A comparative study of minimum inhibitory concentrations (MICs) of antibacterial agents - Cephalosporin, Cloxacillin and Sulbactum on gram-ve and gram+ve bacterial organisms *Salmonella typhi* and *Staphylococci*

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Abstract

Antibiotics act on several and disrupt their cell wall and their components. They also inhibit their protein synthesis and finally kill the bacteria. Most of the β lactums are the antibacterial agents. A minimum inhibitory concentration is the method which is used to estimate the drug sensitivity against the bacteria or microorganisms. The lowest concentrations of the antibiotics that will inhibit the visible growth of the microorganism are the MIC value of that substance. In the present investigation pharmaceutical products like the cephalosporin, cloxillin, sulbactum, and amoxicillin are tested for their antibacterial activity against the bacteria like gram negative bacteria *Salmonella typhi* and gram positive bacteria *Staphylococci* species. In these present investigations orderly cloxicillin showed highest inhibitory zone on *Salmonella typhi* and *Staphylococci* than the cephalosporin followed by sulbactum.

Keywords: Antibiotics, MICs (Minimum inhibitory concentrations), Cephalosporin, Cloxacillin, Sulbactum.

Introduction

Antibiotics are the types of medications that destroy or slow down the growth of bacteria are also known as antibacterial drugs. Antibiotic can be used to treat a wide range of infections to prevent infection. Penicillin is the first antibiotic has the broad spectrum activity on several bacteria like *Salmonella typhi* and *Staphylococci.*[1] Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing against which other methods, such as disk dilution, are calibrated. MIC methods are widely used in the comparative testing of new agents. In clinical laboratories they are used to establish the susceptibility of organisms that give equivocal results in disk tests, for tests on organisms where disk tests may be unreliable, and when a more accurate result is required for clinical management. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in micro plate wells of broth (broth micro dilution) containing dilutions of the antimicrobial agent. The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism; known as the MIC. Although several

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susceptibility testing media are available, a clear choice for a reference medium remains to be determined. Mueller Hinton (MH) agar shows no performance advantages over some other media but is probably the most widely used medium internationally and there is a USA National Committee for Clinical Laboratory document which describes procedures for evaluating MH agar.[3, 4] MH agar which meets the requirements of the NCCLS standard is considered the reference medium.[6] Supplements should not be used unless necessary for growth of the organisms.

Material and Methods

Antibacterial Agents

Obtain antimicrobial powders directly from the manufacturer or from commercial sources. The agent must be supplied with a stated potency (mg or International Units per g powder, or as percentage potency), an expiry date and details of recommended storage conditions. Store powders in sealed containers in the dark at 4 °C with a desiccant unless otherwise recommended by the manufacturer. Ideally, hygroscopic agents should be dispensed into aliquots, and one aliquot used on each test occasion. Allow containers to warm to room temperature before opening them to avoid condensation of water on the powder.

Preparation of stock and working solutions

Use an analytical balance when weighing agents. Allowance for the potency of the powder can be made by use of the following formula:

$$\text{Weight of powder mg} = \frac{\text{Volume of solvent (mL) x Concentration (mg/L)}}{\text{Potency of powder (mg/g)}}$$

Alternatively, given a weighed amount of antimicrobial powder, the volume of diluents needed may be calculated from the formula:

$$\text{Weight of solvent ml} = \frac{\text{Weight of powder (mg) x Potency of powder (mg/g)}}{\text{Concentration (Mg/L)}}$$

Concentrations of stock solutions should be 1000 mg/L or greater, although the solubility of some agents will be limiting. The actual concentrations of stock solutions will depend on the method of preparing working solutions. Manufacturer's recommendations for solvents and diluents should be followed, but where possible agents should be dissolved and diluted in sterile distilled water.

Sterilization of solutions is not usually necessary. If required, sterilization should be by membrane filtration, and samples before and after sterilization must be compared by assay to ensure that adsorption to the membrane has not occurred. Unless otherwise instructed by the manufacturer, store stock solutions frozen in aliquots at 20° C or below. Most agents will keep at 60°C for at least 6 months. Stock solutions must be frozen as soon as possible after preparation, used promptly on defrosting and not refrozen. The range of concentrations tested will depend on the organ-isms and antimicrobial agent being tested, but a two-fold dilution series based on 1mg/L is conventionally used. Twenty milliliter volumes of agar are commonly used in 9 cm Petri dishes for agar dilution MICs. Both schemes involve adding 19-mL volumes of molten agar to 1mL volumes of antimicrobial solution.[6] The more conventional method is based on diluting a 10 240 mg/L stock solution. Always measuring the 1 ml volumes of antimicrobial solution. The other method is based on diluting a 10000 mg/L antimicrobial solution by the use of high-precision variable volume micropipettes, which are now widely available. An alternative to the method is to omit the distilled water added to make antimicrobial volumes up to 1mL and instead to add a variable volume of molten agar to make the total volume 20 mL.

