

# Evaluate Impact of Dual Antibiotics on Growth of *Staphylococcus Aureus* and *Streptococcus Pyogenes*

Vishal Kumar Deshwal<sup>1</sup>, Harpreet Singh<sup>2</sup>, Pooja Bisht<sup>2</sup>

Head, Department of Microbiology, BFIT Group of Institutions, Dehradun, India<sup>1</sup>

PG student, Department of Microbiology, BFIT Group of Institutions, Dehradun, India<sup>2</sup>

**ABSTRACT:** Both *Staphylococcus aureus* and *Streptococcus pyogenes* are pathogenic gram-positive bacteria. Multi drug resistant strains are difficult to treat by mono antibiotic drug so aim of present study is to evaluate the impact of different combination of ceftriaxone and gentamicin against *Staphylococcus aureus* and *Streptococcus pyogenes*. Different treatments of ceftriaxone and gentamicin were prepared and disc diffusion method is used for analysis of present study. The highest inhibition zone was observed against *Staphylococcus aureus* SA-3 in treatment of ceftriaxone (75%) + gentamicin (25%) which is 116 and 100% more as compared to individual ceftriaxone (100%) and gentamicin (100%) respectively. Treatment ceftriaxone (75%) + gentamicin (25%) showed 58.82 % more inhibition zone as compared to gentamicin (100%) but did not show more inhibition zone as compared to ceftriaxone (100%).

**KEYWORDS:** *Staphylococcus aureus*, *Streptococcus pyogenes*, ceftriaxone, gentamicin, Dual antibiotics

## I. INTRODUCTION

*Staphylococcus aureus* and *Streptococcus pyogenes* are gram-positive bacteria. *Staphylococcus* is Gram-positive cocci bacteria and due to divide in more than one plane it forms grape-like clusters [1]. *S. aureus* shows the golden colour of the colony on nutrient agar medium and such pigmentation of *S. aureus* colonies is caused by the presence of carotenoids [2]. Staphylococci are facultative anaerobes capable of generating energy by aerobic respiration, and by fermentation which yields mainly lactic acid. *Staphylococcus* sp. is catalase-positive, a feature differentiating them from *Streptococcus* sp. [3].

Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections [4]. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are especially easily spread throughout a hospital and without implementation of a special surveillance program with control procedures, a risk of an epidemic in such a hospital is high [5]. So *Staphylococcus aureus* is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [6].

*S. pyogenes* is Group A- beta-hemolytic *Streptococcus*. It is a facultative anaerobe, Gram positive extracellular bacterium [7]. It is nonmotile, non-sporing, spherical or ovoid cocci that are less than 2 µm in length and that form chains and large colonies greater than 0.5 mm in size [8]. The life-threatening infections caused by *Streptococcus pyogenes* (group A streptococcus) include scarlet fever, bacteremia, pneumonia, necrotizing fasciitis, myonecrosis, and streptococcal toxic shock syndrome (StrepTSS) [9], *S. pyogenes* causes disease such as pharyngitis [8], impetigo [10], bacteremia, cellulitis and rarely pharyngitis [11], erysipelas [12], otitis media, mastitis, septic arthritis, meningitis [13]. Multiple antibiotic resistant *Staphylococcus aureus* is more the concern these days as they are one of the common causes of severe nosocomial infections. *Staphylococcus aureus* showed percentages of resistance against ampicillin (68.4%), doxycycline (60.5%), cefoxitin (34.2%), vancomycin (36.8%), erythromycin (34.2%), and gentamicin (5.3%) [14]. Group A *Streptococcus pyogenes* showed resistance against penicillin (8%), cefotaxime (4.2%), erythromycin (83%), tetracycline (51%), levofloxacin (8.9%), clindamycin (40%) and ceftriaxone (5.3%) [15-16]. So the aim of the present study is to evaluate impact of dual antibiotics (ceftriaxone + gentamicin) against growth of *Staphylococcus aureus* and *Streptococcus pyogenes*.



## II. MATERIALS AND METHODS

(i) **Pathogens:** Three characterized *Staphylococcus aureus* strains SA-1, SA-2, SA-3 and three *Streptococcus pyogenes* SP-1, SP-2, SP-3 were elected for present study. These characterized strains were collected from Department of Microbiology, BFIT Dehradun.

(ii) **Antibiotics sensitivity test:** Bacterial culture was activated by inoculating a loopful of the strain in Muller Hinton broth (30 ml) and incubated at 37°C for 24 h at 120 rpm until it achieves or exceeds the turbidity of the 0.5 McFarland standard. The turbidity of the actively growing broth culture is adjusted with sterile Muller Hinton broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. This standard is equal to 1 to 2 x 10<sup>8</sup> CFU/ml. Add 0.2 ml inoculum into molten Muller Hinton agar media. Mixed well and it was poured into sterilized Petri plates. Placed prepared antibiotic disc on inoculated plates and were incubated at 37°C for 24 h. The inhibition zone was determined by measuring the diameter of the zone of inhibition around the antibiotics disc.

