World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Combination of gentamicin and clindamycin as a promising therapy for multidrug-resistant (MDR) methicillin-resistant *Staphylococcus aureus* isolated in Enugu State

Miriam Goodness Udochukwu Nwaneri [1], Ijeoma Ngozi. Ebenebe [1], Chinenye Henrietta Nedum [2], Vincent Chima Okore [3], Anthony Amaechi Attama [4], Udochi Anayo Ugo [5,*], Lilian Ngozi Chukwuma [6] and Chukwunonso Marbel Eduzor [7]

[1] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[2] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[3] *Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.*
[4] *Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.*
[5] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.*
[6] *Department of Medical Laboratory Science, Microbiology unit, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.*
[7] *Department of Science Laboratory Technology (SLT), Federal Polytechnic Oko, Anambra State, Nigeria.*

World Journal of Advanced Research and Reviews, 2024, 21(03), 2233–2239

Publication history: Received on 12 February 2024; revised on 24 March 2024; accepted on 27 March 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.21.3.0907

Abstract

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is prevalent in Nigeria. Due to its increasing resistance to treatments with single antibiotics, such as vancomycin and others, it has become necessary to identify appropriate combination of antibiotics for treatment. This research tested the proneness of MRSA isolated in southeastern Nigeria to a combined treatment of selected antibiotics (vancomycin, clindamycin, gentamicin, and ceftriaxone), to identify the best combination for the treatment of MRSA isolated in the region.

Methods: Various samples from patients were obtained in three laboratories in Enugu, Nigeria and cultured for isolation and purification. Minimum inhibitory concentration of vancomycin, clindamycin, gentamicin, and ceftriaxone antibiotics against the purified isolates was determined and the combined activity of the drugs on the isolates was also evaluated using Checkerboard-technique.

Results: MRSA isolates were found to be highly prevalent in the clinical samples. They also showed multidrug-resistant traits. The different combinations of antibiotics against different species of MRSA indicated synergistic, indifferent, additive, and antagonistic effects. The best combination that could treat muti-drug resistant MRSA was found to be gentamycin and clindamycin. MRSA has developed resistance to many drugs, making its infections difficult to treat. The combination of gentamicin and clindamycin is a promising strategy for treating MRSA in the region.

Keywords: MRSA; Gentamicin; Clindamycin; Combination; Synergy

* Corresponding author: Udochi Anayo Ugo

1. Introduction

Multi-resistant *Staphylococcus aureus* (MRSA), is a microbiome that causes skin and soft tissue infection, wound, pneumonia, osteomyelitis, endocarditis, septicemia and meningitis in humans [1,2]. It has been a problem in hospitals, in the community, and in livestock worldwide [2]. It is among the three most important millennium public health threats. Individuals who are healthy may carry it without indications for many years while individuals with low immunity may suffer from secondary infection with symptom.

The infections are mostly treated with cephalexin, trimethoprim-sulfamethoxazole, vancomycin, doxycycline, clindamycin, teicoplanin and gentamycin [3-5]. It could be eliminated with substances like hydrogen peroxide, tobramycin, chlorhexidine digluconate, honey and levofloxacin [6-8].

MRSA resistance to vancomycin, clindamycin, and other drugs [9-11] has been observed. The tendency that MRSA strains will continue to evolve new antibiotic resistances with a single target of action in future makes it an important subject of continuous studies. Since few antibiotics can treat infection by this organism, combination therapy is considered to revive the healing potentials of existing antibiotics, improve the spectrum of drug activity, and prevent the advent of resistance.

This study therefore aims at evaluating the efficacy of the combinations of gentamicin and clindamycin, ceftriaxone and clindamycin, ceftriaxone, and vancomycin against these isolates and to identify the best combinations that can be useful in the management of (MDR) MRSA in the southeast of Nigeria. Currently, there is no investigation on combinations of these drugs against MRSA in Enugu.

2. Materials and Methods

2.1. Materials

A hundred isolates of MRSA obtained from clinical samples at the Safety Molecular Pathology Laboratory Services Ltd, University of Nigeria Teaching Hospital (UNTH), and Annunciation Specialist Hospital, in Enugu, eastern Nigeria were used in this research. The samples included skin scrapings, urine, semen, sputum, catheter tips, and swab from urethra, vagina, endocervix, throat, ears, eyes, and wounds.