Preparation of Inoculum plates

The nutrient agar medium plates were prepared by pouring 15ml of nutrient agar media into sterile Petri plates.[3] The plates were allowed to solidify for 5 minutes and dry the plates so that no drops of moisture remain on the surface of the agar. Do not over dry plates. Plates should not be stored unless the agents have been shown to be stable on storage. Clavulanic acid and carbapenems are particularly unstable.

In agar disc diffusion method by[7] in this paper disc method, discs with 2.5mm diameter were prepared using No1 what man filter paper and sterilized by autoclaving. Then, the discs had been impregnated with various concentrations of the plant extract and introduced onto

upper layer of the seeded agar plates. The plates were then incubated at 37°C for 24 hours. Triplicates were maintained and the averages of the zones of inhibition were calculated. Use the apparatus to transfer the inocula to the series of agar plates, including a control plate without antimicrobial agent. If the incubation period is extended for slow-growing organisms, the stability of the agent over the incubation period must be assessed by the inclusion of control strains with known MICs. Avoid incubation in an atmosphere containing 5% CO_2 unless necessary for growth of the organisms.

Preparation of Inoculum

Standardize the density of inoculums to give 104 colony forming units (CFU) per spot on the agar. Use four or five colonies of a pure culture to avoid selecting an atypical variant. The inoculum may be prepared by emulsifying overnight colonies from an agar medium or by diluting a broth culture. The broth used must not be antagonistic to the agent tested. A 0.5McFar- land standard may be used for visual comparison to adjust the suspension to a density equivalent to approximately 108 CFU/ ml. alternatively, inoculum can be adjusted photometrically. Dilute the suspensions of organisms in 0.85% saline or broth to give 107 CFU/ml. Plates must be inoculated within 30 min of standardizing the inoculum, to avoid changes in inoculums density.

Inoculation of plates

Mark the plates so that the orientation is obvious. Transfer diluted bacterial suspensions to the wells of inoculums replicating apparatus. Replicator pins 2.5 mm in diameter will transfer about 1 ml, i.e. an inoculums of 104 CFU/spot.

Alternatively, a micropipette or standard loop may be used to inoculate plates. Allow the inoculum spots to dry at room temperature before inverting the plates for incubation. Incubate plates at 35+/-37°C in air for 18 h. In order to avoid uneven heating, do not stack plates more than five high.

Quality control

Routine quality control is achieved by the use of control strains, e.g. as shown in Working cultures of control strains may be stored on digest agar slopes and sub cultured weekly. Working cultures should be replaced monthly from lyophilized or frozen cultures (60°C or below).Test colonies of control cultures as recommended for test cultures. Include control cultures with each batch of tests, and test new lots of agar before they are used routinely. In general, MICs of control organisms should be within one two-fold dilution step of the target values given in Table 4. As well as target values for tests on the reference medium, MH agar, tar- get values for control strains tested on ISO Sensitest agar are included for comparison. In addition:

1. The control plate without antimicrobial agents must show adequate growth of both test and control strains.

2. Plate a sample of inoculum prepared for each strain on a suitable agar medium to ensure that the inoculums are a pure culture.

3. Occasionally check that the method of producing the correct inoculum density is working by counting the number of organisms in the inocula.

