Bacterial Sensitivity of Serratia Marcescens against Antibiotics

Ayesha Ali

Abstract— Serratia Marcescens, a well-known human pathogen, was tested with a large number of antibiotics belonging to the following groups: Glycopeptides, Tetracyclines, and Amphenicols. It was found that Serratia marcescens was susceptible to following antibiotics: Chloramphenicol, and Tetracycline, and resistive to following antibiotic: Vancomycin. Values of minimum inhibitory concentrations were then obtained for Chloramphenicol and Tetracycline and were 120 µg and 90 µg respectively.

Index Terms— Antibiotics, chloramphenicol, minimum inhibitory concentration, relative susceptibility, tetracycline, vancomycin, zone of inhibition

1 Introduction

Serratia Marcescens, a well-known human pathogen, belongs to the *Serratia* genus and *Enterobacteriaceae* family [1]. It is a gram-negative bacterium which is primarily characterized by a membrane present around the cell wall, further increasing risk of toxicity to the host. Due to this feature, gram-negative bacteria are more difficult to treat as compared to gram-positive bacteria. Gram-negative bacteria also contain porin channels which can prevent entry of harmful chemicals and antibiotics. These channels also force out any antibiotics present within the bacterium, resulting in increased difficulty of treatment. Serratia Marcescens was tested with antibiotics belonging to the following groups: Glycopeptides, Tetracyclines, and Amphenicols. Tetracyclines are broad-spectrum antibiotics which work as protein synthesis inhibitors. They are used to treat a wide variety of infections – most commonly acne. The absorption of tetracycline antibiotics is impaired if taken with calcium, aluminum salts, and magnesium salts [2]. Glycopeptides, such as Vancomycin, are responsible for inhibition of cell wall synthesis and are bactericidal in nature. Ristocetin, a bactericidal like Vancomycin, was discontinued due to aggregation of blood platelets [3]. This unfavorable attribute made it helpful in diagnosing von Willebrand's disease. Chloramphenicol is also a broad-spectrum antibiotic which is derived from Streptomyces venequelae and is primarily bacteriostatic in nature [4]. Its mechanism comprises of blocking bacterial protein synthesis by binding to the 50S bacterial ribosomal subunit. These antibiotics were tested against Serratia marcescens to test if it was susceptible against these particular antibiotics. The following will cover the methods utilized to reach the necessary results.

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2 Methods

Since results were both qualitative and quantitative, different methods took place for the obtaining of results. For both tests, a culture medium was formed, either in solid or liquid form; upon formation of culture medium, antibiotics were added and their effects on Serratia marcescens were observed and recorded.

For the preparation of Luria-Bertani (LB) agar, distilled water was added to a flask along with 2.5g of Tryptone, 1.25g of yeast extract, 1.25g of Sodium Chloride and 3.75g of agar, to give rise to a solution of a total volume of 250 ml. The flask was then plugged with cellophane and autoclaved at a temperature of 121℃ for 15 minutes, allowing for sterilization of culture media. After sterilization, the mixture was allowed to cool until the flask reached a temperature of 50-60℃; this normally took around 45 minutes to an hour. The cooled mixture was then poured into dried autoclaved petri dishes and were allowed to solidify for 15-20 minutes; the pouring was done in a laminar flow cabinet to prevent contamination. The petri dishes were then inverted after solidification. With the help of an inoculating loop, Serratia marcescens was streaked onto the petri dishes and incubated in the incubator at 37℃ for 24 hours.

After cultures of Serratia marcescens had been obtained, prepared solutions of antibiotics were added. For the solid media, a cotton swab was used to streak a new petri dish with the previously obtained culture of Serratia marcescens. Two antibiotic discs of the respective antibiotic were placed on this petri dish and were incubated for 24 hours at 37℃. This gave rise to zones of inhibition on the petri dish, signifying if Serratia marcescens was susceptible to that particular antibiotic. All antibiotics used throughout experimentation were of the same concentration, 30 µg/disc.

For the preparation of Luria-Bertani (LB) broth, 1.0g of Tryptone was added to a flask along with 0.5g of yeast extract, 0.5g of Sodium Chloride, and distilled water to give rise to a solution of total volume 100 ml. The flask was then plugged with cellophane and autoclaved at a temperature of 121℃ for 15 minutes. Once the flask had cooled down, 100 µl of Serratia marcescens was added with the help of a micropipette and incubated at 37℃ for 24 hours. 5 ml of the mixture was then placed into test tubes along with one antibiotic disc of each antibiotic. The test tubes were then incubated at 37℃ for 24 hours. After a time period of 24 hours, the optical density of the test tubes was measured through the use of a spectrophotometer. The spectrophotometer measures amount of light scattered by the bacteria culture; the wavelength at which the OD was measured was 600 nm. A high OD value will signify resistivity to the particular antibiotic, whereas a low value will indicate sensitivity.

