

COMPARATIVE STUDY OF ANTIMICROBIAL EFFICACY OF AZITHROMYCIN AND CEFIXIME AGAINST *SALMONELLA TYPHI* INFECTION

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ABSTRACT

Introduction: Enteric fever is systemic infection caused by the *Salmonella enteric serovars typhi* and *para typhi* A B and C. It is the significant cause of morbidity and mortality. It occurs in all parts of the world where water supplied and sanitation is substandard. Annually, it is estimated that more than 10 million cases and 100000 deaths are caused by typhoid fever. Regarding to the strains, a high prevalence of *S. typhi* and *S. paratyphi*. A strains in Nepal that showed resistance against the quinolone nalidixic acid (MIC> 256 mcg/ml with a corresponding decreased susceptibility against fluoroquinolones such as ciprofloxacin (MIC>0.125 mcg/ml)

Objectives: The main objective of study was to compare the efficacy of Azithromycin and Cefixime in treatment of typhoid fever.

Methodology: The in vitro antibacterial activity of azithromycin and Cefixime against 4 isolated colonies of *Salmonella typhi* from reference of *salmonella typhi* ATCC no. 14028 and blood culture isolates from three different hospitals was evaluated by disc diffusion (well) method. 0.25 ppm, 0.5 ppm, 4 ppm, 8 ppm, 32 ppm, 128 ppm concentration of both Azithromycin and Cefixime was used. The zone of inhibition was measured and data was analyzed using Excel.

Results: In all isolates of *Salmonella typhi*, the zone of inhibition shown by both Azithromycin and Cefixime is same at low concentration (0.25ppm, 0.5ppm) but with increasing in concentration there is increase in difference in zone of inhibition shown by them. The zone of inhibition shown by Cefixime is greater in high concentration as compared to zone of inhibition shown by Azithromycin

Conclusion: Our result indicate Cefixime is better than Azithromycin in therapeutic option for enteric fever.

Nepal, *Salmonella typhi*, Azithromycin, Cefixime, Antibiotic resistance

INTRODUCTION

Enteric fever is systemic infection caused by the *Salmonella enteric serovars typhi* and *paratyphi A, B and C*. It is the significant cause of morbidity and mortality¹. It occurs in all parts of the world where water supplied and sanitation is substandard. Annually, it is estimated that more than 10 million cases and 100000 deaths are caused by Typhoid fever.^{2,3,4}

In 2008, the WHO recommended in using the vaccines for enteric fever as to control and outbreak the enteric fever as an endemic disease. As per one study more than 190,000 patients were died of enteric fever in 2010 deaths.⁵ The incidence of enteric fever is higher than 7.6 per 1000 per year (India).Case fatality rate due to typhoid is varied between 1.1 and 2.5%. It is one of the killer diseases in Nepal.⁶

In Nepal, all the enteric fever related data has been generating from the Kathmandu valley instead of remote area of Nepal as about 90% of the Nepali population lives outside the region or in rural areas [7]. In summer season most of the hospital will be occupied with the patients of enteric fever. Even emergency department will be also being occupied with it. Children and adult are almost equally affected by this leading febrile bacterial disease.^{8,9}

Because of the lack of proper treatment guidelines and poor surveillance of DDA to retails shop and Nursing home, most of the antibiotics are resistance (resistance of *Salmonella* to antibiotics as well).^{10,11} Still there is no reserved antibiotic list of antibiotics in Nepal as Azithromycin and Ceftriaxone are the drug of choices for enteric fever. Now time has come to find the next generation of drugs as option.^{12,13}

In early 1990s, Chloramphenicol, Ampicillin and Cotrimoxazole were being used as the first line treatment for enteric fever. Unfortunately, *S. typhi* resistant to all first line drugs as mentioned. Fluoroquinolone were widely regarded as the most effective drug for the treatment of typhoid fever. [14]. Ciprofloxacin and Ofloxacin were being still used but also developing resistance or reduced susceptibility to fluoroquinolones.^{15,16,17} A review conducted by Thapa and Khanal¹⁸ the treatment paradigm is shifted to third generation cephalosporin. Another research conducted by the same authors Ceftriaxone had more cure rate in comparison to Azithromycin.¹⁹