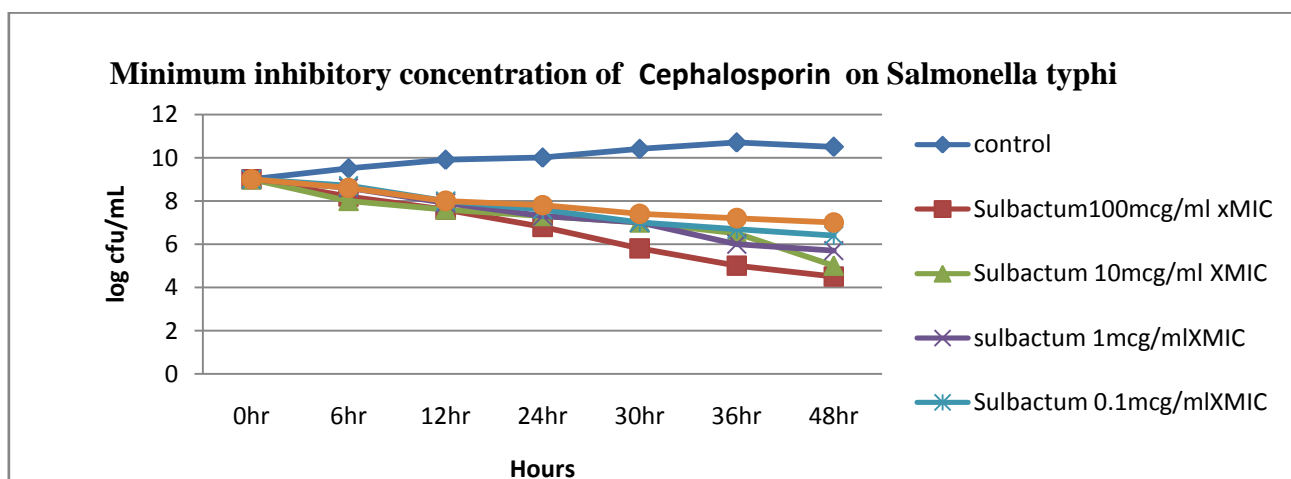4. Check that endpoints are read consistently by all stat independently reading a selection of tests.

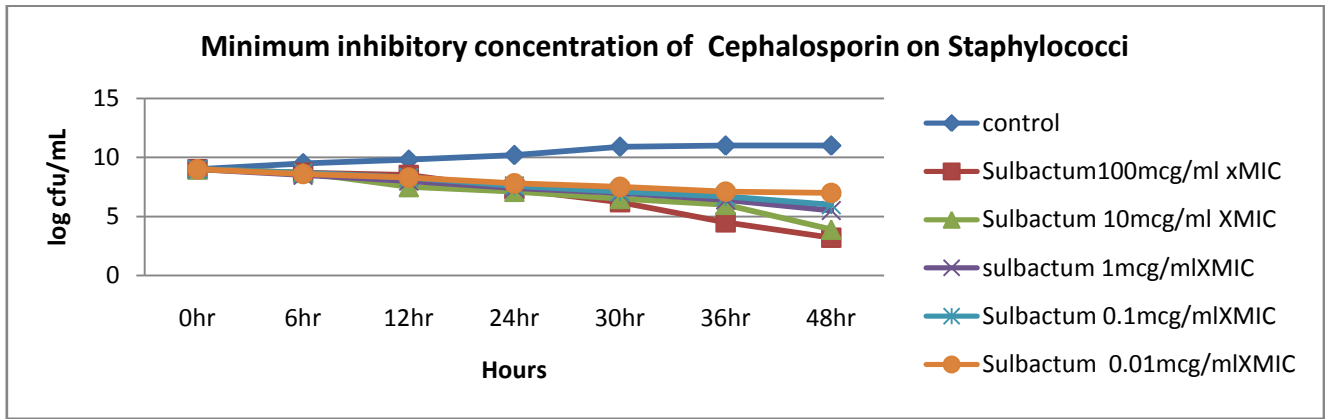**Figure 1:** Effectiveness of cephalosporin on *Salmonella typhi*
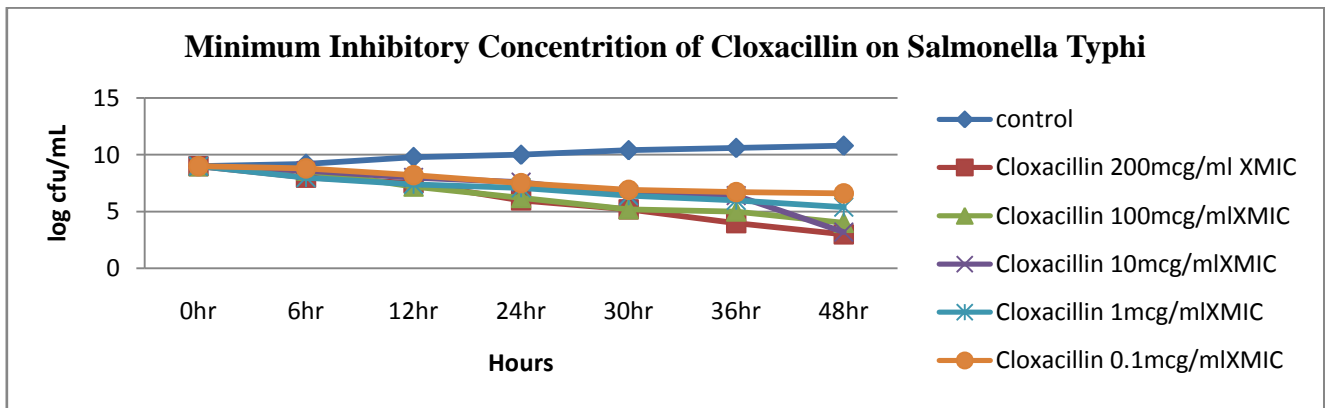
Figure 2: Effectiveness of cephalosporin on *Staphylococci*

