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Assessment of Different Brands of Cefadroxil for Their In Vitro Antibacterial Activity against Staphylococcus aureus and Escherichia coli

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ABSTRACT: *The surprising progression of pharmaceutical industry in Pakistan has elevated certain queries for drug regulatory authorities considering their efficacy and quality. The present study was aimed on assessing the in-vitro antimicrobial activity of six brands of cefadroxil 500 mg tablets/capsules against clinical isolates as well as standard cultures of micro-organism i.e. Staphylococcus aureus and Escherichia coli. The resistance pattern of clinical cultures against cefadroxil was also determined. Broth dilution method was adopted for determining the anti bacterial activity as well as minimum inhibitory concentration (MIC) of cefadroxil. Different concentrations of cefadroxil i.e. 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 µg/ml was prepared. Dilutions of cefadroxil brands were also prepared in the same manner. Microbial growth was observed visually and using spectrophotometer at 546 nm. ANOVA was applied to compare MICs of cefadroxil (reference standard) and its five brands against clinical isolates. Student t-test was also performed to compare mean MIC of cefadroxil (reference standard) against clinical isolates and standard cultures. Level of significance was set at* 0.05. *Clinical isolates of Staphylococcus aureus and Escherichia coli showed pronounced resistance of 71.43% and 85.71% to cefadroxil, respectively. Minimum inhibitory concentration was 2-128µg/ml against S. aureus and 8-256µg/ml against E. coli. The MIC of most of the brands was significantly higher than cefadroxil (reference standard) against Staph. aureus (F = 6.165, p=0.001). MICs of cefadroxil against E. coli and Staph. aureus were significantly different from its MIC against standard cultures of these organisms (p<0.0001). The organisms have developed resistance to cefadroxil due to excessive or irrational use and substandard drugs with reduced antibiotic quantity. It is strongly recommended to develop antibiotic prescribing policies and antibiotic surveillance program to combat the situation.*

KEY WORDS: *Cefadroxil, clinical isolates, broth dilution method, minimum inhibitory concentration.*

I. INTRODUCTION

Cephalosporins are beta-lactum antibiotics, which inhibit cell wall synthesis and frequently prescribed for bacterial infections. Cephalosporins have β-lactum ring fused to dihydrothiazine ring as in penicillin. These are classified into different groups depending upon their molecular structure, activity, stability to beta-lactamase enzyme but the most commonly used classification divides them in generations according to their anti-bacterial spectrum [1]. Cephalosporin first derived from *Cephalosporium acremonium*. The structural modification results in development of a large number of semi-synthetic cephalosporin. These cephalosporins are 7^{th}amino cephalosporanic acid. Alteration on 7^{th} position of lactum ring and 3^{rd} position of dihydrothiazine ring affect antibacterial spectrum and pharmacokinetics, respectively. These are bactericidal, but resistance to these agents has acquired due to many factors like alteration in target protein, lack of penetration to bacterial cell and production of β-lactamase enzyme [2].

First generation cephalosporins include cephradine, cephalexin, cefadroxil, cefazolin and cephalothin. Cefadroxil is para-hydroxy derivative of cephalexin and prescribed for the treatment of mild to moderate infections of soft tissues like skin, upper respiratory tract and urinary tract in the daily dose of 500 to 1000 mg/day [3]. Its spectrum includes many Gram positive and Gram negative organisms, including *Staphylococcus aureus, Escherichia coli, Proteus spp, Bacillus spp*, more potent to *Klebsiela spp* and less sensitive to β-lactamase produced by *Staphylococcus aureus* and *Bacillus subtilis* as compared to penicillins [4]. It has advantage over other members of this group due to its pronounced oral absorption and 85% bioavailability.

Molecular formula is $C_{16}H_{17}N_3O_5S.H_2O$ and molecular weight is 381.4. It remains as free drug in plasma (<20% protein binding) and 90% excreted unchanged in urine. Structure of cefadroxil is given in figure-1.

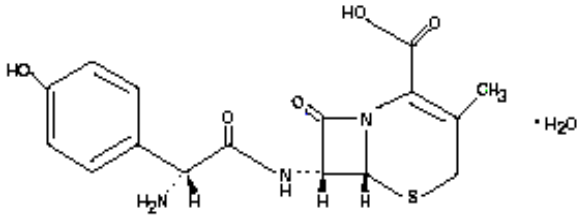

Figure -1 Structure of cefadroxil

Incidence of bacterial resistance creates a massive problem in treating bacterial infections. In developing countries, this incidence is more pronounced. Use of substandard or counterfeit drugs [5], over prescribing of broad spectrum antibiotics [6], low standard of microbiological assay of antibiotics [7], self medication of antibiotics [8-10] resulted in the high prevalence of resistance in clinical isolates to first line, broad spectrum and less expensive antibacterial agents. These reasons contribute to the failure of antibacterial treatment. This does not only increase MIC (minimum inhibitory concentration) but also reduce antibacterial activity of such agents due to development of pronounced bacterial resistance. The problem is getting worst because of lack of antibiotic surveillance program especially in developing countries [11]. It is important to analyze susceptibility of bacterial isolates to antibiotics in order to rationalize the use of these antibiotics. Literature has many published reports which demonstrated the emergence of multidrug resistance among clinical isolates from Pakistan [12-17]. In this scenario, the present study was designed to comparatively evaluate the antibacterial activity of cefadroxil and its different brands marketed in Karachi. Another aim of the study was to establish the resistance pattern of clinical isolates of *Staphylococcus aureus* and *Escherichia coli* to cefadroxil and also determine the MIC of cefadroxil against these clinical isolates

