JMBR: A Peer-review Journal of Biomedical Sciences
December 2015, Vol. 14 No. 2 pp 63-72

EFFICACY OF RIFAMPICIN COMBINATION THERAPY ON SELECTED NON-MYCOBACTERIA ORGANISMS ISOLATED FROM A TERTIARY HEALTH INSTITUTION IN BENIN CITY.

[1]G. C. Josephs, [2]I. M. Moses-Otutu and [1]B. M. Omayone

ABSTRACT
Combination therapy or polytherapy is the use of more than one antibacterial agent in the treatment of a single infection. In this study, rifampicin that was mainly used in the treatment of mycobacterial infections has been used in combination with other agents in non mycobacterial organisms. Clinical isolates of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* strains were exposed to antibacterial agents namely, Gentamicin, Cefuroxime, Ceftriaxone and Norfloxacin singly and also in combination with Rifampicin. Each minimum inhibitory concentration (MIC) as well as agar diffusion assays was determined. No antagonism was confirmed between rifampicin and any of the combination antibiotics evaluated. The study illustrated that synergy was most often observed with Staphylococcus aureus. The most potent combinations for Staphylococcus aureus were rifampicin and gentamicin, rifampicin and norfloxacin. However, Synergy was less often noted in the combination of 20μg rifampicin with Cefuroxime and ceftriaxone at varied concentration. At 10μg, there was no synergistic effect when compared with the use of Cefuroxime and ceftriaxone alone. At 20μg, Staphylococcus aureus and Escherichia coli were effectively inhibited, while at 30μg all isolates were inhibited. This study has also shown that MICs were lowest with norfloxacin, followed by cefuroxime, ceftriaxone than gentamicin. Moreso, cefuroxime, has minimal activity against Pseudomonas aeruginosa strain. However, the zones of inhibition of the drugs when the organisms were cultured in medium containing 20μg rifampicin had improved antibacterial outcomes as seen from the greater diameters of inhibition. Also, the results shows that the MIC of gentamicin monotherapy on Staphylococcus aureus gave 20µg, but combination with rifampicin reduced the MIC to 10µg, however the progeny of this test retained the MIC of the combined therapy (10µg) on *Staphylococcus aureus. Rifampicin combination therapy has enhance effect on some non–mycobacterial organisms; Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and Pseudomonas aeruginosa compared to the single use of each drug.

INTRODUCTION

Antibiotic combination therapy for treatment of infections caused by Gram-negative organisms was initially used to exploit the synergy seen in in-vitro studies between two antibacterial agents. Compared to clinical outcome of using a single antibacterial agent, combination was used to overcome the possible development of resistance during antibiotic usage and ultimately to have a broader effect of two or more antibacterial agent on the pathogen within different spectra of activity[1,2]. The combination antibiotic therapy used to treat infections with Gram-negative bacilli includes two agents to which an organism shows in-vitro susceptibility, that is a β- lactam and an aminoglycoside[3,4] or a fluoroquinolone[5,6]. Moreso, the Checkerboard technique was previously used to demonstrate synergy between a β- lactam and an aminoglycoside combination in in-vitro

KEYWORDS: *Rifampicin, Combination therapy, Non-Mycobacterial organisms.*

[1]G. C. Josephs, [2]I. M. Moses-Otutu and [1]B. M. Omayone
[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin,
[2]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences,University of Benin
PMB 1154, Benin City, Nigeria.

**** Correspondence***
Email: *ifueko.moses-otutu@uniben.edu*
Phone: +234-8036868229

studies and animal models. It was observed that combination allows for a different mechanism of bacteria death[7,8,9]. While the β- lactam acts by disturbing the cell wall of Gram-negative bacteria, thereby allowing the passage of aminoglycoside into the periplasmic space[10]. Also, penicillin was shown to enhance the uptake of aminoglycoside in enterococcal endocarditis in in-vitro models[11]. However, clinical studies showing evidence of synergism between a β- lactam and an aminoglycoside is rare[12,13,14,15]. Reasons are that clinical cure is defined as the resolution of signs and symptoms of infections.

Synergy has also been established in β-lactam and fluoroquinolone combination[16,17]. The synergy ranges from 17% to 82%[18]. Fluoroquinolones are known to have excellent tissue penetration into the lungs, meninges and bone marrow, with low nephrotoxicity when compared to aminoglycosides.

