resistance against antimicrobials [22]. Mahua Sinha indicated that the most common site of isolation of Acinetobacter is respiratory tract especially *A. baumannii*. Large no. of isolates were resistant to tested antibiotics except Carbapenems and Cefoperazone-Sulbactam [23]. Double Disc Synergy was used by different researchers to observe the trend of synergistic activity by different antimicrobial combinations. One of similar studies stated that the combination of Imipenem-Ethylenediaminetetraacetic acid was very effective against *Acinetobacter spp.* The study highly advocated the usefulness of Double Disc Synergy method [24]. Studies of Irene Galanialso indicated that the antibiotic which exhibited prominent activity against *Acinetobacter spp.* was Colistin [25].

MATERIALS AND METHODS

During the months of Feb 2014 till March 2014, we collected 52 samples from different infectious sites of patients admitted to hospital in Karachi. The isolates were identified and confirmed by conventional biochemical and differential techniques. The sensitivity /resistance pattern of all the isolates of *Acinetobacter spp.* was obtained by Kirby Bauer's Method [26]. Standard Oxoid discs of Fosfomycin 50µg, Colistin 10 µg, Polymixin B 300 IU, Imipenem 10 µg were used for determination of susceptibility pattern of bacterial isolates. The results were evaluated as per CLSI (Formerly NCCLS) recommendations [27, 28]. The bacterial isolates were then tested by double Disc Synergy method to observe the synergy against resistant strains of Gram-ve bacteria. The inoculum was grown in Mueller Hinton broth (Oxoiduk) for 4-6 hrs. at 37^{0}C and then lawn culture was made on Mueller Hinton Agar plate. After drying of inoculum the antibiotic discs are placed at a distance of sum of zone radii for each antimicrobial's zone of inhibition, which was obtained

when antimicrobials were tested alone and incubated for 24 hr at 37 ^{0}C. The data were analysed according to CLSI standards for the antagonism, indifference and synergism after visualization of the pattern of inhibited zone [29]. The procedure was performed with 52 isolates of Acinetobacter spp. in duplicate and repeat. *E. coli* 25922 and*S. aureus* 25923 were used as Q. C strains [33].

RESULTS

Our previous study showed prevalence of resistant strainsof *Acinetobacter*.(Table4, fig. 1) Isolates were largely collected from tracheal aspirate and found associated with respiratory disorders and the second highest collection site was through sputum sampling.(table 2),

However, table 3 shows the susceptibility pattern of individual antibiotics against isolates from different site of infection. After investigating the effectiveness of the combination of same antimicrobial together the most useful and effective combination was Fosfomycin-Polymixin, which was successful in inhibiting more than 90% of multidrug resistant isolates as shown in table 5, fig. 2. It was also obvious from our efforts that the combination of Colistin-Fosfomycin was not as much effective compared to Fosfomycin-Polymixin but still over 50 % of isolates were susceptible (fig. 3). It is also indicated in fig. 4 that the least potent combination was Polymixin-Imipenem, It inhibited synergistically only 38.46% of*Acinetobacterspp.* Moreover, it was observed that the tested combinations were highly effective against *Acinetobacter*isolates obtained from tracheal aspirate (table 6).

Table 1: Zone diameter Interprative standards for *Acinetobacter spp.* CLSI standards table of antibiotics for *Acinetobacter spp.*

Antibiotics	Zone of inhibition diameter (mm)			
	Disc content	Resistance	Intermediate	Sensitive
Colistin*	10µg	≤11	-	≥17
Fosfomycin*	50 µg	≤12	13-15	≥16
Imipenem	10 µg	≤13	14-15	≥16
Polymixin B*	300units	≤13	-	≥19

*Since the interpretive standards for Colistin, Fosfomycin and Polymixin B against Acinetobacter is not established in CLSI 2013mannual therefore Zone diameter interperative standards for Enterobacters and E. coli are used. (30)

Table 2: Distribution of *Acinetobacter*isolates in different sites of infection (N= 52)

Site of infection	Distribution of acinetobacter isolates		
	Male (n)	Female (n)	Total
Tracheal aspirate	17	12	29
Pus	02	02	04
Sputum	05	05	10
Blood	02	00	02
Pleural fluid	01	00	01
CVP-Tip	01	01	02
Wound	01	02	03
Peritoneal fluid	01	00	01
	32(61.5%)	**20(38.46%)**	**52**