II. EXPERIMENTAL

Materials

Cefadroxil (reference standard), Nutrient agar (Oxoid), Muller Hinton broth (MHB) and agar (Oxoid), barium chloride (Merck), sulfuric acid (Merck). Five brands of cefadroxil were purchased from local market of Karachi and coded as CFD-1, CFD-2, CFD-3, CFD-4 and CFD-5.

Collection of isolates

Seventy clinical isolates including, *Staphylococcus aureus* (n=35) and *Escherichia coli* (n=35) were collected from different pathological laboratories of Karachi. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were also analyzed as a quality control test. These isolates where inoculated on Nutrient agar plates at 37°C for 24 hours and preserved at 2-4°C [18].

Preparation of antibiotic solutions

Stock solution of cefadroxil (1000µg/ml) was prepared in phosphate buffer (pH=6.0) and diluted in series with double distilled autoclaved water to get the required drug concentrations. Solutions of drug from different brands (n=5) were also prepared in the tightly closed containers and stored at 20°C [19].

Preparation of McFarland (0.5) Index

McFarland (0.5) index was prepared by adding 0.5 ml of 1.175%w/v barium chloride solution to 99.5ml of 1.0% sulphuric acid solution and mixed carefully. This index was matching to approximate bacterial cell density of 1.5×10^8 CFU/ml. The absorbance of this index was 0.136 as noted by spectrophotometer (Spekol 2000 series, Analytikjena). The solution was stored in screw capped test tubes at room temperature in dark and check absorbance after storage.

Preparation of Muller Hinton broth

Mueller Hinton broth (MHB) was prepared as the method mentioned by manufacturer (OXOID, USA) i.e. 21grams in 1 liter purified water. MHB powder was dissolved in distilled water on water bath and then autoclaved.

Preparation of inoculum and standardization

The turbidity of inoculum corresponding bacterial cell density in MHB is an important factor which may affect the result interpretation of sensitivity test. McFarland Index (0.5) was used to standardize inoculated MHB [20]. Adjustment in the turbidity of inoculated broth was done by noting absorbance using spectrophotometer.

Broth dilution susceptibility testing

Broth dilution method was used to observe sensitivity pattern of clinical isolates and also pure cultures. Eleven screw capped sterile tubes were used. 2 ml inoculated MHB was introduced in tube 1 till 10 and 2 ml cefadroxil solution (512 µg/ml) in tube 1. Then 2 ml from tube-1 was transferred to tube 2, 2 ml from tube 2 to tube 3 and so on to make serial dilutions. 2 ml from tube-10 was discarded. Positive and negative control tubes were also prepared using 2ml inoculated and noninoculated broth in two separate tubes and 2 ml distilled and autoclaved water in both tubes. Tubes were incubated aerobically at 37°C for 24 hours [21]. Next day visible growth was noted with reference to McFarland index and turbidity was also observed using spectrophotometer. The minimum concentration of cefadroxil that inhibit the bacterial growth was MIC [22]. Development of resistance was defined as fourfold increment in MIC of antibiotics [23, 24].

Statistical analysis

Data was double entered in Statistical Software for Social Sciences (SPSS 20.0). One way ANOVA was adopted to compare MIC_{90} of cefadroxil (reference standard) and its five brands against *Escherichia coli* and *Staphylococcus aureus*. MIC_{90} of cefadroxil standard against seventy clinical isolates was matched up with its MIC_{90} against standard culture of *E. coli* (ATCC 25922) and *Staph. aureus* (ATCC 25923) by carrying out t-test using 0.05 level of significance.

III. RESULTS

During the present study, antibacterial activity of cefadroxil (reference standard) and its different brands (purchased from different areas of Karachi) against *Escherichia coli* and *Staphylococcus aureus* (two common pathogens of soft tissues infections) were assessed. MIC_{90} was also observed against clinical isolates of the organisms. Clinical isolates of *Staphylococcus aureus* (n = 35) and *Escherichia coli* (n = 35) showed pronounced resistance of 71.43% and 85.71% to cefadroxil, respectively (table 1). Minimum inhibitory concentration was 2-128 µg/ml against *S. aureus* and 8-256 µg/ml against *E. coli*. All tested clinical isolates of *E coli* (n=5) were resistant to three brands of cefadroxil, whereas only one isolate of *Staph. aureus* (n=5) was sensitive to four different brands assessed (table 2 and 3). The MIC of most of the brands was significantly higher than cefadroxil (reference standard) against *Staph. aureus* ($F = 6.165$, $p = 0.001$) (table 4). The t-test revealed that the MIC of cefadroxil (reference standard) against clinical isolates were also found significantly different from MIC against standard cultures ($p<0.0001$).