Unfortunately, Gram-negative bacteria's resistance to antibiotic routinely used for treatment is on the increase[19]. This has given rise to urgent need for the use of antimicrobial combination therapy to prevent or rather delay the development of resistance during therapy[20].

Rifampicin, a semisynthetic compound derived from Streptomyces mediterranei, is seen to possess excellent tissue penetration, oral bioavailabiliy and unique bacteria activity demonstrated in biofilms growing on the surfaces of prosthetic devices[21]. However, emergence of bacteria resistance still occurred, which constitutes a major setback in rifampicin monotherapy. Rifampicin mutations occur within bacteria due to the alteration in the rpo B gene[22]. The aforementioned has given rise to the need for rifampicin combination with other antimicrobial agents. Rifampicin previously used as a major part of antituberculosis treatment is an obvious example for the successful use of antimicrobial combination therapy to prevent resistance in the treatment of infections caused by Mycobacterium tuberculosis[21]. Its combination with other anti-tuberculosis drugs effectively decreased the rate of development of resistance of Mycobacterium tuberculosis to rifampicin.

Rifampicin has found use in combination with other groups of antibiotics; infections due to Staphylococci were one of the first non-mycobacterial diseases treated with rifampicin therapy. Importantly, previous research has shown that the combined therapy had better treatment responses for infections with "lower organism burden"; an example, urinary tract infections[22]. Colistin and rifampicin combination is also an effective and safe combination therapy for severe infections with multi-drug resistant Acinetobacter baumanii[28] or multi drug resistant *Pseudomonas* aeruginosa[25].

The rifampicin MICs against many bacterial organisms were determined in the late 1960s. Previous researches have shown that rifampin is active in vitro against Staphylococcus. aureus, Staphylococcus albus (Staphylococcus epidermidis), Streptococcal organisms (including Streptococcus pneumoniae), Clostridium welchii, Neisseria meningitidis, and Pasteurella multocida. Rifampin has also demonstrated some in vitro activity against Haemophilus influenza and Bacteroides sp. but was inconsistent for the treatment of Salmonella, and Shigella infections[22].

This study is aimed at investigating the efficacy of sub-inhibitory concentration of rifampicin in combination with β-lactam, aminoglycoside or fluoroquinolone combination therapy compared with each monotherapy, on selected non Mycobacterial organisms *(Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and Pseudomonas aeruginosa).

MATERIALS AND METHODS

Bacterial isolates

Clinical strains of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* organisms were isolated between January and June 2015 from the Bacteriology Laboratory of the University of Benin Teaching Hospital (UBTH) Benin City, Nigeria. All strains were identified using the Cheesbrough[23] and Kolhathar[24] recommended methods. Each isolate was prepared to the density of 0.5 McFarland Standard.

Antibiotics

All antimicrobial agents were purchased at microbiological or analytical grade from their respective manufacturers (Gentamicin (Gentalek® -Lek), Cefuroxime (Emzor Pharmaceuticals), Ceftriaxone (Rocephin®-GSK), Norfloxacin (Sam pharmaceuticals), Rifampicin (Sigma, Gmb) and were used without further purification. 1mg/ml stock solution of each, rifampicin, cefuroxime, gentamicin, ceftriaxone and norfloxacin were prepared on the day of usage.

Media

Nutrient broth (LAB M, England) was prepared according to manufacturer's instructions and used for minimum inhibitory concentration within 24hours after preparation. Nutrient agar (LAB M, England) by pour plate method was used for colony counts. Concentrations were made to the final volume of 20ml agar.

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the various drugs was determined using agar dilution method. Each inoculum was obtained by adjusting overnight culture of the organism to 0.5 Mc Farland Standard. The corresponding volume of the drug was pipetted into Petri dish, and the required volume of molten nutrient agar was added to make up to 20ml. The MIC was defined as the lowest concentration of antibiotic with no visible growth. "+" represents growth of the isolate, while "_" represents no growth of isolate. A standard sensitive strain of *Escherichia coli* (CW 3310) was included as the control organism. Measurement in millimeters of diameters of zones of inhibition was determined. Microdilution test procedure was carried out to ensure that MIC values of the antibiotics studied were within the accuracy range as stated by the Clinical and Laboratory Standards Institute (CLSI). Agar diffusion method was used for the determination of zones of inhibition.