The agar media used were Mannitol salt agar (MSA), Mueller Hinton Agar (MHA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) Agar Base (Acumedia, Michigan, USA), while Brain Heart Infusion (BHI), Sucrose broth, Lactose broth and Glucose broth were the broth media used. These media were prepared in accordance with the manufacturer's instructions. Vancomycin (Hospira, Inc. Lake Forest, USA), gentamycin (Greenfield Pharm (Jiang Su) Co. Ltd China), ceftriaxone (Nemel Pharm. Ind. Ltd, Nigeria), cloxacillin sodium and clindamycin (Nichben Pharm. Co Nig Ltd) were the pure drugs used.

2.2. Methods

Isolates were inoculated onto MRSA agar plates that contained cloxacillin sodium 600 µg/ml in 1 litre agar and incubated at 37 ^{o}C for 24 h. The colonies were subjected to Gram staining, coagulase, catalase, lactose, sucrose, glucose, and mannitol fermentation tests for identification up to species level [13]. Each colonial growth was stored in glycerol stock (15 % of distilled water and 85 % of glycerol) at 4 ^{o}C. Before use, an aliquot of the colonies was cultured onto MRSA agar containing 600 µg/ml of cloxacillin sodium in 1 litre agar and incubated at 37 ^{o}C for 24 h to reactivate them. The cultures were standardized by aerobically growing them in a shaker water bath at 37 ^{o}C for 16 ±2 h to a cell density of 2.0 x 10^{8}cfu/ml [14].

Minimum inhibitory concentration (MIC) of vancomycin, gentamycin, clindamycin, and ceftriaxone was evaluated using macro-broth dilution method. The standardized drugs in BHI were prepared in two-fold serial dilutions and mixed with 0.1 ml standardized MRSA cultures in tubes. After 20 mins, the tubes were incubated at 37^{o}C for 24 h. The microbial growths were examined visually and the lowest concentration of the drugs that inhibited the growth of MRSA after 24 h incubation period was recorded as MIC [15]. These were repeated two times, and the mean was taken.

Different solutions of vancomycin, gentamicin, clindamycin, and ceftriaxone containing twice the MIC of each drug were prepared with injection water. These drugs solutions were combined in ratios in a checkerboard model [16]. Each combination was diluted (in triplicates) with normal saline in two-fold serial dilution up to 7 dilutions in sterile test tubes. Then, 0.1 ml of 0.5 McFarland standard MRSA cultures were added into each tube and incubated at 37^{o}C for 24 h.

The Fractional Inhibitory Concentration (FIC) of all the combination ratios of the drugs was determined while the FIC index for each drug was computed using the formula [16]:

$$FIC_A = \frac{MIC\ of\ \ drug\ A\ in\ combination\ with\ drug\ B}{MIC\ of\ \ drug\ A\ alone}$$

$$FIC_A = \frac{MIC\ of\ \ drug\ \mathrm{B}\ in\ combination\ with\ drug\ \mathrm{A}}{MIC\ of\ \ drug\ \mathrm{B}\ alone}$$

$$FIC\ Index\ = FIC_A + \ FIC_B$$

The FIC index is interpreted as: "FIC index < 1.0 means Synergism, 1 means additivity, > 1, but less than 2 means indifference while ≥ 2 means antagonism" [16].

3. Results

The result of MRSA isolated from the clinical specimens indicates that it is one of the most prevalent pathogens found in these clinical samples. The Gram-stained result showed that they were Gram-positive cocci while the biochemical result indicated that they were catalase and coagulase positive and fermented glucose, lactose, sucrose, and mannitol. The MIC result when compared with the MIC values of Clinical Laboratory Standard Institute (CLSI) breakpoints for *S. aureus* [17] revealed that MRSA isolates were resistant to the test drugs except ceftriaxone (Table 1). Our result showed high resistance of the isolates to the drugs.