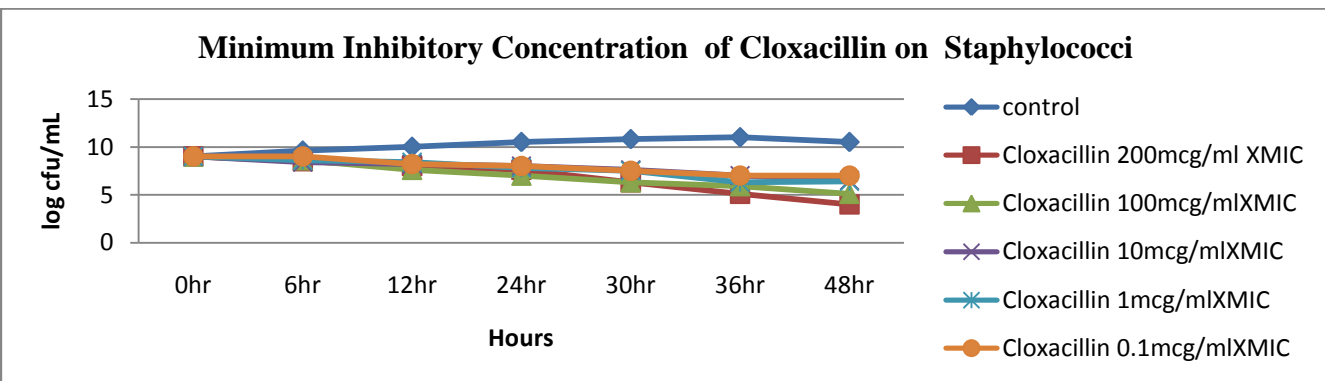**Figure 3:** Effectiveness of Cloxicillin on *Salmonella typhi*

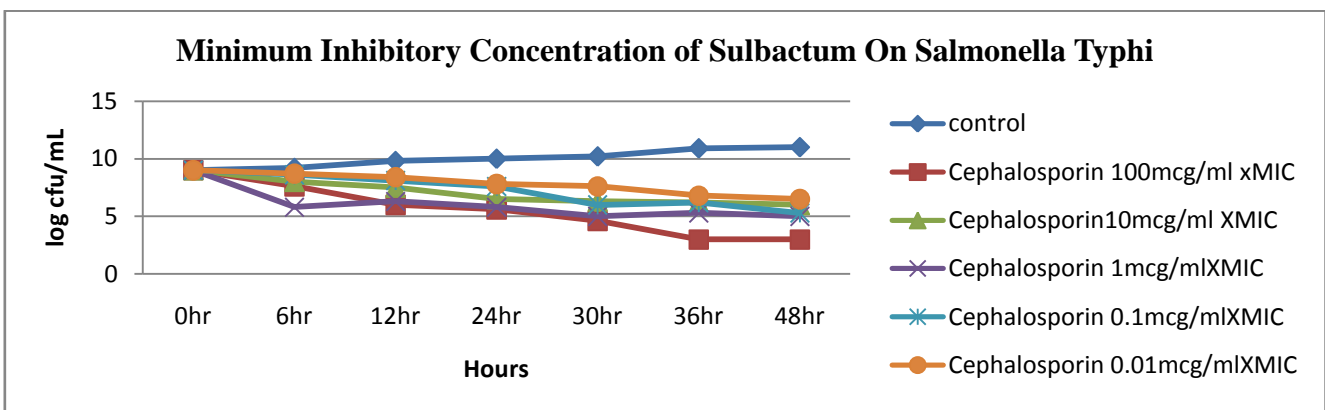**Figure 4:** Effectiveness of Cloxicillin on *Staphylococci*