MIC of the combination of sub inhibitory concentration (20μg) of rifampicin was added to the different concentrations (10μg, 20μg, 30μg and 40μg) of the different drugs (namely, gentamicin, cefuroxime, ceftriaxone and norfloxacin) against each of the isolates *(Staphyloccocus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa).*

The progeny was subcultured and the MIC was determined.

RESULTS

A total of 4(four) isolates were obtained and they comprised of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*. The isolates were exposed to the following antibacterial agents, gentamicin, cefuroxime, ceftriaxone and norfloxacin singly and in combination with rifampicin. Single use of each antibacterial agent had an inhibitory effect depending on the concentration used on the organisms. At 20μg, it was only *Staphylococcus aureus and Escherichia coli* growth that were inhibited by gentamicin whereas at 30μg and 40μg all the organisms growth were inhibited by gentamicin. The cephalosporins (cefuroxime and ceftriaxone) inhibited the growth of *Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae but had no effect on Pseudomonas aeruginosa* at the various concentrations. Norfloxacin had inhibitory effect on all isolates at 40μg (Table1).

No antagonism was observed between rifampicin with any combination antibacterial agent evaluated (Table 2). The result shows that the combination of 20μg rifampicin was synergistic with cefuroxime, ceftriaxone and norfloxacin at various concentrations respectively. However, synergy was less often noted in the combination of 20μg rifampicin with the cephalosporins (cefuroxime and ceftriaxone) at varied concentration. At 10μg, there was no synergistic effect when compared with the single use of the cephalosporins (cefuroxime and ceftriaxone) whereas, at 20μg, *Staphylococcus aureus* and Escherichia coli were effectively inhibited, while at 30μg all isolates were inhibited (Table 2). The combination of 20μg rifampicin with various concentrations (10μg, 20μg, 30μg) of gentamicin and norfloxacin greatly inhibited the growth of Staphylococcus aureus when compared with single use of the drugs.

The various antibacterial agents had different levels of activity against the various bacterial isolates. The MICs were lowest with norfloxacin, followed by cefuroxime, ceftriaxone then gentamicin. While, cefuroxime, like other cephalosporin, had minimal activity against Pseudomonas aeruginosa isolate (Table 3).

The results in table 4 shows that the sub inhibitory concentration of rifampicin (20µg) combined with the other drugs in the presence of the clinical isolates reduced the MIC to 10µg. The progeny of these test on the organisms also retained the MIC of the combined therapy (10µg).

Table 1: Growth of each isolate after exposure to the various concentration of each drug.

A	Gentamicin				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	_	_	+	+
	30 µg	_	_	_	_
	40 µg	_	_	_	_
B	Cefuroxime				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	+	+	+	+
	30 µg	_	_	+	+
	40 µg	_	_	_	+
C	Ceftriaxone				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	+	+	+	+
	30 µg	_	_	+	+
	40 µg	_	_	_	+
D	Norfloxacin				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	+	+	+	+
	30 µg	_	_	+	+
	40 µg	_	_	_	_
E	Rifampicin				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	+	+	+	+
	30 µg	_	_	+	+
	40 µg	_	_	_	_

Table 2: Growth of each isolate after exposure to the various concentrations of combined antibiotics

A	Gentamicin+Rifampicin(20µg)				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	_	+	+	+
	20 µg	_	_	+	+
	30 µg	_	_	_	_
B	Cefuroxime+Rifampicin(20µg)				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	_	_	+	+
	30 µg	_	_	_	_
C	Ceftriaxone+Rifampicin(20µg)				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	+	+	+	+
	20 µg	_	_	+	+
	30 µg	_	_	_	_
D	Norfloxacin+Rifampicin(20µg)				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
	10 µg	_	+	+	+
	20 µg	_	_	+	+
	30 µg	_	_	_	_

