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Research Article

EVALUATION OF BACTERIAL RESISTANCE OF CEFADROXIL, CEPHALEXIN AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE MICRO-ORGANISMS

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ABSTRACT

Objective: This study was aimed to overcome the problems of bacterial resistance to antibiotics used for the treatment of diseases.

Methods: In this aspect, two antibiotics were selected such as cefadroxil and cephalexin for the evaluation of antibiotic resistance against selected three Gram-positive microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Bacillus cereus* and three Gram-negative microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens.* The selected antibiotics were evaluated by the determination of minimum inhibitory concentration and the agar disk diffusion method.

Results: According to the results obtained, cefadroxil was resistant against *Bacillus cereus* (25.6 µg/ml, 10 mm) and *Serratia marcescens* (25.6, 6 mm), sensitive against *Pseudomonas aeruginosa* (6.4 µg/ml, 18 mm), *Bacillus subtilis* (6.4 µg/ml, 30 mm), intermediate against *E. coli* (12.8 µg/ml, 16 mm), and *Staphylococcus aureus* (12.8 µg/ml, 15 mm). Similarly, cephalexin was resistant against *Serratia marcescens* (25.6 µg/ml, 10 mm), intermediate against *E. coli* (12.8 µg/ml, 14 mm), *Pseudomonas aeruginosa* (25.6 µg/ml, 14 mm), *Bacillus subtilis* (12.8 µg/ml, 15 mm), *Staphylococcus aureus* (6.4 µg/ml, 16 mm), and sensitive against *Bacillus cereus* (6.4 µg/ml, 18 mm) according to the CLSI report.

Conclusion: The increased illness of human beings by the usage of antibiotics frequently without prescriptions is a serious problem that dramatically raises the cost of health-care worldwide. Hence, the study was useful and effective for the determination of antibiotic resistance of cephalexin and cefadroxil for the future treatment in more efficacies with fewer side effects.

Keywords: Bacterial resistance, Cefadroxil, Cephalexin, Agar disk diffusion.

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INTRODUCTION

Antibiotics are known as antimicrobial drugs used in the treatment, and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. They are not effective against viral infections such as the common cold or influenza and inappropriate use allows the emergence of resistant organisms. Antibiotic resistance is the ability of a microorganism to develop resistance against antibiotics. Today, almost all bacterial infections in India and throughout the world are becoming resistant to antibiotics. Antibiotic resistance is one of the world's worst effects on public health concerns. The balanced use of antibiotics is the key to controlling the spread of resistance. Antibiotics that are used incorrectly may cause several health problems including the increase of health cost, antibiotic resistance, and potential side effects [1].

At present, antibiotic therapy is often misused as a result of the ridiculous use and ease of people to obtain antibiotics without a prescription. It triggers high multi-drug resistance. Resistance bacterial infection to antibiotics will lead to increase morbidity and mortality that required second or even third-line antibiotics that have lower effectiveness, may have more side effects, and are more expensive. The use of antibiotics repeated and improper is the main cause of the increase in the number of bacteria that are resistant to antibiotics [2]. There is a need to ascertain the chemical and biological equivalence of antibiotics due to global health problems posed by antibiotic and multi-drug resistance [3]. The selection and use of appropriate antibiotics will determine the success of treatment and can avoid the occurrence of antibiotic resistance [4].

Cephalexin, a semisynthetic derivative of cephalosporin, is known to have antibacterial action against Gram-positive and Gram-negative bacteria. Cephalexin is a potent cephalosporin and exhibits a broad spectrum of antibiotic activity, low toxicity and to be rapidly absorbed following oral administration to give a high serum level and urine concentration. The drug, therefore, is widely used for clinical chemotherapy [5,6]. Cephalexin may be administered by the parenteral or oral routes and provide an immediate or sustained release of the drug, thus prolonging the duration of the antibacterial activity (long-acting formulations). Cephalexin pharmacokinetics have been described in several domestic species, such as dogs [7,8], horses, and ruminants.