Global antimicrobial resistances are the worldwide problems. Most of the polyclinics are still using fluoroquinolones although it is not working as well.^{20,21} Regarding to the strains, a high prevalence of *S. typhi* and *S. paratyphi*. A strains in the quinolone Nepal that showed resistance against nalidixic acid (MIC> 256 mcg/ml with a corresponding decreased susceptibility against fluoroquinolones such as ciprofloxacin (MIC>0.125 mcg/ml).^{22, 23}. Nowadays third generation cephalosporin are being used as if it were a first line agent for typhoid fever in many countries. Azalides are another class of antibiotics which have shown promise in the treatment of typhoid fever. Azithromycin is first line drug of azalides class. It has been found that Azithromycin reduces the clinical failures rate and duration of hospital

stay in comparison to fluoroquinolones and relapse rate in comparison to treatment of multidrug resistant typhoid fever.²⁴

In two tertiary care hospitals of Kathmandu Valley (Patan Hospital and Civil Hospital) Azithromycin (1000mg once a day for 5 days) is being prescribed for OPD and Inj Ceftriaxone (2 gm once a day) for the in patients of typhoid fever respectively on the base of evidence. Ceftriaxone is for in patients up to this point. The initial results of Azithromycin in enteric fever are reported to be encouraging. Studies comparing the efficacy of Azithromycin with Cefixime in adults and children with typhoid fever have reported to be safe and efficacious.^{25, 26}

Azithromycin

Azithromycin is an azalide antimicrobial, acts by inhibiting ribonucleic acid (RNA)-dependent protein synthesis by binding to the receptor at the bacterial 50S ribosomal subunit.²⁷ It is available in various dosages form like capsules, film-coated tablets and as well as powder for oral suspension.

Cefixime

Cefixime is an antibiotic useful to treat a wide variety of bacterial infections. It lies in third generation cephalosporin which only cure the bacterial infections not viral infections. Unnecessary use or overuse of any antibiotic can lead to its decreased effectiveness²⁸

MATERIALS AND METHOD

The reference of *salmonella typhi* ATCC no. 14028 was obtained from Vega pharmaceuticals Pvt Ltd and other samples of *salmonella typhi* from patient's blood culture were taken from the three hospitals viz; Siddhi Memorial hospital, Summit hospital and Norvic hospital

Kirby Bauer Susceptibility Test Method:

The Kirby-Bauer test for antibiotic susceptibility (also called the disc diffusion) test is used to determine the resistance or sensitivity of aerobes or facultative anaerobes to specific chemicals, which can forward for the clinical practice. The zone of inhibition shows the efficacy of specific chemical towards the bacteria.

RESULT

Zone of inhibition shown by Cefixime and Azithromycin in *Salmonella typhi* isolates from different area.

Table 1; Zone of inhibition shown by different concentration of Cefixime

| Concentration(ppm) | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|--------------------|------|-----|------|------|------|------|
| Vega | 6mm | 6mm | 13mm | 15mm | 21mm | 36mm |
| Siddhi | 6mm | 6mm | 13mm | 15mm | 21mm | 35mm |
| Norvic | 6mm | 6mm | 13mm | 14mm | 20mm | 36mm |
| Summit | 6mm | 6mm | 13mm | 15mm | 20mm | 35mm |

As shown in table, the zone of inhibition shown by Cefixime is almost same in all the isolates collected from different places.

Table 2: Zone of inhibition shown by different concentration of azithromycin

| Concentration(ppm) | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|--------------------|------|-----|-----|-----|------|------|
| Vega | 6mm | 6mm | 8mm | 9mm | 12mm | 26mm |
| Siddhi | 6mm | 6mm | 7mm | 8mm | 11mm | 25mm |
| Norvic | 6mm | 6mm | 8mm | 9mm | 11mm | 26mm |
| Summit | 6mm | 6mm | 7mm | 9mm | 12mm | 26mm |

As shown in table no 2, the zone of inhibition shown by azithromycin is almost same in all the isolates collected from different places.