To deduce which antibiotics Serratia marcescens was sensitive to, values of optical density were compared to a control. The control comprised of 100 µl of Serratia marcescens, which had been incubated alongside the test tubes containing antibiotic discs. After it had been deduced which antibiotics Serratia marcescens was susceptible to, further investigation was conducted to obtain values for minimum inhibitory concentration. This was done by placing 5 ml of LB broth mixture containing 100 µl of Serratia marcescens into test tubes. Then, varying number of antibiotic discs were placed within the test tubes to give rise to concentrations of 30 µg, 60 µg, 90 µg, and 120 µg, and 150 µg. The following represents how the number of antibiotic discs required by each antibiotic to reach the necessary concentration was calculated:

$$\textit{Concentration of Antibiotic} = x\ \mu g$$
$$\textit{Desired Concentration} = y\ \mu g$$
$$\textit{Number of Required Antibiotic Discs} = \frac{y}{x}$$

The test tubes were then incubated at 37℃ for 24 hours. After a time period of 24 hours, the optical density of the test tubes was measured through the use of a spectrophotometer. The values were then plotted on a graph to obtain values of minimum inhibitory concentrations; comparison of minimum inhibitory concentrations allows for the comparison of relative susceptibility of each antibiotic.

3 Results and Discussion

The following results were obtained:

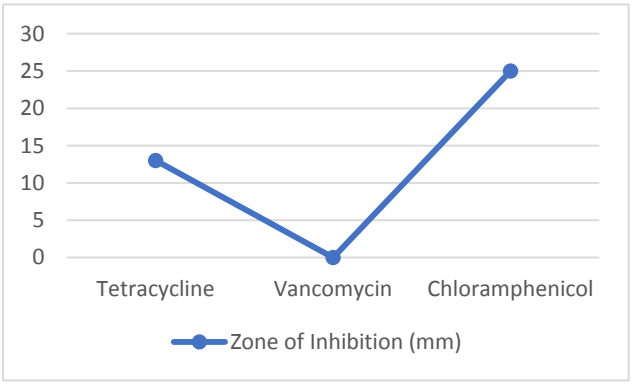

Fig 1: Measurement of zones of inhibition obtained with Tetracycline, Vancomycin, and Chloramphenicol. Measurements of these zones in mm are on the y-axis and the first letter of each antibiotic on the x-axis.

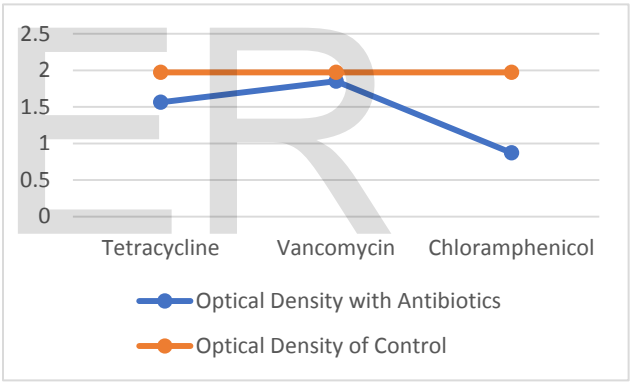

Fig 2: Measurement of bacterial growth with Chloramphenicol, Tetracycline, Vancomycin, and Chloramphenicol alongside a control; this was achieved through the measurement of optical density by spectrophotometers. This measurement is based on amount of light scattered by the bacteria culture, signifying if any bacterial growth has taken place. OD measurements are on the y-axis and first letter of each antibiotic on the x-axis.

Deducing whether the bacteria was susceptible or not to the antibiotic, both zone of inhibition and values of optical density were considered. Some antibiotics had low values of optical density and large zones of inhibition, as in the case with Chloramphenicol. Others had moderate values of optical density and zones of inhibition, as in the case with Tetracycline. On the other end of the spectrum, antibiotics had no effect, with a high value of optical density, and no zone of inhibition, as was the case with Vancomycin. All these factors were considered when concluding which antibiotics, the bacteria was susceptible or resistant to and values obtained were compared with the control. After it had been concluded that Chloramphenicol and Tetracycline are susceptible to Serratia marcescens, values for minimum inhibitory concentration were obtained. The following represents the results achieved:

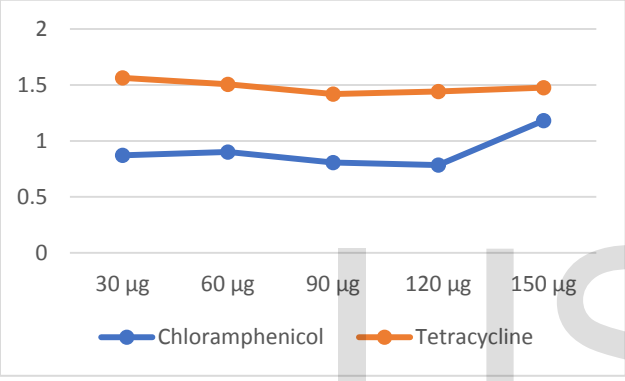

Fig 3: Measurement of optical density with varying number of antibiotic discs to obtain ranges of minimum inhibitory concentration of Chloramphenicol and Tetracycline.

In the case of Chloramphenicol, optical density first increases and then decreases to a minimum value of 0.785 when the concentration of antibiotic is 120 µg. The optical density then sharply increases when concentration is 150 µg to 1.182. In the case of Tetracycline, the optical density gradually decreases to value of 1.419 when antibiotic concentration is 90 µg and then increases at a moderate rate for later concentrations.

4 Conclusion

The results attained demonstrate sensitivity of Serratia marcescens towards the following antibiotics: Chloramphenicol, and Tetracycline, and resistivity towards Vancomycin. The relative susceptibility of Chloramphenicol and Tetracycline was observed through comparison of ranges of minimum inhibitory concentrations which were 120 µg and 90 µg respectively. Determining relative susceptibility of antibiotics towards bacteria is highly essential and greatly aids towards development of treatment plans.

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References

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