Table 3: MIC of each antibiotic alone and in combination with Rifampicin

	Staphylococcus aureus	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
Gentamicin alone	20µg	20µg	30µg	30µg
Gentamicin+Rifampicin	10µg	20µg	30µg	30µg
Rifampicin alone	30µg	30µg	40µg	40µg
Cefuroxime alone	30µg	30µg	40µg	40µg
Cefuroxime+Rifampicin	<20µg	<20µg	<30µg	<30µg
Rifampicin alone	30µg	30µg	40µg	40µg
Ceftriaxone alone	30µg	30µg	40µg	40µg
Ceftriaxone+Rifampicin	<20µg	<20µg	<30µg	<30µg
Rifampicin alone	25µg	25µg	30µg	30µg
Norfloxacin alone	30µg	30µg	40µg	40µg
Norfloxacin+Rifampicin	10µg	20µg	30µg	30µg
Rifampicin alone	30µg	30µg	40µg	40µg

Table 4: MIC of each organism's progeny

	Staphylococcus aureus(progeny)	***Escherichia coli(progeny)***	***Klebsiella pneumonia(progeny)***	***Pseudomonas aeruginosa(progeny)***
Gentamicin alone	20µg	20µg	30µg	30µg
Gentamicin+Rifampicin	10µg	20µg	30µg	30µg
Gentamicin on progeny	10µg	20µg	30µg	30µg
Cefuroxime alone	30µg	30µg	40µg	40µg
Cefuroxime+Rifampicin	20µg	20µg	30µg	30µg
Cefuroxime on progeny	20µg	20µg	30µg	30µg
Ceftriaxone alone	30µg	30µg	40µg	40µg
Ceftriaxone+Rifampicin	20µg	20µg	30µg	30µg
Ceftriaxone on progeny	20µg	20µg	30µg	30µg
Norfloxacin alone	30µg	30µg	40µg	40µg
Norfloxacin+Rifampicin	10µg	20µg	30µg	30µg
Norfloxacin on progeny	10µg	20µg	30µg	30µg

DISCUSSION

Antimicrobial combination therapy is used primarily to extend spectrum coverage, prevent the emergence of resistant mutants and gain synergy between antimicrobials. No antagonism was observed between rifampicin and any of the other antibiotics it was combined with. This study shows that synergy was most often observed with *Staphylococcus aureus.* The most potent combination for Staphylococcus aureus was rifampicin and gentamicin, rifampicin and norfloxacin. The combination of 20μg rifampicin with various concentrations (10μg, 20μg, 30μg) of gentamicin and norfloxacin greatly inhibited the growth of Staphylococcus aureus when compared to a single use of the drugs. Similarly, a study conducted by combining rifampicin with daptomycin at various concentrations also observed a positive synergistic effect between both drugs[25]. It was also observed that Staphylococcus aureus was eradicated more commonly in rifampicin combination regimen (rifampicin with ciprofloxacin, minocycline, cloxacillin, and vancomycin) compared to monotherapy with other systemic agents[26].

The result also shows that the combination of 20μg rifampicin (sub inhibitory concentration) was synergistic with cefuroxime, ceftriaxone and norfloxacin at various concentrations respectively. Moreover, synergy was less often noted in the combination of 20μg rifampicin with the cephalosporins (cefuroxime and ceftriaxone) at varied concentration. At 10μg, there was no synergistic effect when compared with the single use of the cephalosporins (cefuroxime and ceftriaxone) whereas, at 20μg, Staphylococcus aureus and Escherichia coli were effectively inhibited, while at 30μg all isolates were inhibited. However, in a study conducted on carbapenem-resistant Acinetobacter baumanii isolate, antagonism was observed between rifampicin and polymixin combination therapy[27]. Moreover, in in-vitro study of colistin combinations against extensively drug-resistant Acinetobacter baumanii producing OXA-23 carbapenemase, observed that combinations between colistin and rifampicin, colistin and imipenem were more active than the combinations of colistin and fosfomycin, colistin and levofloxacin[28].

The various antibacterial agents have different levels of activity against the various bacterial isolates. This result have shown that MICs were lowest with norfloxacin, followed by cefuroxime, ceftriaxone then gentamicin. Cefuroxime, like other cephalosporin, has minimal activity against *Pseudomonas aeruginosa.*

The results also show that the sub inhibitory concentration of rifampicin (20μg) combined with the other drugs in the presence of the clinical isolates reduced the MIC to 10μg. The progeny of these test on the organisms also retained the MIC of the combined therapy (10g). This may have been achieved by the removal or deletion of the resistance factor which may have been an R-plasmid. This agrees with the findings on rifampicin curing of plasmids in Escherichia coli K_{12}-rifampicin resistant host[29]. This means that a combination of these drugs can apply after in-vivo investigation.