Table 3: %Distribution of resistance pattern of Acinetobacter isolated from different sites by DDM

S. No.	Isolation Site	Fosfomycin			Imipenem			Colistin			Polymixin		
		R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)
1.	CVP-Tip	50	50	0	50	0	0	0	100	0	0	100	0
2.	TrachealAsp.	100	0	0	74.41	0	27.58	0	100	0	0	100	0
3.	Sputum	50	0	50	100	0	0	90	10	0	30	70	0
4.	Blood	50	0	50	50	50	0	0	100	0	50	50	0
5.	Pus	100	0	0	100	0	0	0	100	0	0	100	0
6.	Pleural fl.	0	0	100	100	0	0	0	100	0	0	100	0
7.	Peritoneal fl.	0	0	100	100	0	0	0	100	0	0	100	0
8.	Wound	33.33	0	66.66	100	0	0	0	100	0	0	100	0

Table 4: Total % efficacy of different antibiotics among *Acinetobacter spp.* isolated (N= 52)

S. No.	Antibiotics	Disc code	Resistance n (%)	Intermediate n(%)	Sensitive n (%)
1.	Colistin	CT	01(1.9)	00	51(98)
2.	Fosfomycin	FOS	34(65.38)	17(32.69)	01(1.9)
3.	Imipenem	IPM	51(98)	00	01(1.9)
4.	Polymixin B	POLB	01(1.9)	00	51(98)

Table 5: Double disc synergy results for different antimicrobial combinations checked against *acinetobacter spp.* isolated (N= 52)

S. No.	Drug combination	Synergy N (%)	Indifferent N (%)	Antagonism N (%)
1.	Colistin-fosfomycin	27(51.92)	25(48.08)	0(0)
2.	Polymixin-imipenem	20(38.46)	32(61.54)	0(0)
3.	Fosfomycin-polymixin	47(90.38)	05(9.62)	0(0)

Table 6: % synergy pattern of *Acinetobacter* isolated from different sites by double disc synergy method:

S. No.	Isolation source	Name antimicrobial combinations tested		
		Fosf-Polym. B (%)	Col-Fosf (%)	Polym. B-Imip (%)
1.	TrachAspi.	53.19	40.74	50
2.	Sputum	19.14	29.62	25
3.	Pus	8.51	3.70	5
4.	Wound	6.38	11.11	5
5.	CVP-Tip	4.25	7.40	5
6.	Blood	4.25	3.70	5
7.	Pleural Fluid	2.13	0	0
8.	Perit. Fluid	2.13	3.70	5

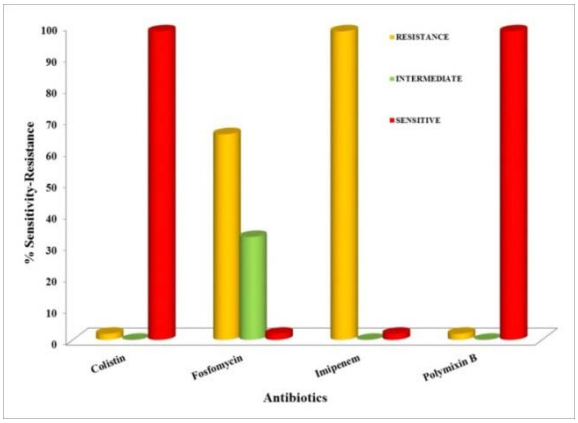

Fig. 1: Sensitivity pattern of *Acinetobacter spp.* against four broad spectrum antibiotics