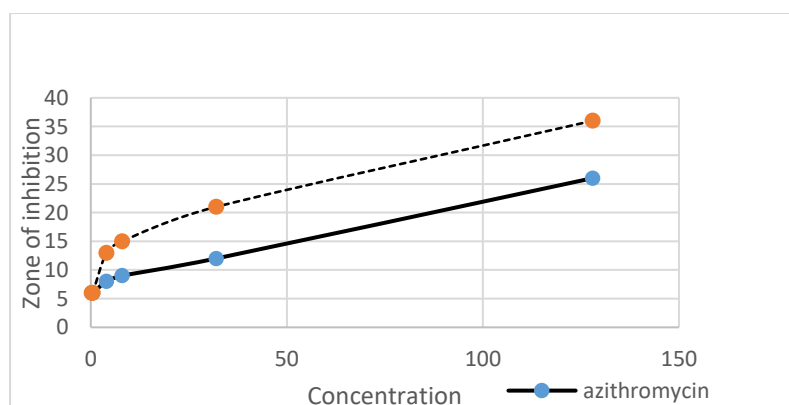
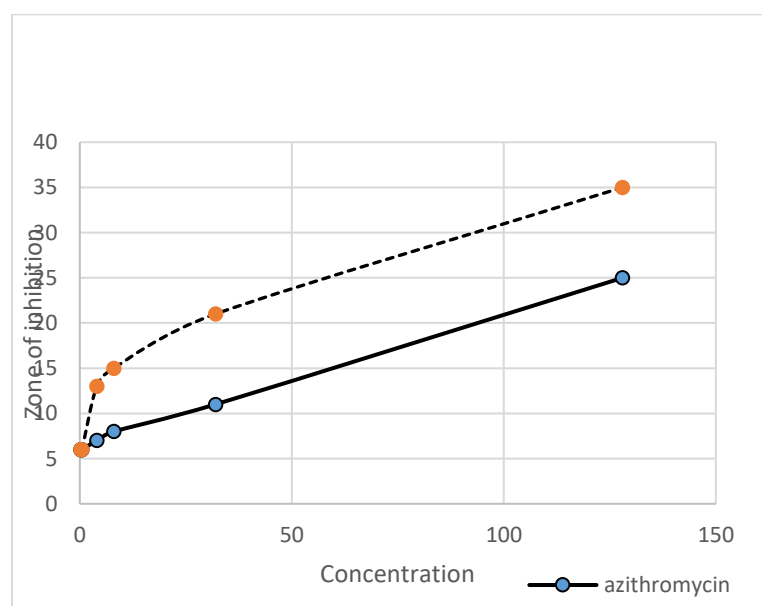
**Figure 1: Concentration Vs Zone of inhibition (Vega pharmaceutical Pvt Ltd)**

Table 3: Ratio of zone of inhibition shown by different concentration of cefixime and azithromycin

| Sampling area | Concentration | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|---------------|-----------------------|------|-----|-------|-------|-------|------|
| Vega | Cefixime/azithromycin | 1 | 1 | 1.733 | 1.685 | 1.782 | 1.38 |

As comparing the ratio between Cefixime and Azithromycin, it showed that in concentration 0.25 ppm and 0.5 ppm, both drugs showed same zone of inhibition. On increasing the concentration in both drugs (4ppm, 8ppm, 32 ppm), it showed that zone of inhibition of Cefixime is increasing as compared to Azithromycin (1.63, 1.67, 1.75). However, the zone of inhibition decreased by 1.38 folds when concentration is increased to 128 ppm.

**Figure 2: Concentration Vs Zone of inhibition (Siddhi Polyclinic)****Table 4: Ratio of zone of inhibition shown by different concentration of cefixime and azithromycin**

| Sampling area | Concentration | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|-------------------|-------------------------|------|-----|------|------|------|-----|
| Siddhi polyclinic | Cefixime / azithromycin | 1 | 1 | 1.85 | 1.87 | 1.90 | 1.4 |

As comparing the ratio between Cefixime and Azithromycin, it showed that in concentration 0.25 ppm and 0.5 ppm, both drugs showed same zone of inhibition. On increasing the concentration in both drugs (4ppm, 8ppm, 32 ppm), it showed that zone of inhibition of Cefixime is increasing as compared to Azithromycin (1.85, 1.87, 1.90). However, the zone of inhibition decreased by 1.4 folds when concentration is increased to 128 ppm.

