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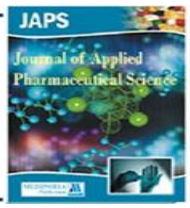

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Comparative in vitro antibacterial analysis of different brands of cefixime against clinical isolates of *Staphylococcus aureus* and *Escherichia coli*

Hafiz Muhammad Arshad, Omair Anwar Mohiuddin and Muhammad Bilal Azmi

Hafiz Muhammad Arshad,
Omair Anwar Mohiuddin
Dow College of Pharmacy,
Dow University of Health Sciences,
Karachi, Pakistan 74200.

Muhammad Bilal Azmi
Quality Enhancement Cell,
Dow University of Health Sciences,
Karachi, Pakistan 74200.

For Correspondence
Hafiz Muhammad Arshad,
Dow College of Pharmacy,
Dow University of Health Sciences,
Karachi, Pakistan 74200.

ABSTRACT

The objective of this study was to compare the antibacterial activity of standard and different brands of *Cefixime*, against standard samples and clinical isolates of *E. coli* and *S. aureus* collected from different hospitals. Standard samples and isolates of *E. coli* and *S. aureus* were separately cultured in *Mueller Hinton* broth. After the bacterial incubation, 5 ml solution each of standard *Cefixime* and its different brands were added to the test tubes containing bacterial culture. *Cefixime* samples were added in the concentration of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128µg/ml to separate test tubes. The cultures were again incubated and then the culture samples were analyzed by UV-spectrophotometer, and minimum inhibitory concentrations of all samples were determined. The analysis and interpretation of results were done by single factor ANOVA. An MIC of 0.75µg/ml and 8µg/ml of standard *Cefixime* was found for standard *E. coli* and *S. aureous* respectively. Standard *Cefixime* and its six selected brands exhibited a higher MIC range for clinical isolates of *S. aureus* than the clinical isolates of *E. coli*. Higher MIC values of standard *Cefixime* and its brands were observed for clinical isolates of *E. coli* and *S. aureus*. Higher MIC values for the clinical isolates of E. *coli* and *S. aureus* indicated that both the organisms have developed resistance to *Cefixime* in comparison to standard microorganisms acquired from ATCC.

Keywords: Antibacterial, Clinical Isolates, Cefixime, E. coli, S. aureus, Mueller Hinton broth.

INTRODUCTION

Antibiotics are the group of medicines that are produced by microorganisms or formulated synthetically; they have dynamic property of inhibiting bacterial growth or completely suppressing the toxic effects of microorganisms. Accessibility of commercially available broad spectrum antibiotics causing multi drug resistance remains a key global health issue (Khan *et al.*, 2011). Antibiotics have been used for decades but especially in Asia region due to frequent administration of antibiotics in humans, common environmentally existing bacteria are quickly becoming resistant to treatment with these drugs (Hawser *et al*., 2007). Broad spectrum antibiotics have a comprehensive range of coverage that contributes to the effectiveness of these medicines against both gram positive and gram negative bacteria. *S. aureus* (Gram positive bacteria) has been a cause of various infections of skin, soft tissues, bones and joints, abscesses and normal heart valves, which is globally becoming a leading cause of death and disability (Hannan *et al*., 2008), while among gram negative species, *E. coli* is considered lethal and virulent organism as it causes several infections which are intestinal or extra-intestinal and is becoming a big contributor in morbidities and mortalities worldwide (Hammerum and Heuer, 2009).

Hence in this study the drug used for investigation is *Cefixime*, which is characterized as a broad spectrum antibiotic (Anacona and Estacio, 2006). *Cefixime* is a β-lactamase stable third generation cephalosporin, which is a semi synthetic compound and was the first orally active and effective antibiotic with longest half life (Rafal'skii *et al.,* 2011, Wilson and Gisvold's, 1998 and Wu, 1993). *Cefixime* has very significant biological properties, as it exhibits potent antibacterial activity against a varied range of different strains of bacteria. Previous researches have reported *Cefixime* as a nontoxic and effective oral therapeutic especially in case of multidrug-resistance (Memon *et al.,* 1997). The chemical structure of *Cefixime* (Figure 1.) having molecular formula $C_{16}H_{15}N_5O_7S_2$, with molecular weight ***453.4***, consists of the *Cephem* nucleus, in which a ring of β-lactam is fused to a 6-membered di-hydro-thiazine ring (Gelone and O'Donnell, 2005). The basic ring structure incorporates two major modifications; *Cephem* nucleus conatins Vinyl group at 3rd position which is responsible for appropriate absorption in the intestine, the permeation of the drug occurs by a carrier mediated transport mechanism (Naqvi *et al.,* 2011). The antibacterial activity of *Cefixime* is due to aminothiazole ring and the R-OXy amino group present on the side chain at the 7-position in its chemical structure (Rafal'skii *et al.,* 2011). Evidences also report that *Cefixime* produces the antibacterial activity by inhibiting peptidoglycan synthesis in the bacterial cell wall (Petri, 2006).