Table 1 Minimum Inhibitory Concentration of antibiotics against isolates of MRSA

Isolates	MIC(µg/ml)			
	Va	Cl	Ge	Ce
1	250.0	125.0	42.1	2.0
2	125.0	62.5	42.1	4.0
3	125.0	125.0	42.1	2.0
4	250.0	31.3	42.1	0.5
5	125.0	62.5	675.0	2.0
6	250.0	31.3	675.0	2.0
7	125.0	31.3	42.1	0.5
8	250.0	187.5	13330.0	4.0
9	125.0	375.0	675.0	2.0
10	125.0	250.0	42.1	0.5
11	62.5	23.4	42.1	0.5
12	250.0	125.0	675.0	2.0
13	62.5	250.0	42.1	4.0
14	125.0	62.5	42.1	4.0
15	125.0	62.5	42.1	2.0
16	125	31.25	675	2.0
17	62.5	62.5	42.12	2.0
18	125	31.25	42.12	0.5

19	62.5	125	42.12	4
20	62.5	62.5	42.12	2

Key: Va = vancomycin, Cl = clindamycin, Ge = gentamicin, Ce = ceftriaxone

The results of clindamycin and gentamycin combination on some MRSA isolates are shown in Fig. 1. It follows from this figure that 24 % are indifferent, 42 % exhibited synergistic effect, and 18 % was additive (10:0/0:10) while 16 % was antagonistic. The distinctive finding in this result is that each ratio showed some levels of synergism on at least one of the isolates irrespective of the controls.

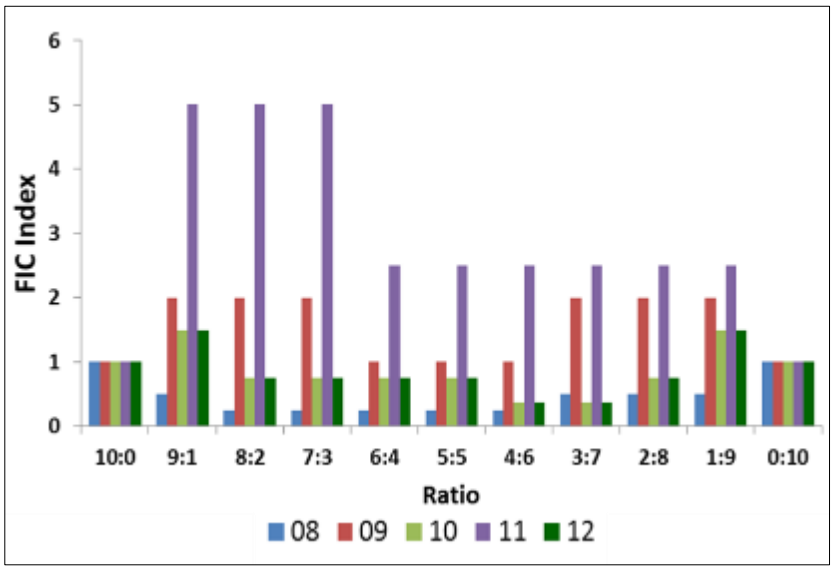

Figure 1 Clindamycin and gentamicin combination on some MRSA isolates

Combinations of clindamycin and ceftriaxone on some species of MRSA exhibited antagonistic and indifferent effects. Only 7 % of the ratios are synergistic and should be taken as insignificant (Figure 2).