CONCLUSION

Rifampicin combination therapy has enhanced effect on some non-mycobacterial organisms; *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* compared to the single use of the drugs. Rifampicin can now be used in

combination with other antibiotics other than the anti-tuberculous drugs for the treatment of resistant bacteria especially in Escherichia coli and Staphylococcus aureus infections.

RECOMMENDATION

The fact that in-vitro test does not include the drugs hepatic and renal metabolism, serum protein binding, drug distribution, half-life as well as other drug interactions, in-vivo, and clinical studies should be done to determine these drugs administration, the regime and dosing interval.

REFERENCES

1. Elphick, H. E. and Tan, A. (2001). Single versus combination intravenous antibiotic therapy for people with cystic fibrosis. Cochrane Database Syst. Rev.:CD002007. doi:10.1056/NEJM198712313172703.

2. Micek, S. T., Lloyed, A. E., Ritchie, D. J., Reichley, R. M., Fraser, V. J. and Kollef, M. H. (2010). Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to Gram-negative bacteria: a retrospective analysis. Antimicrob. Agents Chemother. 54: 1742–1748.

3. Paul, M. and Leibovici, L. (2005). Combination antibiotic therapy for Pseudomonas aeruginosa bacteraemia. Lancet Infect. Dis. 5: 192–194.

4. Gasink, L. B., Fishman, N. O., Weiner, M. G., Nachamkin, I., Bilker, W. B. and Lautenbach, E. (2006). Fluoroquinolone-resistant Pseudomonas aeruginosa: assessment of risk factors and clinical impact. Am. J. Med. 119(526): e519-e525.

5. Bhat, S., Fujitani, S., Potoski, B. A., Capitano, B., Linden, P. K., Shutt, K. and Paterson, D. L. (2007). Pseudomonas aeruginosa infections in the intensive care unit: can the adequacy of empirical beta-lactam antibiotic therapy be improved? Int. J. Antimicrob. Agents 30: 458–462.

6. Hyle, E. P., Lipworth, A. D., Zaoutis, T. E., Nachamkin, I., Bilker, W. B. and Lautenbach, E. (2005). Impact of inadequate initial antimicrobial therapy on mortality in infections due to extended-spectrum beta-lactamase-producing Enterobacteriaceae: variability by site of infection. Arch. Intern. Med. 165: 1375–1380.

7. El Amari, E. B., Chamot, E., Auckenthaler, R., Pechere, J. C. and Van Delden, C. (2001). Influence of previous exposure to antibiotic therapy on the susceptibility pattern of Pseudomonas aeruginosa bacteremic isolates. Clin. Infect. Dis. 33: 1859–1864.

8. Giske, C. G., Monnet, D. L., Cars, O. and Carmeli, Y. (2008). Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob. Agents Chemother. 52: 813–821.

9. Soo Hoo, G. W., Wen, Y. E., Nguyen, T. V. and Goetz, M. B. (2005). Impact of clinical guidelines in the management of severe hospital-acquired pneumonia. Chest 128: 27780–27787.

10. Guner, R., Hasanoglu, I., Keske, S., Kalem, A. K. and Tasyaran, M. A. (2011). Outcomes in patients infected with carbapenem-resistant Acinetobacter baumannii and treated with tigecycline alone or in combination therapy. Infection. 39: 515–518.

11. Moellering, R. C. (1979). Antimicrobial synergism an elusive concept. J. Infect. Dis. 140: 639-641.

12. Kett, D. H., Cano, E., Quartin, A. A., Mangino, J. E., Zervos, M. J., Peyrani, P., Cely, C. M., Ford, K. D., Scerpella, E. G. and Ramirez, J. A. (2011). Implementation of guidelines for management of possible multidrug-resistant pneumonia in intensive care: an observational, multicentre cohort study. Lancet Infect. Dis. 11: 181–189.

13. Bouza, E. and Munoz, P. (2000). Monotherapy versus combination therapy for bacterial infections. Med. Clin. North Am. 84: 1357–1389.