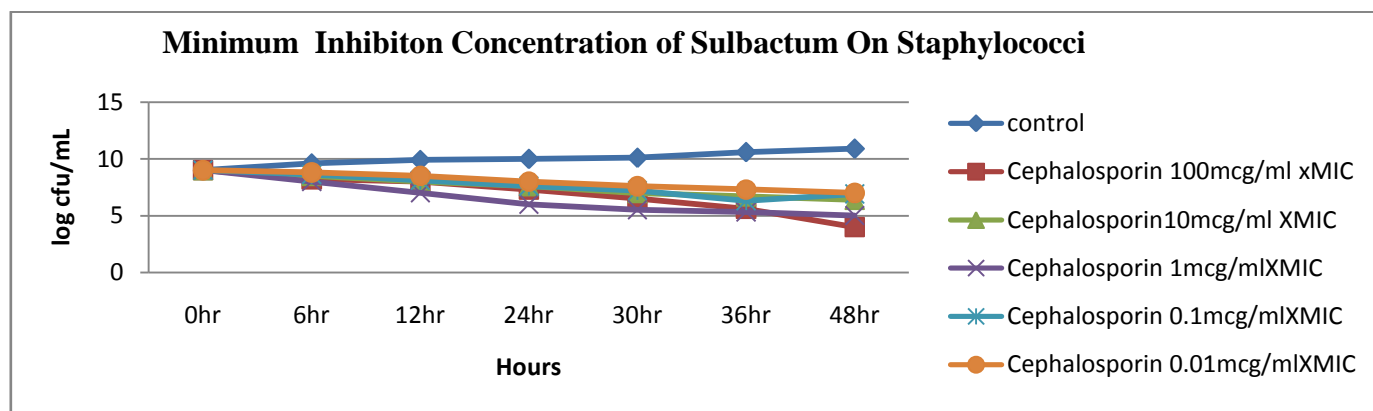**Figure 5:** Effectiveness of Sulbactum on *Salmonella typhi*

Figure 6: Effectiveness of Sulbactum on *Staphylococci*

Results

Antibacterial activity of cephalosporin, cloxillin, sulbactum, and amoxicillin on the gram negative bacteria *Salmonella typhi* and gram positive bacteria *Staphylococci* was assayed and data on effect of cephalosporin on the growth of series of bacteria presented in Figure 1-6. The data revealed that significant reduction in growth of bacteria. In this investigation we studied the effectiveness of drugs like cephalosporin, cloxillin, sulbactum, and amoxicillin on the gram negative bacteria *Salmonella typhi* and gram positive bacteria *Staphylococci*. In these present investigations orderly cloxicillin showed highest inhibitory zone on *Salmonella typhi* and staphylococci than the cephalosporin followed by sulbactum, and amoxicillin.

Discussion

The MIC is the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot. A trailing endpoint with a small number of colonies growing on concentrations several dilutions above that which inhibits most organisms should be investigated by subculture and retesting. Such trailing endpoints may indicate contamination, resistant variants, b-lactamase producing organisms, or, if incubation is prolonged, re growth of susceptible organisms following deterioration of the agent. With sulfonamides and trimethoprim, endpoints may be seen as a reduction in growth, and a haze of growth may be seen at several dilutions above the actual MIC. When testing organisms that produce extracellular β lactamases, the MICs of penicillin and cephalosporin may be markedly ejected by the density of inoculum. The standard inoculum may result in MICs only slightly higher than those obtained with susceptible strains. β lactamase production

can be more reliably detected in staphylococci, gonococci, Haemophilus in£uenzae and Moraxella catarrhal is by definitive tests such as nitrogen based techniques.[8] Among Gram negative organisms, the elect of the inoculum depends on the amount of enzyme produced, and the activity of the enzyme against the particular penicillin or cephalosporin under test. Standardization of the inoculum is necessary to avoid large variations in MIC results. A minimum inhibitory concentration of antimicrobial drugs is the reference method to test the antimicrobial susceptibility of a drug.[9] Result of the MIC is at the lowest concentration of drug that inhibits more than 99% of the bacterial population.[10] Previously some other methods like radiometric method allow to the completion of the test in 8 days and gives lower probability of drug. So the radiometric method is the time taking processes. MIC is the very effective method to determine the lowest concentration of the drug inhibits the growth of bacterial colonies. In the present investigation we studied the effectiveness of drugs like cephalosporin, cloxillin, sulbactum, and amoxicillin on the gram negative bacteria *Salmonella typhi* and gram positive bacteria *Staphylococci*.[11]

Conclusion

In these present investigations orderly cloxicillin showed highest inhibitory zone on *Salmonella typhi* and *Staphylococci* than the cephalosporin followed by sulbactum, and amoxicillin. Here we conclude that the broad spectrum antibacterial activity exhibited by cephalosporin, cloxillin, sulbactum, and amoxicillin results the study have justified the use of the how much concentrations of the antibiotics are to curing the diseases.

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