One of the antibiotics used for the treatment of acute respiratory infection is Cefadroxil. Cefadroxil is highly active against Gram-positive *Coccus*, such as *Pneumococcus*, *Streptococcus*, and *Staphylococcus*. Cefadroxil has given in doses of 0.5–1 g twice daily excretion primarily through glomerular filtration and tubular secretion into the urine [9]. The mechanism of Cefadroxil is inhibiting bacterial cell wall synthesis. After oral absorption, it has good tissue penetration and exerts more sustained action at the site of infection [10]. Although, its plasma half-life is 1.5 h; dose. Various studies have shown a comparison between topical and oral antimicrobial agents for superficial skin infections and derived that topical antimicrobial is as effective as oral agents in treating the infections [11,12]. In these studies, two antibiotics, such as Cefadroxil and Cephalexin, were selected for the antibiotic resistance analysis by the determination of minimum inhibitory concentration, and by the agar disk diffusion method.

METHODS

Standard drug

Two drugs were selected such as Cefadroxil and Cephalexin in the brand name of Cefadrox (Aristo Pharmaceuticals), Phexin (Glaxo Smithkline Pharmaceuticals), respectively, and it was purchased from the local medical store, Vijayawada.

Microorganisms

Three Gram-negative microorganisms such as *E. coli* (NCIM 2256), *P. aeruginosa* (NCIM 2037), *Serratia marcescens* (NCIM 2078), and three Gram-positive microorganisms such as *Bacillus cereus* (NCIM 2914), *Bacillus subtilis* (NCIM 2710), and *Staphylococcus aureus* (NCIM 2794) were used in these studies. All the test microorganisms were obtained from National Collection of Industrial Microorganisms (NCIM).

Instruments and chemicals

The instruments used in this analysis are Autoclave, Hot air oven, Laminar air-flow chamber, Incubator, Refrigerator, Precision electric balance, Micropipette (100 to 1000 µl), Inoculating loop, etc. The chemicals were used for this work are ethanol, peptone, sodium chloride, and beef extract (Research Lab Fine Chem. Industries), Mueller-Hinton Agar (Titan Biotech Ltd).

Agar disk diffusion test

In this method, the standardized bacterial isolate was spread on an agar plate, and then paper discs containing a specific concentration of antibiotics were placed and incubated at 37°C overnight. If the isolate is sensitive to the antibiotic, it does not grow around the disk thus forming a zone of inhibition. Resistant strains to an antibiotic grow up to the margin of the disk. The diameter of the zone of inhibition must be measured and the result read from the Kirby Bauer chart as sensitive, intermediate, or resistant [13].

Preparation of inoculum

A loopful of inoculum was taken from a pure culture of *E. coli* bacteria and inoculated into 10 ml of Mueller-Hinton broth (Hi-Media, Mumbai, India). A similar procedure was adopted to prepare the inoculum of other bacterial species, that is, *P. aeruginosa, Serratia marcescens, Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus*. The broth suspension was then incubated at 37°C for 24 h and utilized for MIC determination.

Media for test organisms

19 g of Mueller-Hinton agar medium was mixed with 250 ml of distilled water and sterilized in an autoclave at 121°C for 15 min at 15 lbs. Medium gets cooled to room temperature, and then it was inoculated with both Gram-positive and Gram-negative microorganisms and poured into sterile Petri plates and set aside until it gets solidified.

Mueller-Hinton broth (O.D. 600=0.08) was used to dilute the bacterial cultures to obtain a bacterial suspension of 10^8 CFU/ml. Diluted cultures (200 µl) were inoculated into the Petri plates containing 20 ml of Mueller-Hinton agar media by the spread plate technique. A 20 µl of the Cefadroxil and Cephalexin (30 µg/ml) were loaded onto the filter paper discs (Whatman No. 1–5 mm diameter) and were allowed to dry completely. Filter paper discs were placed on the inoculated agar surface. Plates were incubated at 37°C for 24 h. The antibiotic sensitivity was determined by measuring the inhibition zone.

The diameter of the zone of inhibition is measured in millimeters either manually or using an automated system and then is compared with standardized CLSI-interpretive criteria to designate the isolate as sensitive or resistant to the drug.