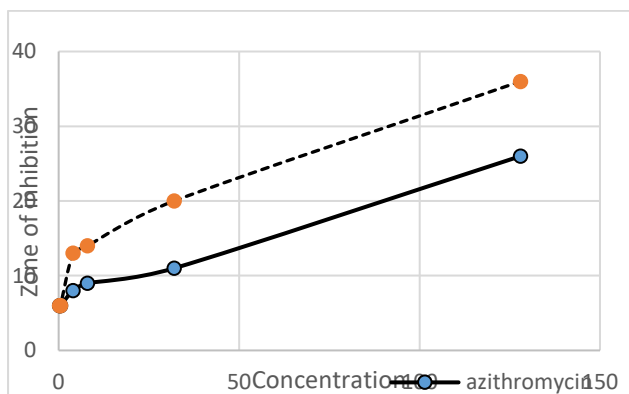


Figure 3: Concentration Vs Zone of inhibition (Norvic)

Table 5: Ratio of zone of inhibition shown by different concentration of cefixime and azithromycin

| Sampling area | concentration | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|-----------------|-----------------------|------|-----|------|------|------|------|
| Norvic hospital | Cefixime/azithromycin | 1 | 1 | 1.63 | 1.78 | 1.81 | 1.38 |

As comparing the ratio between cefixime and azithromycin, it showed that in concentration 0.25 ppm and 0.5 ppm, both drugs showed same zone of inhibition. On increasing the concentration in both drugs (4ppm, 8ppm, 32 ppm), it showed that zone of inhibition of Cefixime is increasing as compared to Azithromycin (1.63, 1.78, 1.81). However, the zone of inhibition decreased by 1.38 folds when concentration is increased to 128 ppm.

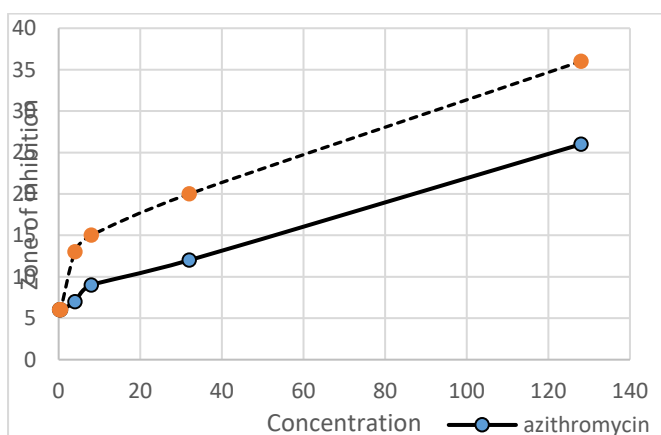


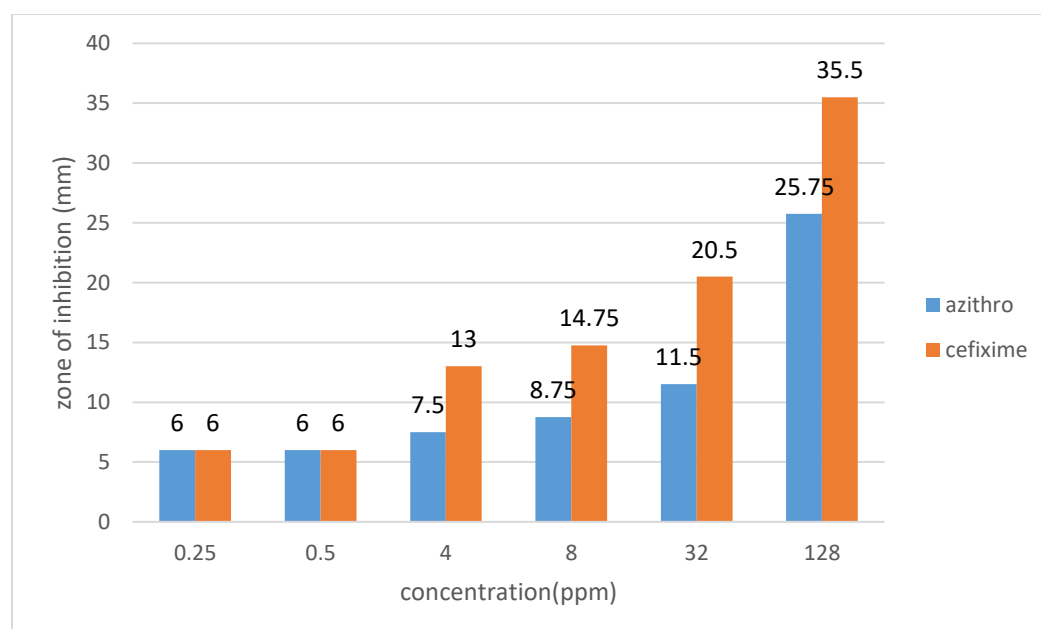
Figure 4: Concentration Vs Zone of inhibition (Summit hospital)