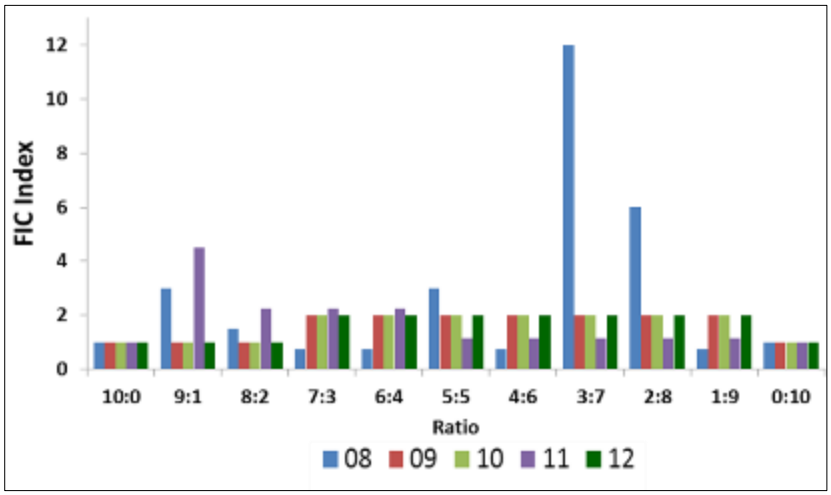

Figure 2 Clindamycin and ceftriaxone combination on some MRSA isolates

Combinations of vancomycin and ceftriaxone result presented in Fig. 3 indicated that 64% of the ratios were indifferent, 18 % are additive, 3 % was antagonistic, and 15% exhibited synergistic effect on the isolates. Synergism did not occur in most of the isolates tested. Therefore, clindamycin and gentamycin remain the best combination.

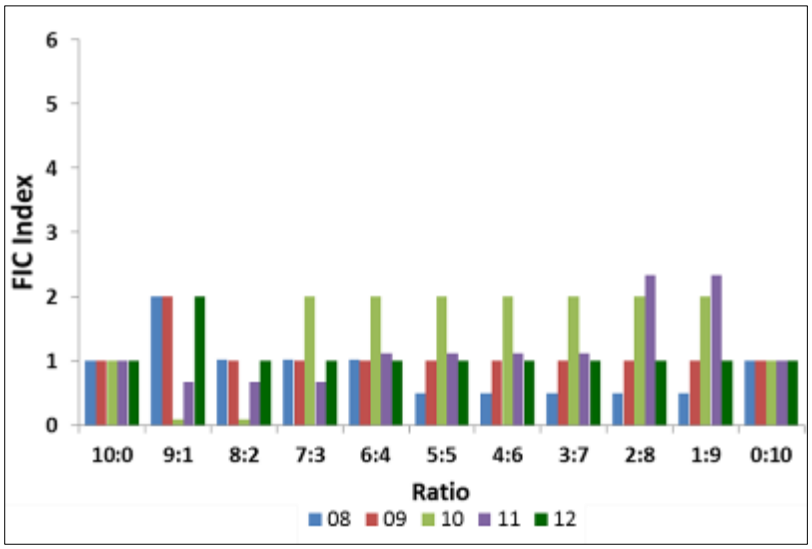

Figure 3 Ceftriaxone and vancomycin combination on some MRSA isolates

4. Discussion

Methicillin-resistant *Staphylococcus aureus* strains represent a worldwide threat because they are widely distributed and highly virulent [18,19]. This is buttressed by this study. The biochemical tests result confirmed the isolates to be *S. aureus.*[20]. In order not to compound the problem of antibiotic resistance through wrong reporting of other species as *S. aureus* especially, in poor and developing country, it is necessary to categorize the organisms epidemiologically and clinically to the specie level. The MIC results were in concordance with the report of some researchers which stated that the MRSA strains are often resistant to β-lactam agents, vancomycin, fluoroquinolones, chloramphenicol, clindamycin, tetracyclines, gentamycin and aminoglycosides [10,21,22]. However, they disagree with earlier works [23], which reported that vancomycin, clindamycin, and gentamycin are used to treat MRSA infections. The reason for the observed multi- drug resistance could be mutation of the MRSA strains over the years. This makes their infections difficult to treat.

Combining antibiotics could be the best approach to handle infections caused by muti-drug resistant MRSA. Another advantage offered by combination of antibiotics is that it is used to boost the effect of single antimicrobials by means of synergistic interactions; reduce the dosage of individual drugs to minimize chances of occurrence and adversative effects; overcome problems that are harmful and prevent treatment failure [24-27]. Combinations of gentamycin can inflate the scope of treatment, quicken the clearance of bacteria and reduce the resistance to antibiotics [28]. It is inferred therefore, that gentamicin and clindamycin combinations improved on the efficacy of the respective drugs and will be effective in the treatment of infection involving multidrug-resistant MRSA. According to some findings, clindamycin and gentamycin combination is effective for all the surgical wounds contaminants, such as *Pseudomonas aeruginosa*, *S. aureus*, and anaerobic organisms. Others indicated that they are used in the treatment of pelvic inflammatory disease (PID) and post cesarean endometritis [29,30]. Because they have different mechanisms of neuromuscular blockades, they can interact synergistically to reduce the concentrations of each drug [31]. During this research, we discovered that the combination can effectively be used to treat multidrug-resistant MRSA as well.