Minimum inhibitory concentration

100 ml nutrient broth was prepared and sterilized by autoclaving at 121°C/15Ibs pressure for 20 min. Cool the sterilized broth at room temperature and dispense exactly 9.9 ml quantities in 7 sterile tubes. A stock solution (1000 µg/ml) of cephalexin and cefadroxil in sterile water was prepared. Carried out dilution of the stock to obtain a range of concentrations (3.2–25.6 µg/ml). Added 0.1 ml of each concentration to the first five tubes of the broth and label them as 3.2, 6.4, 12.8, and 25.6 µg/ml. Inoculated 0.1 ml of washed cells of *Staphylococcus aureus* culture having an OD of 0.01 at 530 nm into the first six tubes. Label the sixth tube as a positive control. Left the last tube as uninoculated and label it as medium control. Incubated all the test tubes at 37°C for 24 h.

A similar procedure was followed for the remaining two Gram-positive microorganisms such as *Bacillus subtilis*, *Bacillus cereus,* and three Gram-negative microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens.* Turbidity in the test tubes must be checked carefully in full light. For easy interpretation, hold the test tube between the PC and MC tubes and compared the turbidity. Check for growth in the form of turbidity in the tubes and report the results**.** The concentration of the drug in the first tube shows that no growth is the MIC value of the drug for that culture.

RESULTS AND DISCUSSION

Antibiotic susceptibility testing of cefadroxil, cephalexin was analyzed against the different gram-positive {*Bacillus cereus* (NCIM 2914), *Bacillus subtilis* (NCIM 2710), and *Staphylococcus aureus* (NCIM 2794)} and Gram-negative microorganisms {*E. coli* (NCIM 2256), *P. aeruginosa* (NCIM 2037), *Serratia marcescens* (NCIM 2078)} by disk diffusion method. The zone of inhibition of cefadroxil against different microorganisms was measured in millimeters is tabulated below in Table 1, and graphical representation in Fig. 1.

Similarly, antibiotic susceptibility testing of cephalexin was analyzed against the Gram-positive and Gram-negative microorganisms by the disk diffusion method. The zone of inhibition of cephalexin against different microorganisms was measured in millimeters is tabulated below in Table 2, and graphical representation in Fig. 2.

Quantitative analysis of antibiotic susceptibility testing was carried out by the determination of minimum inhibitory concentration of cephalexin and cefadroxil against selected Gram-positive and Gram-

Table 1: Diameter of zone of inhibition of cefadroxil against different microorganisms

S. No.	Name of microorganisms	Diameter of zone of Inhibition [mm]
1.	*Escherichia coli*	16±0.7 mm
2.	*Bacillus subtilis*	30±0.5 mm
3.	*Bacillus cereus*	10±0.3 mm
4.	*Staphylococcus aureus*	15±0.6 mm
5.	*Pseudomonas aeruginosa*	18±0.5 mm
6.	*Serratia marcescens*	06±0.4 mm

Table 2: Zone of inhibition of cephalexin against different microorganisms

S. No.	Name of microorganism	Diameter of zone of inhibition (mm)
1.	*Escherichia coli*	14±0.5 mm
2.	*Bacillus subtilis*	15±0.9 mm
3.	*Bacillus cereus*	18±0.6 mm
4.	*Staphylococcus aureus*	16±0.7 mm
5.	*Pseudomonas aeruginosa*	14±0.6 mm
6.	*Serratia marcescens*	10±0.3 mm

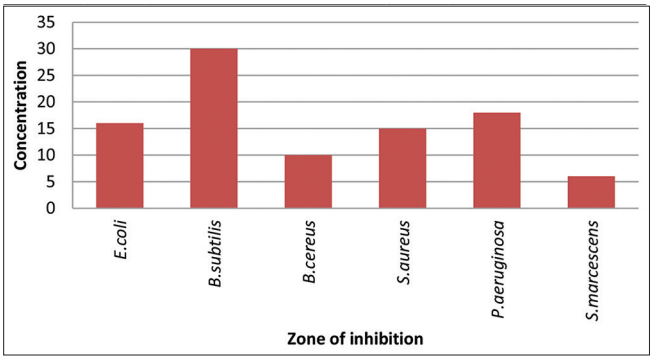

Fig. 1: Diameter of zone of inhibition of cefadroxil

negative microorganisms. The MIC of cefadroxil was analyzed against different microorganisms with different concentration ranges of antibiotic and reported in the given below Tables 3 and 4, Figs. 3 and 4.