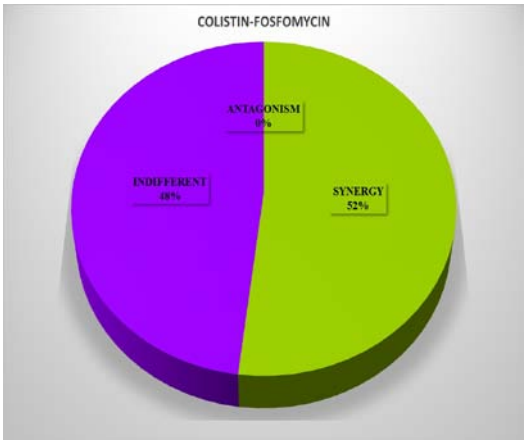

Fig. 2: Synergy between Col-Fosf

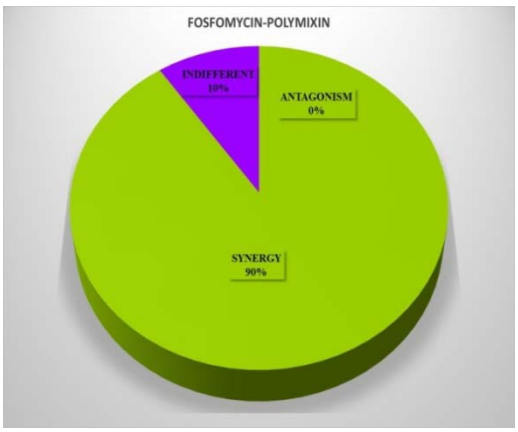

Fig. 3: Synergy between Fosf-Poly. B

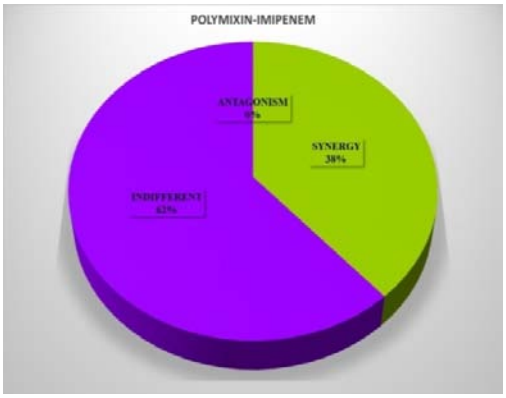

Fig. 4: Synergy between Poly. B-Imip

Fig. 2-4: Double Disc Synergy results for different antimicrobial combinationsChecked against*Acinetobacter spp.* Isolated

DISCUSSION

Not much data is available on an evaluation of synergy between broad spectrum antibiotics against *Acinetobacter spp.* by Disc Diffusion Method. Therefore an effort is made to study the current trend in the resistance pattern of *Acinetobacter spp.* As well as determination of an efficient combination of different antibiotics to inhibit the growth of bacterial isolates.

Our study was substantiated by two resembling studies conducted byThanaporn*et al* and Irene Galani. Thanaporn*et al*established through their finding that Colistin successfully inhibited 100% bacterial isolates when tested by E-test method while comparatively disc diffusion method showed 11-14 mm zone of inhibition.[15,35] We also observed that except one sample all remaining isolates displayed ranges of inhibition zone between 12-16 mm. Our results are in confirmation with the work done by EnasADaef*et al* who detected the high rate of resistance against Ciprofloxacin (64.7%) similar to our finding that Ciprofloxacin was least effective against *Acinetobacter spp.* and failed to inhibit94.23% isolates while 92.3 % isolates were resistant against CeftrioxoneEnas A Daef*et al* observed 56.9% inhibition. Our results were strongly contraindicated the findings of Enas A Daef who reported low rates (31.4%) of resistance against imipenem as compared to our studies showing 98% resistance.[14]

Our results are further supported by the findings of Wareham DW *et al* which indicated effectiveness of using combination and synergy between combination of Colistin and teicoplanin that significantly inhibited multidrug resistant *Acinetobacter spp.* [16]We also inspected that over 51% isolates were successfully inhibited by combine use of Colistin-Fosfomycin. One of another resembling study by Gordon NC *et al* who noticed the significant synergy between Vancomycin and Colistin when evaluation was done by three standard methods against Multidrug –Resistant strains.[31]Although the breakpoints for zone of inhibition for Acinetobacter has not been established yet for some third generation cephalosporin but we tested the sensitivity of Ceftrioxone alone which showed 0 mm inhibition depicting 92.3% resistance of *Acinetobacter spp.* One of related study conducted by

Alparabadia*et al* indicated that Cefotaxime if used in combination with Terminaliachebula extract can effectively inhibit the growth of *Acinetobacter spp.* [32]

CONCLUSION

Many traditionally used broad spectrum antibiotics are losing their effectiveness if used alone. However many combinations have been investigated and also resulted in the successful inhibition of resistant strains. Therefore, it is suggested that instead of opting monotherapy clinicians should adopt combination therapy. Moreover, those antibiotics should be chosen that complement each other and effectively inhibits the multidrug resistant *Acinetobacter spp*.

