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Impact Combination of Amoxicillin and Gentamicin on Growth of *Staphylococcus Aureus* and *Streptococcus Pyogenes*

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ABSTRACT

Aim of the present study is to evaluate the impact of different combinations of Amoxicillin and Gentamicin against *Staphylococcus aureus* and *Streptococcus pyogenes*. These two strains are responsible for nosocomial infection. Different treatments of Amoxicillin and Gentamicin were prepared and the disc diffusion method is used for the analysis of present study. All *Staphylococcus aureus* strains namely SA-1, SA-2, and SA-3 showed maximum inhibition zone in treatment Amoxicillin (50%) + Gentamicin (50%) which is 6.66% more as compared to individual Amoxicillin (100%) and Gentamicin (100%) respectively. All *Streptococcus pyogenes* strains SP-1, SP-2, and SP-3 showed maximum inhibition zone in treatment Amoxicillin (75%) + Gentamicin (25%) which was slightly higher than Gentamicin (100%). All results suggested that various combinations of drugs can be effective to control MDR pathogens.

Key-words: *Staphylococcus aureus*, *Streptococcus pyogenes*, Amoxicillin, Gentamicin, Dual antibiotics

INTRODUCTION

Staphylococcus aureus and *Streptococcus pyogenes* are known as pathogenic gram-positive cocci and responsible for nosocomial infection. Nosocomial infections can be defined as those occurring within 48 hours of hospital admission, 3 days of discharge, or 30 days of operation. They affect 1 in 10 patients admitted to the hospital.[1] Hospital-acquired infections (HAIs) are a major safety concern for both health care providers and patients. Considering morbidity, mortality increased length of stay and the cost, efforts should be made to make the hospitals as safe as possible by preventing such infections.[2]

The *Staphylococcus aureus* is divide in more than one plane it forms grape-like clusters.[3] The name of *S. aureus* strains shows the golden colour of the colony on nutrient agar medium.[4] These Methicillin-resistant *Staphylococcus aureus* (MRSA) is spread throughout a hospital.[5] *Staphylococcus aureus* is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections.[6]

Streptococcus pyogenes is one of the members of Group A- beta-hemolytic Streptococci. *S. pyogenes* is a facultative anaerobe.[7] *Streptococcus pyogenes* is responsible for scarlet fever, bacteremia, pneumonia, necrotizing fasciitis, myonecrosis, and streptococcal toxic shock syndrome (StrepTSS).[8] S. *pyogenes* causes disease such as pharyngitis[9], impetigo[10], bacteremia, cellulitis and rarely pharyngitis[11], erysipelas[12], 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.[14-16] So the aim of the present study is to evaluate impact of dual antibiotics (Amoxicillin + Gentamicin) against growth of *Staphylococcus aureus* and *Streptococcus pyogenes.*

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 Group of Insitutions, Dehradun (India).

(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 Huller Hinton broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. This standard is equal to 1 to 2 x 10^8 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 37ºC for 24 h. The inhibition zone was determined by measuring the diameter of the zone of inhibition around the antibiotics disc.

(iii) *Treament:* Two antibiotic drug Amoxicillin and Gentamicin antibiotics were slected for present stydy. Treament I- Amoxicillin (0%) + Gentamicin (100%), Treament II- Amoxicillin (25%) + Gentamicin (75%), Treament III- Amoxicillin (50%) + Gentamicin (50%), Treament IV- Amoxicillin (75%) + Gentamicin (25%), Treament V- Amoxicillin (100%) + Gentamicin (0%) were prepared. The stock concentration of Amoxicillin and Gentamicin was 25, 8mg/ml respectively.