According to the analysis of antibiotic susceptibility testing of cefadroxil and cephalexin against selected Gram-positive and Gram-negative microorganisms reveals the MIC of cefadroxil against *Serratia marcescens* and *Bacillus cereus* was higher value such as 25.6 µg/ml, similarly the lowest MIC value against the *Pseudomonas aeruginosa* and *Bacillus subtilis* was 6.4 µg/ml. MIC of cephalexin against *Serratia marcescens* and *Pseudomonas aeruginosa* were higher value such as 25.6 µg/ml, similarly, the lowest MIC value against the *Staphylococcus aureus and Bacillus cereus* was 6.4 µg/ml. The remaining microorganism such as *E. coli* and *Bacillus subtilis* produced the MIC value of 12.8 µg/ml.

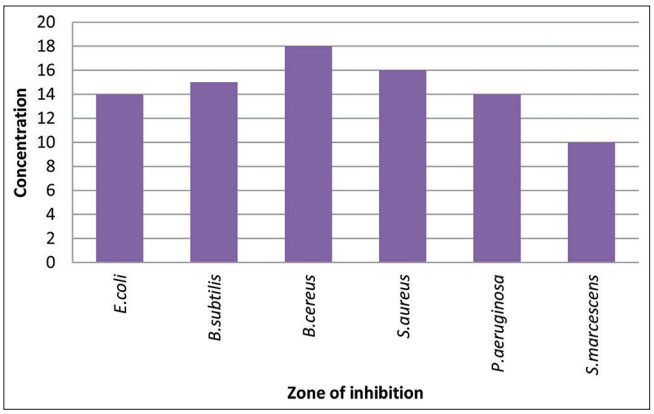

Fig. 2: Diameter of zone of inhibition of cephalexin

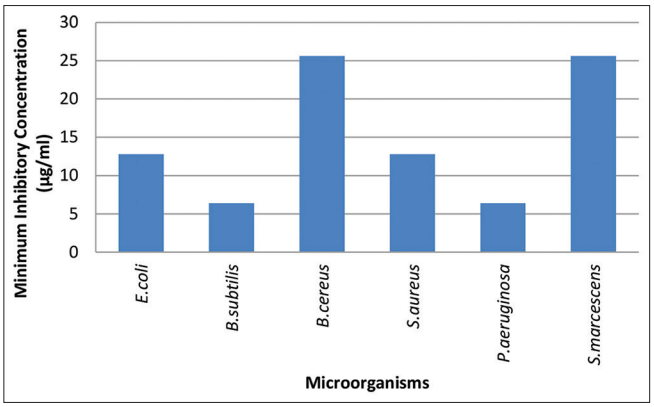

Fig. 3: Minimum inhibitory concentration of cefadroxil

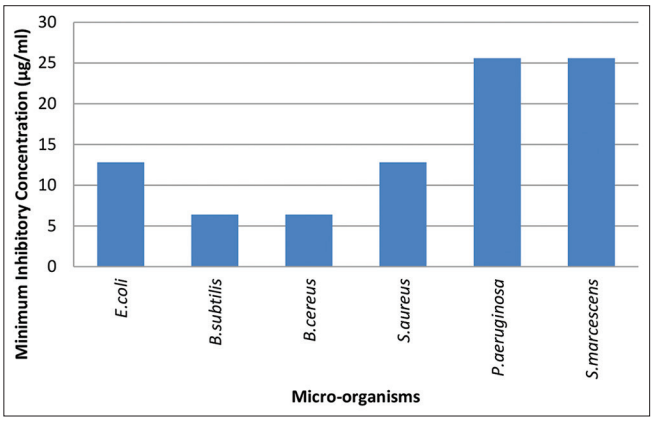

Fig. 4: Minimum inhibitory concentration of cephalexin

The higher zone of inhibition produced by the cefadroxil against *Bacillus subtilis* was 30 mm and the lowest zone of inhibition against *Serratia marcescens* was 6 mm and *E. coli, Pseudomonas aeruginosa* produced the same zone of inhibition was 16 mm and 18 mm, respectively. The moderate value for *Bacillus cereus* and *Staphylococcus aureus* was 10 mm and 15 mm, respectively.