CONFLICT OF INTERESTS

Declared None

REFERENCE

1. Zhang Y, Chen F, Sun E, Ma R, Qu C, Ma L. *In vitro* antibacterial activity of combinations of fosfomycin, minocycline and polymyxin B on pan-drug-resistant *Acinetobacterbaumannii* ExpTher Med. 2013;5:1737-39.
2. Principe L, D'Arezzo S, Capone A, Petrosillo N, Visca P. *In vitro* activity of tigecycline in combination with various antimicrobials against multidrug resistant *Acinetobacterbaumannii.* Ann Clin Microbiol Antimicrob 2009;8:18.
3. Charles R Bonapace, Roger L White, Lawrence V Friedrich, John A Bosso. Evaluation of antibiotic synergy against *Acinetobacterbaumannii*: a comparison with Etest, time-kill, and checkerboard methods. Diagn Microbiol Infect Dis 2000;38:43-50.
4. Dai XT, Sun FJ, Chen ZH, Luo GM, Feng W, Xiong W, *et al*. The Epidemiology and Resistance Mechanisms of Acinetobacterbaumannii Isolates from the Respiratory Department ICU of a Hospital in China. Microb Drug Resist. 2014; 20(6):618-22.
5. Mahzounieh M, Khoshnood S, Ebrahimi A, Habibian S, Yaghoubian M. Detection of antiseptic-resistance genes in pseudomonas and acinetobacter spp. isolated from burn patients. Jundishapur J Nat Pharm Prod 2014;9:e15402.
6. Dettori M, Piana A, Deriu MG, Lo Curto P, Cossu A, Musumeci R, *et al*. Outbreak of multidrug-resistant Acinetobacterbaumannii in an intensive care unit. New Microbiol 2014;37:185-91.
7. Poulikakos P, Tansarli GS, Falagas ME. Combination antibiotic treatment versus monotherapy for multidrug-resistant, extensively drug-resistant, and pandrug-resistant *Acinetobacter* infections: a systematic review. Eur J Clin Microbiol Infect Dis 2014;33:1675-85.
8. David W Wareham, David C Bean. *In-vitro* activity of polymyxin B in combination with imipenem, rifampicin and azithromycin versus multidrug resistant strains of *Acinetobacterbaumannii* producing OXA-23 carbapenemases. Ann Clin Microbiol Antimicrob 2006;21:5:10.
9. Robert A. Bonomo1 and dora szabo mechanisms of multidrug resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa.* Clin Infect Dis 2006;43 Suppl 2:S49-S56.
10. Lee JH, Choi CH, Kang HY, Lee JY, Kim J, Lee YC, *et al*. Differences in phenotypic and genotypic traits against antimicrobial agents between *Acinetobacterbaumannii* and Acinetobacter genomicspecies 13TU. J Antimicrobial Chemother 2007;59:633–9.
11. Jana M Swenson, George E Killgore, Fred C. Tenover Antimicrobial Susceptibility Testing of *Acinetobacter*spp. By NCCLS broth microdilution and disk diffusion methods. J Clin Microbiol 2004;42:5102-08.
12. Spoorthi N Jain, Vishwanatha T, Reena V, Divyashree BC, Aishwarya Sampath, Siddhalingeshwara KG, Venugopal N, *et al*. Antibiotic synergy test: checkerboard method on multidrug resistant *Pseudomonas Aeruginosa* IRJP 2011;2:196-8.
13. Tan TY, Lim TP, Lee WH, Sasikala S, Hsu LY, Kwa AL. *In vitro* antibiotic synergy in extensively drug-resistant *Acinetobacterbaumannii*: The effect of testing by time-kill, checkerboard, and etest methods *Antimicrob.* Agents Chemother 2011;55:436.
14. Enas A Daef, Ismail S Mohamed, Ahmed S Ahmed, Nahla M Elsherbiny, Ibrahim M. Sayed Evaluation of different phenotypic assays for the detection of metallo-β-lactamase production in*carbapenem*susceptible and resistant *Acinetobacterbaumannii*isolates. J Am Sci 2012;8:292-9.
15. Hortiwakul T, Chayakul P, Ingviya N, Chayakul V. *In vitro* activity of colistin, Fosfomycin, and Piperacillin/tazobactam Against *Acinetobacterbaumannii*and *Pseudomonas aeruginosa*in Songklanagarind Hospital, Thailand. J Infect Dis Antimicrob Agents 2009;26:91-6.