(iii) **Treatment:** Two antibiotic drug ceftriaxone and gentamicin antibiotics were selected for present study. Treatment I- ceftriaxone (0%) + gentamicin (100%), Treatment II- ceftriaxone (25%) + gentamicin (75%), Treatment III- ceftriaxone (50%) + gentamicin (50%), Treatment IV- ceftriaxone (75%) + gentamicin (25%), Treatment V- ceftriaxone (100%) + gentamicin (0%) were prepared. The stock concentration of ceftriaxone and gentamicin was 100, 40mg/ml respectively.

## III. RESULTS AND DISCUSSION

All *Staphylococcus aureus* strains SA-1, SA-2, and SA-3 showed maximum inhibition zone in treatment ceftriaxone (75%) + gentamicin (25%). The highest inhibition zone was observed against *Staphylococcus aureus* SA-3 in treatment of ceftriaxone (75%) + gentamicin (25%) which is 116 and 100% more as compared to individual ceftriaxone (100%) and gentamicin (100%) respectively. Second highest inhibition zone was observed in ceftriaxone (50%) + gentamicin (50%) (Table 1). All *Streptococcus pyogenes* strains SP-1, SP-2 and SP-3 showed maximum inhibition zone in treatment gentamicin (100%). The highest inhibition zone was observed against *Streptococcus pyogenes* SP-2 in treatment of ceftriaxone (100%) + gentamicin (0%). Treatment ceftriaxone (75%) + gentamicin (25%) showed 58.82 % more inhibition zone as compared to gentamicin (100%) but did not show more inhibition zone as compared to ceftriaxone (100%) (Table 2). ceftriaxone is a one of the effective broad spectrum third generation cephalo-sporin and effective against certain gram negative and positive bacteria [17]. Similarly, previous study suggested that combination therapy with a beta-lactam plus a macrolide or doxycycline or monotherapy with a “respiratory quinolone” (i.e., levofloxacin, gatifloxacin, moxifloxacin, or gemifloxacin) are optimal first-line therapy for patients hospitalized with community-acquired pneumonia [18]. Another report suggested that combination antibiotic therapy for invasive infections with Gram-negative bacteria is employed in many health care facilities, especially for certain subgroups of patients, including those with neutropenia, those with infections caused by *Pseudomonas aeruginosa*, those with ventilator-associated pneumonia, and the severely ill [19]. All result suggested that dual antibiotic may be better for hospitalized patients.

Table 1: Impact of ceftriaxone + gentamicin antibiotics against *Staphylococcus aureus* by disc diffusion method.

S. No.	Antibiotic Combination	Inhibition zone (mm)		
		SA1	SA2	SA3
1	ceftriaxone (0%) + gentamicin (100%)	12	13	13
2	ceftriaxone (25%) + gentamicin (75%)	17	16	18
3	ceftriaxone (50%) + gentamicin (50%)	21	20	21
4	ceftriaxone (75%) + gentamicin (25%)	25	25	26
5	ceftriaxone (100%) + gentamicin (0%)	13	12	13

(ceftriaxone = 100mg/ml; gentamicin= 40mg/ml).



Table 2: Impact of ceftriaxone + gentamicin antibiotics against *Streptococcus pyogenes* by disc diffusion method.

S. No	Antibiotic Combination	Inhibition zone (mm)		
		SP1	SP2	SP3
1	ceftriaxone (0%) + gentamicin (100%)	17	17	16
2	ceftriaxone (25%) + gentamicin (75%)	18	19	20
3	ceftriaxone (50%) + gentamicin (50%)	21	19	22
4	ceftriaxone (75%) + gentamicin (25%)	27	26	26
5	ceftriaxone (100%) + gentamicin (0%)	28	30	29

(ceftriaxone = 100mg/ml; Gentamicin= 40mg/ml)

#### IV. CONCLUSION

Two antibiotics ceftriaxone and gentamicin were selected for analysis of the impact of dual antibiotic against *Staphylococcus aureus* and *Streptococcus pyogenes*. We observed that one treatment ceftriaxone (75%) + gentamicin (25%) is effective against *Staphylococcus aureus* as compared to ceftriaxone (100%) and gentamicin (100%) respectively. But this combination showed second highest against *Streptococcus pyogenes*. The author suggested making other combinations of the ceftriaxone and gentamicin for proper analysis of dual antibiotics in the future.