The higher zone of inhibition produced by the cephalexin against *Bacillus cereus* was 18 mm and the lowest zone of inhibition against *Serratia marcescens* was 10 mm. The remaining microorganisms such as *E. coli*, Pseudomonas aeruginosa, *Bacillus subtilis,* and *Staphylococcus aureus* produced the zone of inhibition 14, 14, 15, and 16 mm, respectively.

According to the Clinical and Laboratory Standards Institute (CLSI), the Performance Standards for Antimicrobial Susceptibility Testing of Cefadroxil and Cephalexin (30 µg/ml) by Agar disk diffusion method identified the zone of inhibition produced less than 18 mm is considered as sensitive, 15–17 mm is considered as intermediate, and 14 mm is considered as resistant.

Minimum inhibitory concentration determination of cefadroxil and cephalexin (30 µg/ml) produced more than 8 µg/ml is considered as sensitive, 16 µg/ml is considered as intermediate, and more than 32 µg/ml is considered as resistant according to the CLSI report. In this analysis, Cefadroxil was resistant against *Bacillus cereus* (25.6 µg/ml, 10 mm) and *Serratia marcescens* (25.6, 6 mm), sensitive against *Pseudomonas aeruginosa* (6.4 µg/ml, 18 mm), *Bacillus subtilis* (6.4 µg/ml, 30 mm), intermediate against *E. coli* (12.8 µg/ml, 16 mm), and *Staphylococcus aureus* (12.8 µg/ml, 15 mm). According to the results obtained from this analysis, cephalexin was resistant against *Serratia marcescens* (25.6 µg/ml, 10 mm), intermediate against *E. coli* (12.8 µg/ml, 14 mm), *Pseudomonas aeruginosa* (25.6 µg/ml, 14 mm), *Bacillus subtilis* (12.8 µg/ml, 15 mm), *Staphylococcus aureus* (6.4 µg/ml, 16 mm), and sensitive against *Bacillus cereus* (6.4 µg/ml, 18 mm).

The present study was conducted to know the microbial resistance of cephalexin and cefadroxil against both selected Gram-positive and Gram-negative organisms. The agar disk diffusion method and minimum inhibitory concentration analysis were used in this study. Cephalexin and cefadroxil are the first-line antibiotics under cephalosporin derivatives. Antibiotic resistance is the most common and gives more important for these studies. Depending on the CLSI report cephalexin and cefadroxil resistant, intermediate, and sensitive microorganisms

Table 3: Minimum inhibitory concentration of cefadroxil against different microorganisms

S. No.	Name of microorganism	Minimum Inhibitory Concentration (µg/ml)
1.	*Escherichia coli*	12.8±0.3
2.	*Bacillus subtilis*	06.4±0.4
3.	*Bacillus cereus*	25.6±0.9
4.	*Staphylococcus aureus*	12.8±0.4
5.	*Pseudomonas aeruginosa*	06.4±0.7
6.	*Serratia marcescens*	25.6±0.6

Table 4: Minimum inhibitory concentration of cephalexin against different microorganisms

S. No.	Name of microorganism	Minimum inhibitory concentration (µg/ml)
1.	*Escherichia coli*	12.8±0.6
2.	*Bacillus subtilis*	6.4±0.5
3.	*Bacillus cereus*	6.4±0.4
4.	*Staphylococcus aureus*	12.8±0.6
5	*Pseudomonas aeruginosa*	25.6±0.5
6.	*Serratia marcescens*	25.6±0.7

were identified. Hence, the study was useful and effective for the determination of antibiotic resistance of cephalexin and cefadroxil for the future treatment in more efficacies with less side effects.

CONCLUSION

In the population under investigation bacterial strains are developing resistance against cephalexin and cefadroxil. World Antibiotic Awareness Week should be held from November 13 to 19 every year, during this period create the awareness about the usage antibiotics. It is suggested that physician who prescribe antibiotics with caution and rationality unless no other choice is available. A clinical pharmacist can come in handy in such scenario otherwise it seems that treatment of some infectious diseases may become impossible in the future. From these studies, it is concluded that everyone has responsible for the safe usage of antibiotics for the treatment of diseases.

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AUTHOR'S CONTRIBUTIONS

The author have contributed to manuscript article preparation and editing.

CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest.

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