As presented in above table, the zone of inhibition of Cefixime is greater than the zone of inhibition shown by azithromycin in all sample isolated from different hospital in different concentration. The highest diameter of zone of inhibition of azithromycin (23 mm) and Cefixime (37 mm) was seen in 128 ppm concentration of each antibiotic. The lowest diameter of zone of inhibition of Azithromycin (6mm) and Cefixime (6mm) was seen in 4 ppm and 0.5 ppm concentration of azithromycin and Cefixime respectively.

Table 6: Ratio of zone of inhibition shown by different concentration of Cefixime and Azithromycin

| Sampling area | Concentration | 0.25 | 0.5 | 4 | 8 | 32 | 128 |
|-----------------|-------------------------|------|-----|------|------|------|------|
| Summit hospital | Cefixime / azithromycin | 1 | 1 | 1.85 | 1.67 | 1.67 | 1.34 |

As comparing the ratio between Cefixime and Azithromycin, it showed that in concentration 0.25 ppm and 0.5 ppm, both drugs showed same zone of inhibition. On increasing the concentration in both drugs (4ppm), it showed that zone of inhibition of Cefixime is increasing as compared to Azithromycin (1.85). However, the zone of inhibition is same when concentration is increased to 8 ppm and 32ppm and get decreased by 1.34 folds when concentration is increased to 128 ppm.

**Figure 5: Comparison of Zone of inhibition shown by Azithromycin and Cefixime**

As shown in fig 5 the zone of inhibition shown by both Azithromycin and Cefixime is same at low concentration (0.25ppm, 0.5ppm) but with increasing in concentration there is increase

in difference in zone of inhibition shown by them. The zone of inhibition shown by Cefixime is greater in high concentration as compared to zone of inhibition shown by Azithromycin

DISCUSSION

This study was carried out to compare the antimicrobial efficacy of Azithromycin and Cefixime against *Salmonella typhi* isolate collected from different hospitals and to know either the isolates are sensitive or resistance to them.

The samples were collected from 3 different hospitals and one standard sample of *Salmonella typhi* ATCC no.14028 was collected from Vega pharmaceuticals. The standard Azithromycin and Cefixime was brought from Vega pharmaceuticals and Vijayadeep pharmaceuticals respectively. The antimicrobial activity of Azithromycin and Cefixime were tested against the collected sample and result were compared.

Since the load variation of bacteria and standard of drugs are not same with other research, we cannot compare our data with other research data done by in vitro method. As comparing to the in vitro activity with in vivo activity, it shows the similar type of result. Comparing the efficacy of Azithromycin in the treatment of childhood for a period of one year from January 2011 to December 2011, the clinical cure rate was 87% in azithromycin group and 93% in cefixime group.²⁹ An in vivo study shows that Azithromycin and Cefixime up to 7 days for the treatment of uncomplicated typhoid fever, a total of 31 (91%) of the 34 patients treated with Azithromycin and 29 (97%) of the 30 patient treated with Cefixime were cured.³⁰

An open label, non-comparative study, which evaluated the efficacy and safety of Azithromycin for the treatment of uncomplicated enteric fever, found that azithromycin cured 93% of the subjects whereas the Cefixime cured 100% of the subjects.³¹

Azithromycin was said to have been effective in 92.4% of the 1039 reports which included an opinion about effectiveness whereas Cefixime was said to have been effective in 94.29% of the 1039.³²

CONCLUSION

Salmonella typhi is more sensitive to Cefixime than Azithromycin. From the above data it can be concluded that for the treatment of typhoid fever Cefixime is more effective than the Azithromycin. The certain stamps the *Salmonella typhi* shows resistance. So, regular monitoring of emergence of resistance is highly recommended and specific antibiotic should be identified after antibiotic susceptibility test and results for the effective management of disease caused by *Salmonella typhi*.