#### REFERENCES

- [1] Z. Ahmad, S. Agarwal, A.S. Narwal and V.K. Deshwal, “Comparative study of antibiotics resistance of Coagulase Negative Staphylococci”, *Dev. Micro. Mol. Biol.*, 4(1): 29-33, 2013.
- [2] G.Y. Liu, A. Essex, J.T. Buchanan, V. Datta, H.M. Hoffman, J.F. Bastian, J. Fierer and V. Nizet, “*Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity”, *J. Exp. Med.*, 202: 209-215, 2005.
- [3] K. Plata, A.E. Rosato and G. Wegrzyn, “*Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity”, *Acta Biochim. Pol.*, 56: 597-612, 2009.
- [4] J.A. Lindsay and M.T. Holden, *Staphylococcus aureus: superbug, super genome?*, *Trends Microbiol.*, 12: 378-385, 2004.
- [5] J. Kurlenda, M. Grinholc, K. Jasek and G. Wegrzyn, “RAPD typing of methicillin-resistant *Staphylococcus aureus*: a 7-year experience in a Polish hospital”, *Med. Sci. Monit.*, 13: MT13-MT18, 2007.
- [6] S.Y. Tong, J.S. Davis, E. Eichenberger, T.L. Holland and V.G. Fowler Jr, “*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management”, *Clin. Microbiol. Rev.*, 28(3): 603-661, 2015.
- [7] M.W. Cunningham, “Pathogenesis of group A streptococcal infections and their sequelae”, *Adv. Exper. Med. Biol.*, 609:29-42, 2008.
- [8] J.R. Kenneth and W.D. Lawrence, “The streptococci and Enterococci”, In: Sherris Medical Microbiology, (Eds.), McGraw-Hill, New York, USA., pp: 443-469, 2010.
- [9] A.L. Bisno, M.O. Brito and C.M. Collons, “Molecular basis of group a streptococcal virulence”, *Lancet infect. Dis.*, 3: 191-200, 2003.
- [10] A.L. Bisno and D.L. Stevens, “*Streptococcus pyogenes*”, In Mandel GL, Bennett JE, Dolan R (ed), Mandell, Douglas, and Bennett’s principles and practice of infectious diseases, 7th ed. Livingstone Elsevier, Philadelphia, PA., pp 2593-2610, 2010.
- [11] F. Brooks, J.S. Butel and S.A. Morse, “The streptococci. In: Jawetz, Melnick and Adelberg’s Medical Microbiology”, Lange Medical Brooks/ McGraw-Hill, New York, pp, 25: 195-209, 2004.
- [12] R. Celestin, J. Brown, G. Kihiczak and R.A. Schwartz, “Erysipelas: A common potentially dangerous infection”, *Acta Dermatol. Alpina Pann. Adriat.*, 16: 123-127, 2007.
- [13] R.K. Aziz, R. Kansal, B.J. Aronow, W.L. Taylor, S.L. Rowe, M. Kubal, G.S. Chhatwal, M.J. Walker and M. Kotb, “Micro evolution of Group A streptococci in vivo: Capturing regulatory networks engaged in sociomicrobiology, niche adaptation and hypervirulence”, *PloS. One*, 5: 9798-9800, 2010.
- [14] A. Onanuga and T.C. Temedie, “Multidrug-resistant Intestinal *Staphylococcus aureus* among Self-medicated Healthy Adults in Amassoma, South-South, Nigeria”, *J. Health Popul. Nutr.*, 29(5): 446-453, 2011.



- [15] V. Ayer, W. Tewodros, A. Manoharan, S. Skariah, F. Luo and D.E. Bessen, "Tetracycline Resistance in Group A Streptococci: Emergence on a Global Scale and Influence on Multiple-Drug Resistance", *Antimicrob. Agents Chemother.*, 51(5): 1865-1868, 2007.
- [16] A. Berwal, K. Chawla, S. Shetty and A. Gupta, "Trend of antibiotic susceptibility of *Streptococcus pyogenes* isolated from respiratory tract infections in tertiary care hospital in south Karnataka", *Iran. J. Microbiol.*, 11(1): 13-18, 2019.
- [17] S.K. Nath, G.A. Foster, L.A. Mandell and C. Rotstein, "Antimicrobial activity of ceftriaxone versus cefotaxime: negative effect of serum albumin binding of ceftriaxone", *J. Antimicrob. Chemother.*, 33(6): 1239-1243, 1994.
- [18] F.J. Martinez, "Monotherapy versus Dual Therapy for Community-Acquired Pneumonia in Hospitalized Patients", *Clin. Infect. Dis.*, 38 Suppl 4: 328- 340, 2004.
- [19] P.D. Tamma, S.E. Cosgrove and L.L. Maragakis, "Combination Therapy for Treatment of Infections with Gram-Negative Bacteria", *Clin. Microbiol. Rev.*, 25(3): 450-470, 2012.