5. Conclusion

MRSA is one of the most isolated pathogens in Nigeria and has developed high resistance to many drugs, making their infections difficult to treat. This poses threat to health within the region and the world at large. Nevertheless, a drug combination such as clindamycin and gentamycin are an encouraging strategy that may be adopted in treating MRSA.

Our research *in vitro* study provides preliminary information on the combination of gentamycin and clindamycin but did not state the side effects of the combination.

Compliance with ethical standards

Acknowledgments

We are grateful to the staff of the department in all the hospitals and the laboratory where the research was conducted.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Authors Contributions

MGUN, AAA and VCO conceptualized and designed the study; Data collection was done by MGUN, Data Analysis was done by UAU, LAN and CME while writing, editing, and proofreading was done by MGUN, UAU, INE and CHN. All the authors read and approved the manuscript.

References

[1] Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018 Sep; 31(4): e00020-18.

[2] Baddour LM, Wilson WR, Bayer AS, Fowler VG, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA., American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Stroke Council. Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications: A Scientific Statement for Healthcare Professionals from the American Heart Association. Circulation. 2015 Sep; 13;132(15):1435-86.

[3] Holmes NE, Tong SY, Davis JS, and van Hal SJ. Treatment of methicillin-resistant *Staphylococcus aureus*: vancomycin and beyond. Semin. Respir. Crit. Care Med. 2015 Feb; 36(1), 17–30.

[4] Huang SS, Singh R, McKinnell et al. Decolonization to reduce post discharge infection risk among MRSA carriers. N. Engl. J. Med. 2019 Feb; 380(7):638–650.

[5] Kevin MK, Alisa WS, Timothy RK, and Lynn EC. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016 Jun; 6(6): a027029.

[6] Echague CG, Hair PS, and Cunnion KM. A comparison of antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* and gram-negative organisms for antimicrobial compounds in a unique composite wound dressing. Wound Care 2010 Sep; 23 (9):406–413.

[7] Nolan VC, Harrison J, Wright JEE, and Cox JAG Clinical Significance of Manuka and Medical-Grade Honey for Antibiotic-Resistant Infections: A Systematic Review Antibiotics. 2020 Oct; 9(11), 766.

[8] Kwakman PH, Velde AA, de Boer L, Speijer D, Vandenbroucke-Grauls CM, Zaat SA. How honey kills bacteria. FASEB Journal 2010 Mar; 24 (7):2576–2582.

[9] Yunlei G, Guanghui S, Meiling S, Juan W, and Yi W. Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. Front Cell. Infect. Microbiol. 2020 Mar; 10:107.

[10] Abdullahi N, and Iregbu KC. Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. Ann. Trop. Pathol. 2018 Jun; 9:6-10.

[11] Cennet R, Mehmet P, Yasemin B, Huseyin G, and Nesrin C. Evaluation of Antimicrobial Resistance in *Staphylococcus aureus* Isolates by Years Interdisciplinary Perspectives on Infectious Diseases. 2016 May.

[12] Nitzan O, Supornitzky U, Kennes Y, Chazan B, Raz R, and Colodner R. Is chloramphenicol making a comeback? Israel Medical Association Journal 2010 Jun; 12(6):371–374.

[13] Haroon M, Mark C, and Mohamed NS. Antibacterial Evaluation of Synthetic Thiazole Compounds In-vitro and In-vivo In a Methicillin-Resistant *Staphylococcus aureus* (MRSA) skin infection Mouse Model. Plos one. 2015 Nov.

[14] David N, Clement OT, Edward KE, Justice DA, Felix CMR, Daniel S, Adjoam AB, and Daniel AA. Evaluation of the Modulatory Effect of Annonamuricata Extracts on the Activity of Some Selected Antibiotics against Biofilm-Forming MRSA. Evidence-Based Complementary and Alternative Medicine. 2021Dec: 9342110

[15] Dae-Young, L, Yeon BK, Sang-Won L, Seon-Woo C, Hong-Woo P, Geum-Soog K, Dong-Yeul K, Min-Ho L, and Sin-Hee H. The Mechanism underlying the Antibacterial Activity of Shikonin against Methicillin Resistant *Staphylococcus aureus*. Young-Seob, Evidence-Based Complementary and Alternative Medicine 2015.