14. Daikos, G. L., Petrikkos, P., Psichogiou, M., Kosmidis, C., Vryonis, E., Skoutelis, A., Georgousi, K., Tzouvelekis, L. S., Tassios, P. T., Bamia, C. and Petrikkos, G. (2009). Prospective observational study of the impact of VIM-1 metallo-beta-lactamase on the outcome of patients with Klebsiella pneumoniae bloodstream infections. Antimicrob. Agents Chemother. 53: 1868–1873.

15. Neuhauser, M. M., Weinstein, R. A., Rydman, R., Danziger, L. H., Karam, G. and Quinn, J. P. (2003). Antibiotic resistance among gram-negative bacilli in US intensive care units: implications for fluoroquinolone use. JAMA. 289: 885–888.

16. Klibanov, O. M., Raasch, R. H. and Rublein, J. C. (2004). Single versus combined antibiotic therapy for gram-negative infections. Ann. Pharmacother. 38: 332–337.

17. Yellin, A. E., Hassett, J. M., Fernandez, A., Geib, J., Adeyi, B., Woods, G. L. and Teppler, H. (2002). Ertapenem monotherapy versus combination therapy with ceftriaxone plus metronidazole for treatment of complicated intra-abdominal infections in adults. Int. J. Antimicrob. Agents. 20: 165–173.

18. Ibrahim, E. H., Ward, S., Sherman, G., Schaiff, R., Fraser, V. J. and Kollef, M. H. (2001). Experience with a clinical guideline for the treatment of ventilator-associated pneumonia. Crit. Care Med. 29: 1109–1115.

19. Paterson, D. L. (2008). Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clin. Infect. Dis. 47(Suppl. 1): S14–S20.

20. Mouloudi, E., Protonotariou, E., Zagorianou, A., Losifidis, E, Karapanagiotou, A., Giasnetsova, T., Tsioka, A., Roilides, E., Sofianou, D. and Gritsi-Gerogianni, N. (2010). Bloodstream infections caused by metallo-beta-lactamase/Klebsiella pneumoniae carbapenemase-producing K. pneumoniae among intensive care unit patients in Greece: risk factors for infection and impact of type of resistance on outcomes. Infect. Control Hosp. Epidemiol. 31: 1250–1256.

21. Nadim, C., Jean-Marc, R. and Philippe, B. (2014). A new strategy to fight antimicrobial resistance: the revival of old antibiotics. Frontiers in microbiology. Available from doi: 10.3389/fmicb.2014.00551.

22. Forrest, G.N. and Tamura, K. (2010). Rifampicin combination therapy for non-mycobacterial infections. Clin. Microbiol. Rev. 23: 14–34.

23. Cheesebrough, M. (2006). District Laboratory Practice in Tropical Countries. Cambridge University press, Cambridge, New York, Melbourne, Madrid, Cape Town, Sao Paulo; 2nd edition: 62-70.

24. Kolhatkar, O. (2008). Medical Laboratory Science. Tata McGraw-Hill Publishing Company Limited, 7 West Patel Nagar, New Delhi 110008, Typeset at Anvi composers.

25. Hideharu, H., Tomohiro, T., Kosuke, K., Asuka, S., Kikuko, A.,Koichi, W., Yoshihisa, H. and Fumio, O. (2014). Successful Treatment of Persistent MRSA Bacteremia using High-dose Daptomycin Combined with Rifampicin. Intern Med. 53: 2159-2163.

26. Mathew, E. F. and Konstantinos, N. F. (2007). Oral rifampicin for eradication of Staphylococcus aureus carriage from healthy and sick populations: A systematic review of the evidence from comparative trials. Am. J. Infect. Contrl. 35(2): 106-114.

27. Nageeb, W., Metwally, L., Kamel, M. and Zakaria, S. (2015). Invitro antimicrobial synergy studies of carbapenem-resistant Acinetobacter baumannii isolated from intensive care unit of a tertiary care hospital in Egypt. J. Infect. Public Health. Available from doi: 10.1016/j.jiph 2015.05.007.

28. Wei, W., Yang, H., Liu, Y., Ye, Y. and Li, J. (2015). Invitro synergy of colistin combinations against extensively drug resistant Acinetobacter baumannii producing OXA-23 carbapenemase. J. Chemother. Available at 19739478150000000030.

29. Obaseki-Ebor, E. E. (1984). Rifampicin curing of plasmids in Escherichia coli K_{12}-rifampicin resistant host. J. Pharm. 30: 7467-7470.