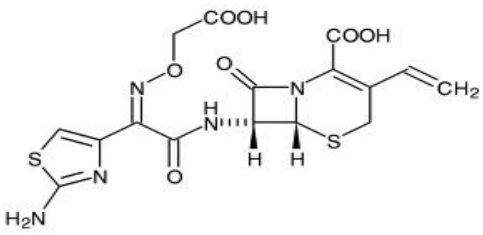

Fig.1: Chemical structure of *Cefixime*.

Conventionally *Cefixime* has been used in the treatment of respiratory tract infections (Kunel'skaya *et al.,* 2008 and Adelstein *et al.,* 1993) urinary tract infections (Rafal'skii *et al.,* 2011 and Dagan *et al.,* 1992) gonorrhea (Ison and Alexander, 2011) and typhoid fever (Barry *et al.,* 1994). *Cefixime* has significant activity against Group A and B hemolytic *Streptococci* and *Streptococcus pneumonia* (Barry *et al.,* 1994). *Neisseria gonorrheae* and *Haemophilus influenzae* are also *Cefixime* sensitive even in the presence of beta-lactamase enzyme (Mortensen and Himes, 1990 and Nash *et al.,* 1991). The Minimum Inhibitory Concentration (MICs) of *Cefixime* is considerably higher against ampicillin-resistant *Haemophilus influenzae* strains, *Enterobacteriaceae, Escherichia coli*, *Klebsiella, proteus* and *providencia spp*. are usually *Cefixime*-sensitive. Most strains of *Serratia macrcescens* and *Citrobacter diversus* are usually sensitive. *Pasteurella multocida* is *Cefixime* sensitive (Mesnard and Donnio PY, 1991) as is *helicobacter pylori* (Ikeda *et al.,* 1990). *Acenobacter spp., Ps. Aerogenosa*, other *Pseudomonas spp*. and *Stenotrophomonas maltophilia* are resistant (Stone *et al.,* 1989). The MIC (Minimum Inhibitory Concentration) of *Cefixime* against *E. coli* has been reported to be 0.5-1μg/ml and the MIC against *S. aureus* has been reported as 16μg/ml (Powell and Williams, 1987 and Bowie *et al.,* 1986). Hence this study was undertaken to determine the comparative antibacterial activity of *Cefixime* standard and different brands commercially available in the market, against clinical isolates collected from different hospitals at Karachi and standard ATCC *(American Type Culture Collection)* bacterial cultures. In future a country wide analysis of different clinical isolates of bacteria with different brands of various broad spectrum antibiotics will help to assess the performance and therapeutic standards.

MATERIALS AND METHODS

Cefixime

Total six (06) *Cefixime* brands were purchased from local pharmacy and were designated with different numbers as *CEF-1, 2, 3, 4, 5 & 6* respectively, the information related to the pharmaceutical preparations with batch identities were also recorded. It was assured that all purchased brands have an expiry date not earlier than one (01) year. *Cefixime* micronized USP reference powder was acquired from *Hilton Pharmaceutical Private Ltd, Karachi*.

Preparation of the McFarland standard

To prepare McFarland standard solution 0.5 ml of 1.17% w/v solution of barium chloride and 9.5 ml of 1%v/v solution of H2 SO4 were prepared and mixed with constant stirring. The mixture was distributed into screw cap tubes of same sizes. The tubes were sealed tightly to prevent any loss by evaporation. They were then stored at room temperature and protected from light.

Bacterial standards and isolates

Standard *E. coli* culture (sample number ATCC-25922) was acquired from ATCC. Standard *S. aureus* culture (sample number ATCC-25923) was also acquired from ATCC. Five samples each of *E .coli* and *S. aureus* were isolated from different hospitals in Karachi, Pakistan. The clinical isolates were then identified by Gram staining and microscopic examination by the expert from Microbiology Department. The five *E. coli* isolates were designated numbers as *E. coli* I, II, III, IV and V, similarly the *S. Aureus* isolates were numbered as SA I, II, III, IV and V.

Mueller Hinton broth

For the inoculation of bacteria Mueller Hinton broth was prepared according to the procedure specification provided by manufacturer (21g in 1 liter distilled water). 9.5 ml each of prepared culture broth was then transferred to several test tubes. The test tubes containing the broth were then autoclaved (Jeong *et al.,* 2009).