[16] Pratiwi W, and Elin YS. Evaluation of the Combined Activity of Kaempferia Pandurata rhizome and Sennaalata leaves against Methicillin-Resistant *Staphylococcus aureus*. Asian Journal of Pharmaceutical and Clinical Research. 2016 Jan; 9(3).

[17] Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial Susceptibility Testing; 21st informational supplement. CLSI document M100-S21 Vol 31 No.1. Clinical and Laboratory Standards Institute, Wayne, Pa, USA 2011.

[18] Ali H, Peter KL, and Bruce F. Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment Critical Care. 2017 Aug; 21: 211(2017).

[19] Grema HA, Geidam YA, Gadzama GB, Ameh JA, and Suleiman A. Methicillin resistant *Staphylococcus aureus* (MRSA): a review. Advances in Animal and Veterinary Sciences. 2015 Jan; 3(2):79–98.

[20] Rasigade JP, Vandenesch F. *Staphylococcus aureus*: a pathogen with still unresolved issues. Infect Genet Evol 2014 Jan; 21:510-514.

[21] Patric EA, Rashida R, and Stefan M. Molecular characterization of antimicrobial resistance genes against *Staphylococcus aureus* isolates from Trinidad and Tobago. Journal of Infection and Public Health. 2017 May-Jun; 10(3):316-323.

[22] Anejionu MGU, Oli AN, Ezeudu CE, Ezejiofor OI, Ezeogu J, Attama AA, and Okore VC. Methicillin-Resistant *Staphylococcus aureus* May Also Be Resistant to Clindamycin and Vancomycin. Journal of Biosciences and Medicines. 2022 Aug; 10:1-3.

[23] Mohammedaman M, Addis A, Kassahun M, Molla T, and Eyerusalem A. Methicillin- and Inducible Clindamycin-Resistant *Staphylococcus aureus* among Patients with Wound Infection Attending Arba Minch Hospital, South Ethiopia. 2019 Apr.

[24] Dejing S, Yue L, Fengquan J, Fangyu J, He W, and Xue H. Synergistic Antibacterial Activity of Designed Trp-Containing Antibacterial Peptides in Combination with Antibiotics Against Multidrug-Resistant Staphylococcus epidermidis Front. Microbiol., 2019 Nov; 10:2719

[25] Nuoyan W, Jing L, Fei D, Yasi H and Hong Z. Antibiotic CombinationTherapy: A Strategy to Overcome Bacterial Resistance to AminoglycosideAntibiotics. Front. Pharmacol. 2022 Feb.

[26] Soren, O, Brinch, S, Patel, D, Liu, Y, Liu, A, Coates, A, et al. Antimicrobial peptide novicidin synergizes with rifampin, ceftriaxone, and ceftazidime against antibiotic resistant Enterobacteriaceae invitro. Antimicrob. Agents Chemother. 2015 Oct; 59(10): 6233–6240.

[27] Ankomah P, Johnson PJT, and Levin BR. The pharmaco –, population and evolutionary dynamics of multi-drug therapy: experiments with *S. aureus* and E. coli and computer simulations. PLOS Pathogens 2013 Apr; 9: e1003300.

[28] Rhodes A, Evans L E, Alhazzani W, Levy M M, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock. Intensive Care Med. 2017 Jan; 43 (3): 304–377.

[29] Li-Yeh C, Tomor H, Yu-Hsun C, Hsuan C, and Dah-Ching D. The Combination of Clindamycin and Gentamicin Is Adequate for Pelvic Inflammatory Disease: A Retrospective Cohort Study. J Clin Med. 2021 Sep; 10(18): 4145.

[30] Zambrano D. Clindamycin in the treatment of obstetric and gynecologic infections: a review. Clin Ther. 1991Jan-Feb; 13(1):58–80.

[31] Ji Hyeon L, Soo-Il L, Chan Jong C, Jong Hwan L, Seung Cheol L, So Ron C, Ji Na O, and Jae Young B. The synergistic effect of gentamicin and clindamycin on rocuronium-induced neuromuscular blockade. Korean J Anesthesiol. 2013 Feb; 64(2): 143-151.