ACKNOWLEDGMENTS

We would like to acknowledge Pro. Dr. Dharma Prasad Khanal (visiting professor, Mewar University, Rajasthan, India), Krishna Govind Prajapati, Sarad Pudasaini, Sharmila Joshi, Alina Shrestha, Puja Adhikari, Ranjit Singh Mahato, Vega pharmaceuticals Pvt Ltd, Siddhi Memorial hospital, Summit hospital and Norvic hospital Pvt. Ltd for their magnanimous support on the study.

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Annex: I Photographs



Annex II: Procedure for Antimicrobial Susceptibility test

Preparation of Nutrient Agar (NA) media and subculture of bacteria

28 gram of NA was weighed and 1000ml of distilled water was added. Then it was boiled in conical flask with occasional stirring. It was sterilized then in autoclave at 121 °C and 15 lb pressure for 15 minutes. It was then poured in the sterilized petriplate and left for sometimes to cool down it. Then after the solidification of the media the bacteria was subcultured with the help of the sterilized loop by streak method and then incubated for 24 hours at 37°C.

Preparation of Nutrient Broth (NB) media and subculture of bacteria

0.312 gm of NB was weighed and 25 ml of water was added in a test tube. Then it was sterilized in autoclave at 121 °C and 15 lb. pressure for 15 minutes and left to cool it. Then bacteria were inoculated in the media and incubated for 4 hours and compared its turbidity with McFarland (0.5).

Preparation of McFarland tube

1% H₂SO₄ (9.95 ml) and 1% BaCl₂. H₂O (0.05 ml) was mixed in a test tube. It had a cloudy look.

Preparation of Muller-Hilton Agar (MHA) media

38 gram of MHA was weighed and kept in a conical flask and then 1000ml of water was added. Then it was boiled in the burner and sterilized in the autoclave at 121 °C and 15 lb pressure for 15 minutes. Then it was poured in the petri plate and cooled down to the room temperature.

Preparation of antibiotic stock solutions

0.25-128 mg/l dilution range for both azithromycin and cefixime was chosen.

Stock solution was prepared by using the formula

$$100/p \times V \times C = W$$

Where p= potency given by manufacturer

V= volume required

C= final concentration of solution and

W= Weight of antibiotic in mg to be dissolved in volume V.

For cefixime

$$W = 1000/1023.7 \times 25 \times 10 = 244.21 \text{ mg}$$

For azithromycin

$$W = 1000/996.8 \times 25 \times 10 = 250.802 \text{ mg}$$

244.21 mg of cefixime and 250.802 mg of azithromycin dissolved in 25 ml of saturated sodium bicarbonate solution and ethanol(70%) respectively = 10,000 mg/l stock solution.

For preparation of further stock solutions, from initial 10,000 mg/l solution, following were prepared

1 ml of 10,000 mg/l + 9 ml distilled water = 1000 mg/l

100 µl of 10,000 mg/l + 9.9 ml diluent = 100 mg/l

Preparation of antibiotic dilution range

Selected concentration: 128 ppm, 32 ppm, 8 ppm, 4 ppm, 1, 0.5 ppm, 0.25 ppm

For 128 ppm and 32 ppm concentration, 256 μ l and 32 μ l was dispensed in beaker having 20 ml sterilized water respectively from the 10,000 mg/l stock solution.

For 8 ppm and 4 ppm concentration, 160 μ l and 80 μ l was dispensed in beaker having 20 ml sterilized water respectively from the 1000mg/l stock solution.

For 0.5 ppm and 0.25 ppm concentration, 100 μ l and 50 μ l was dispensed in beaker having 20 ml sterilized water respectively from 100 mg/l stock solution.

Subculture of bacteria

The two well having diameter 6mm was made in upper and lower part of MHA media and the bacteria from NB media (whose turbidity was same as the McFarland 0.5) were sub cultured in MHA media with cotton swab by spread plate method. Then it was left for sometimes and 70 μ l of different concentration of cefixime and azithromycin was placed in well of upper and lower part of MHA respectively of each 24 petri plates. These plates were then incubated at 37°C for 24 hours. Then the zones of inhibition shown by different concentration were measured.