Inoculum preparation

The standards and 5 isolates each of *E. coli* and *S. aureus* were then added to the test tubes containing Mueller Hinton broth.

The inoculated culture broth was then incubated at 37°C for 8 hours until the turbidity of the broth exceeded that of a 0.5 McFarland standard. These suspension tubes were compared using UV-spectrophotometer to the McFarland standard, if this standard was found to be more turbid than the culture suspension, then the culture was further diluted with broth and incubated for a few more hours.

Preparation of antibiotic solution

First a stock solution of the antibiotic was prepared by dissolving 10 mg of the drug in 100 ml distilled water; this gave us a stock solution of 0.1mg/ml concentration. After preparing the antibiotic stock solution, dilutions were made from it. A two fold dilution strategy was employed such that *Cefixime* was prepared in the concentration of 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128µg/ml. Equal volume of each concentration were then added to the inoculums and then incubated at 37°C for 18 hours.

The same procedure was used for the preparation of standard *Cefixime* solution and the 5 brands of *Cefixime* obtained from the market. After the preparation of a wide range of dilutions of standard *Cefixime* and its different brands, the dilutions were added to culture broth, which had already been inoculated and incubated with bacteria. 5ml of each dilution of *Cefixime* was added to each test tube containing culture broth. The microbial cell culture was again incubated for 18 hours at 37°C.

Spectrophotometric analysis

Spectrophotometric analysis was carried out using *Parken ELMER, λ-20*. After the incubation of microbial culture along with different dilutions of *Cefixime*, the test tubes were examined by UV-spectrophotometer at 546 nm for the determination of concentration of microorganisms present in the broth. The concentration was determined by comparing the absorbance of the sample to standard McFarland solution. Minimum inhibitory concentration was also determined (table 1) (Pfaller *et al*., 2001).

Table. 1: Comparison of Minimum Inhibitory concentrations of standard *Cefixime* and its brands against standard and clinical isolates of *E. coli* .

Microorganisms	STD. - CEF µg/ml	CEF - 1µg/ml	CEF - 2µg/ml	CEF - 3µg/ml	CEF - 4µg/ml	CEF - 5µg/ml	CEF - 6µg/ml
E. coli-Std.	0.75	1	2	4	1	1	2
E. coli-I	8	16	8	32	32	32	16
E. coli –II	8	8	8	32	8	8	8
E. coli –III	64	32	32	32	32	32	32
E. coli –IV	64	32	32	32	32	32	32
E. coli –V	16	16	32	32	16	16	32

Statistical analysis

The data were analyzed by one way ANOVA *(by Graphpad software, Quick calcs online calculator for scientists)*

RESULTS

In the present study, susceptibility test on standard ATCC samples and clinical isolates of *E. coli* and *S. aureus* was conducted using standard *Cefixime* and its different brands. The results are tabulated in Table No. 1 and 3 respectively. The study showed that the MIC of standard *Cefixime* for standard *E. coli* sample is 0.75µg/ml and for standard *S. aureous* sample is 8µg/ml. Standard *Cefixime* exhibited MIC ranging between 8 – 64 µg/ml for 5 clinical isolates of *E. coli* and 8 –128 µg/ml for the 5 clinical isolates of *S. aureus*. The MIC of 6 selected brands for the 5 clinical isolates of *E. coli* was found to be in the range of 8 – 32 µg/ml. The same 6 brands exhibited MIC in the range of 8 – 256 µg/ml for 5 clinical isolates of *S. aureus*. Analysis of variance was performed on the data tabulated in Table 1 and 3 which are mentioned below in Table 2 and 4 respectively. The results obtained from ANOVA indicate no significant variance between the MIC values of standard *Cefixime* and its brands for standard and isolates of *E. coli* and *S.aureus*.