RESULTS AND DISCUSSION

All *Staphylococcus aureus* strains SA-1, SA-2, and SA-3 showed maximum inhibition zone in treatment Amoxicillin (50%) + Gentamicin (50%). The highest inhibition zone was observed against *Staphylococcus aureus* SA-1 and SA-2 in treatment of Amoxicillin (50%) + Gentamicin (50%) which is 6.66% more as compared to individual Amoxicillin (100%) and Gentamicin (100%) respectively (Table 1). All *Streptococcus pyogenes* strains SP-1, SP-2 and SP-3 showed maximum inhibition zone in treatment Amoxicillin (75%) + Gentamicin (25%) which was slightly higher than Gentamicin (100%). The lowest inhibition zone was observed against *Streptococcus pyogenes* SP-2 in treatment of Amoxicillin (100%) + Gentamicin (0%) (Table 2). All results suggested that combination of Amoxicillin (50%) + Gentamicin (50%) is effective against *Staphylococcus aureus* but combination of Amoxicillin (75%) + Gentamicin (25%) was effective against *Streptococcus pyogenes*. Similarly, Combinations of ceftizoxime or other third-generation cephalosporins with aminoclycosides are generally synergistic or indifferent against staphylococci, Gram-negative bacilli and certain β-lactam-β-lactam combinations exhibit striking antagonisms against Gram-negative bacilli, including *Citrohacler freundii, Enterobacter* spp., *Morganella morganii. Serratia marcescens*, and *Pseudomonas aeruginosa*.[17] Similar results has been 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] Further, *In vitro* combination effects of amoxicillin and cotrimoxazole on clinical isolates was investigated using the agar diffusion and macro broth dilution methods and reported that susceptibility of the isolates to the antibacterial combinations showed that they were susceptible in the following order: *Streptococcus pyogenes* (TD2) >*Streptococcus pyogenes* (TD10) >*Streptococcus pneumoniae* (TE10) >*Salmonella typhi* (TC6) >*Salmonella typhi* (TC2).[19] All results suggested that a combination of two antibiotics is a better option for treatment of pathogenic strains. But authors suggested that dual antibiotic maybe more harmful for human because the impact of two types of antibiotics is more negative on the health of the patient as compared to mono antibiotics so it suggests that it should be used only for critically ill patients.

CONCLUSION

Two antibiotics Amoxicillin and Gentamicin were selected for analysis of the impact of dual antibiotic against *Staphylococcus aureus* and *Streptococcus pyogenes*. Results suggested that a combination of Amoxicillin (50%) + Gentamicin (50%) is effective against *Staphylococcus aureus* but a combination of Amoxicillin (75%) + Gentamicin (25%) was effective against *Streptococcus pyogenes.* Both *Staphylococcus aureus* and *Streptococcus pyogesns* showed the different concentrations of Amoxicillin and Gentamicin.

Therefore, the authors suggested preparing different combinations of the above-said drug for proper analysis of dual antibiotics in the future.

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

S.No.	Antibiotic Combination	Inhibition zone (mm)		
		SA1	SA2	SA3
1	Amoxicillin (0%) + Gentamicin (100%)	15	15	14
2	Amoxicillin (25%) + Gentamicin (75%)	13	13	12
3	Amoxicillin (50%) + Gentamicin (50%)	16	16	15
4	Amoxicillin (75%) + Gentamicin (25%)	13	11	11
5	Amoxicillin (100%) + Gentamicin (0%)	15	13	12

(Amoxicillin = 25mg/ml; Gentamicin= 8mg/ml)

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

S. No.	Antibiotic Combination	Inhibition zone (mm)		
		SP1	SP2	SP3
1	Amoxicillin (0%) + Gentamicin (100%)	18	18	16
2	Amoxicillin (25%) + Gentamicin (75%)	15	16	15
3	Amoxicillin (50%) + Gentamicin (50%)	16	17	16
4	Amoxicillin (75%) + Gentamicin (25%)	18	18	18
5	Amoxicillin (100%) + Gentamicin (0%)	15	13	16

(Amoxicillin = 25mg/ml; Gentamicin= 8mg/ml)

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