16. Wareham DW, Gordon NC, Hornsey M. *In vitro* activity of teicoplanin combined with colistin versus multidrug-resistant strains of *Acinetobacterbaumannii.* J Antimicrob Chemother 2011;66:1047–51.
17. Divyashree BC, Vishwanatha T, Spoorthi N Jain, Reena V, Aishwarya Sampath, Siddhalingeshwara KG, *et al*. Valuation Of *In vitro* synergy between amipicillin and kanamycin against. *Staphylococcus Aureus.* J Drug Delivery Ther 2012:2:144-6.
18. Sueke H, Kaye SB, Neal T, Hall A, Tuft S, Christopher M. Parry an *in vitro* investigation of synergy or antagonism between antimicrobial combinations against isolatesfrom bacterial keratitis. Invest Ophthalmol Visual Sci 2010:51:4151-5.
19. Soltani R, Khalili H, Shafiee F. Double-disk synergy test for detection of synergistic effect between antibiotics against nosocomial strains of *Staphylococcus aureus.* J Res Pharm Practice 2012;1:21-4.
20. Tatman-Otkun M, Gürcan S, Ozer B, Shokrylanbaran N. Annual trends in antibiotic resistance of nosocomial Acinetobacterbaumannii strains and the effect of synergistic antibiotic combinations. Microbiol 2004;27:21-8.
21. Robert A. Bonomo and dora szabo clinical infectious diseases september mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. 2006;43 Suppl 2:S49-S56.
22. Lee JH, Choi CH, Kang HY, Lee JY, Kim J, Lee YC, *et al*. Differences in phenotypic and genotypic traits against antimicrobial agents between *Acinetobacterbaumannii* and Acinetobacter genomicspecies 13TU. J Antimicrobial Chemother 2007;59:633–9.
23. Sinha M, Srinivasa H, Macaden R. Antibiotic resistance profile & extended spectrum beta-lactamase (ESBL) production in *Acinetobacter*species. Indian J Med Res 2007;126:63-7.
24. Yan JJ, Wu JJ, Tsai SH, Chuang CL. Comparison of the double-disk, combined disk, and Etest methods for detecting metallo-β-lactamases in gram-negative bacilli. Diagn Microbiol Infect Dis 2004;49:5-11.
25. Galani I, Kontopidou F, Souli M, Rekatsina PD, Koratzanis E, Deliolanis J, *et al*. Colistinsusceptibility testing by Etest and disk diffusion methodsInternational. J Antimicrobial Agents 2008;31:434-9.
26. Bauer AW, Kirby WMM, Sherris JC, Tuck M. Antibiotics susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966;45:493-6.
27. National Committee for Clinical Laboratory Standards. Performance standard for antimicrobial disc susceptibility test. 5th ed. Villanova PA: NCC Document; 1993. p. M2-A5.
28. Clinical and laboratory standards institute, Performance standard for antimicrobial susceptibility testing X. CLSI, Wayne PA. CLSI Document M; 1993;29(3):100-S19.
29. Eliopoulos GM, RC moelleringjr. Antimicrobial combinations. In: V Lorian. ed, Antibiotics in Laboratory Medicines. 4thed. The Williams & Wilkins Co, Baltimore, Md; 1996. p. 330-96.
30. Özseven AG, Çetin ES, Arıdoğan BC, Özseven L. *In vitro* synergistic activity of carbapenems in combination with other antimicrobial agents against multidrug-resistant *Acinetobacterbaumannii* Afr J Microbiol Res 2012;6:2985-92.
31. Gordon NC, Png K, Wareham DW. Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of *Acinetobacterbaumannii*. Antimicrobial Agents Chemother 2010;54:5316–22.
32. Rabadia A, Kamat SD, Kamat DV. Study of synergistic action of cefotaxime and *terminalia chebula* on *acinetobacter baumannii* using checkerboard assay. Int J Pharm Pharm Sci 2013;5:830-2.
33. Clinical and Laboratory Standards Institute, Performance standard for antimicrobial susceptibility testing. CLSI Wayne PA. CLSI Document 2013;33(1):M100-S23.