DISCUSSION

After literature review the concept of our present study was novel as the determination of the use of an antibiotic for treatment of bacterial infection relies upon the information collected by susceptibility test conducted on infecting microorganism (Gennaro, 1985). Analysis of antibacterial activity has traditionally been conducted *In Vitro* quite frequently, because results of these susceptibility tests can be used to determine how a drug would act inside the body (Hannan *et al.*, 2008). Resistance of virulent microorganisms to antibiotics has been a major concern for a long period (Gums, 2002) therefore the purpose of the current study was to evaluate the antibacterial activity of different commercially available brands of *Cefixime* in the market against isolates of selected organisms i.e., *E. coli* and *S. aureus*. The primary objective was to determine that different brands of drug *Cefixime* (available in the market) possess comparable antibacterial activity against the above selected microorganism. For the determination of antimicrobial activity of different brands of *Cefixime*, broth micro-dilution method was employed (Jeong *et al*., 2009). The experiments were carried out on standardized cultures (acquired from ATCC) and isolates of *E.coli* and *S.aureus* collected from different hospitals. The results were observed visually and spectrophotometrically to calculate the concentration of microorganism after respective treatment (Pfaller *et al.*, 2001)**.** Previous studies have reported the MIC of standard *Cefixime* in the range of 0.5 to 1µg/ml for standard *E.coli* cultures (ATTC # 25922) (Neu, 1987), and in the current study the MIC was observed in the same range as reported. The standard *Cefixime* against clinical isolates of *E. coli* has shown MIC ranging between 8 – 64 µg/ml. (Table 1) The MICs of different brands of *Cefixime* against standard *E. coli o*f ATCC # 25922 showed MICs ranging between 1 – 4 µg/ml with an average of 1.8 µg/ml. Out of the six brands, three brands (50%) showed MICs of 1µg/ml, two brand (33%) showed MICs of 2µg/ml, and only one brand showed 4µg/ml MIC. The same six brands were evaluated against clinical isolates of *E. coli* and showed MICs range of 8 – 32 µg/ml. (Table. 1) From the results it is clearly evident that a higher concentration of *Cefixime* is required to kill isolates of *E. coli* as compared to standard *E. coli*. The increase in the MICs of *Cefixime* against clinical isolates

of *E. coli* indicates an emergence of resistance of *E. coli* to *Cefixime* which is also in conformance with the study conducted by Sakata *et al.,* 1992. The reported MIC for standard *Cefixime* is in the range of 4 – 64 µg/ml against S. *aureus* ATTC # 25922 (Guay *et al.,* 1986)**,** which was observed in the same range from the study conducted. The standard *Cefixime* against clinical isolates of *S. aureus* showed MIC ranging between 8 –128 µg/ml. (Table. 3) The MIC of different brands of *Cefixime* for standard *S. aureus* were found to be in the range of 8 – 16 µg/ml with an average of 15 µg/ml, of six brands, five brands (83%) showed MICs of 16µg/ml, and only one brand showed 8µg/ml MIC. The six different brands were evaluated against clinical isolates of S. *aureus* and exhibited MIC in the range of 8 – 256 µg/ml (Table 3). Sample SA-V exhibits highest MIC values of *Cefixime* and therefore can be termed as the isolate with the highest resistance against *Cefixime.* Single factor ANOVA was applied on the determined MIC values of *Cefixime* (standard and brands) against both the microorganisms (Table. 2 and 4). This analysis showed insignificant change of inhibitory concentration range between standard and brands of *Cefixime*. The results obtained after application of ANOVA confirm that the brands of *Cefixime* purchased from the local market produce very similar effect in comparison to the standard drug. The increase in the MIC of all *Cefixime* samples against different clinical isolates of *E. coli* and *S. aureus* indicates the presence of an increasing resistance of the bacteria to the antibiotic. In the past half a century there has been a constant increase in the use of antibiotics for the treatment of bacterial infections. The continuous use and often abuse of the drug have led to an increased resistance to *Cefixime*. The present results are in conformation with work published by Roche, 1989.

Table. 2: Anova single factor of MICs for *E. coli* (from table 1).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3678.5	6	613.08	0.7857	0.587	2.3718
Within Groups	27311	35	780.33			
Total	30990	41				

Table 3: Comparison of Minimum Inhibitory concentrations of standard *Cefixime* and its brands against standard and clinical isolates of *S. aureus.*

Microo-rganisms	Std. - CEF µg/ml	CEF - 1µg/ml	CEF - 2µg/ml	CEF - 3µg/ml	CEF - 4µg/ml	CEF - 5µg/ml	CEF - 6µg/ml
SA-Std.	8	16	16	16	16	8	16
SA-I	16	16	16	16	16	16	32
SA-II	32	64	64	64	64	64	64
SA-III	8	16	16	8	8	8	16
SA-IV	32	64	32	32	32	32	128
SA-V	128	128	128	128	256	128	128

Table. 4: Anova single factor of MICs for *S. aureus* (from table 3).

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2611.81	6	435.302	0.2004	0.9745	2.3718
Within Groups	76053.33	35	2172.95			
Total	78665.14	41				

CONCLUSION

From the results it is concluded with no reservation that the 6 brands of *Cefixime* (purchased from the market) possess similar antibacterial activity as compared to standard *Cefixime.* While in context with the same results, higher MIC values of *Cefixime* (standard and brands) have been observed for the clinical isolates of *E. coli* and *S. aureus* in comparison to the MIC values for standard microorganisms. This indicates that the clinical isolates of both *E. coli* and *S. aureus* have developed resistance to antibacterial effect of *Cefixime*

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