IV. DISCUSSION

Bacterial resistance among clinical isolates has been developed due to excessive and irrational use of antibiotics. Treatment failure of antibiotic therapy resulted from emergence of such resistance. In developing countries, mostly broad-spectrum antibiotics are prescribed without analyzing reports of antibiotic susceptibility test which is again a reason for resistance expansion. Once clinical isolates develop resistance, it always amplified despite of restricting use of such antibiotic [25]. It is obligatory to develop antibiotic surveillance program to monitor emergence of such resistance regularly and continuously because percentage of resistant organisms may fluctuate over time [26]. During the present study, antibacterial activity of cefadroxil (reference standard) and its different brands purchased from different areas of Karachi against *Escherichia coli* and *Staphylococcus aureus* (two common pathogens of soft tissues infections) were assessed. $MIC_{90}$was also observed against clinical isolates of the two organisms to examine the activity of cefadroxil in-vitro which can be applied to correlate its activity within the human body.

MIC_{90} of cefadroxil reference standard against standard culture of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) was 16 and 4 µg/ml, respectively.MIC_{90} of cefadroxil (reference standard) against clinical isolates was also noted in the range of 8-256 µg/ml for *E coli* with mean as 149.26 µg/ml and 2-128µg/ml for *Staph. aureus* with mean as 28.51 µg/ml (table-1). MICs of cefadroxil against *E. coli* and *Staph. aureus* were significantly different from MIC against standard cultures of these organisms ($p<0.0001$). All tested clinical isolates of *E coli* (n=5) were resistant to three brands of cefadroxil, whereas only one isolate of *Staph. aureus* (n=5) was sensitive to four different brands assessed. MIC_{90} of different brands of cefadroxil against clinical isolates of *E. coli* and *Staph.aureus* was ranged between 8 to 256µg/ml and 8 to

64µg/ml, respectively. Although standard culture were sensitive to different brands (tablets and capsules) of cefadroxil but clinical isolates showed resistance (Table-2 and 3).

The reasons for this emergence of resistance may include substandard or counterfeit drugs, exposure of microorganisms to sub inhibitory concentrations, reduced potencies or ignorance to microbiological assays of brands after approval from regulatory authorities. Most of the clinical isolates were resistant to cefadroxil i.e. 85.71% of *E. coli* and 71.43% of *Staph. aureus* as summarized in table (1). This finding is an affirmation of some other work from different part of the world and from Pakistan. Harris, et al reported 90% resistant strain of *Staphylococcus aureus*,[15] whereas Tambekar, et al observed 92% and 100% resistance among *Staph* and *E coli* to cefadroxil, respectively [27]. Kumar, et al, also noted that all isolates of *E. coli* (n=43) analyzed were resistant to cefadroxil [28]. This increase in resistance results from excessive use of broad spectrum antibiotics without any control program [29]. Many published reports have an evidence of drastic decline in antibacterial activity of first generation cephalosporins in our community [14]. Other researchers also reported resistance among clinical isolates to augmentin, ampicillin, cefotaxime, ceftazidime, etc in Pakistan [30]. Resistance to cefadroxil was very low (<5% among *E. coli*) in developed countries like USA, UK, Canada, Sweden, Finland, Germany, Belgium, resulted from implementation of an antibiotic policy to control and rationalize its use [31]. There is prerequisite of nationalized surveillance program especially in this part of the world. The development of new antibiotic molecules is also a necessity of this modern era to successively treat bacterial infections [31].

V. CONCLUSION AND RECOMMENDATIONS

It was concluded that irrational or misuse of broad spectrum antibiotics have been resulted in resistance among clinical isolates of this part of the world. Antibiotic surveillance program development and implementation by government agencies is a critical requirement of our community. Irrational use of broad spectrum antibiotics can be restricted by controlling antibiotic prescribing, educating medical practitioners and public, restricting antibiotic selection. Antimicrobial resistance will continue to rise in developing countries and in near future organisms will develop 100% resistance to many broad spectrum antibiotics until and unless these curative measuresare adopted.

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Table-1: Distribution of MICs of cefadroxil (reference standard) against clinical isolates (n=70)

Clinical isolates		Concentration of cefadroxil (µg/ml)									Resistance (%)
		≥256	128	64	32	16	8	4	2	1≥	
***Escherichia coli* (n=35)**	S	16	7	5	4	4	1	0	0	0	85.71
	R	19	28	30	31	31	35	35	35	35	
***Staphylococcus aureus* (n=35)**	S	29	18	10	9	8	6	1	1	0	71.43
	R	6	17	25	26	27	29	34	34	35	

where, S=susceptible, R=resistant.

Table-2: MICs of cefadroxil (reference standard) and its five brands against *Escherichia coli* standard and clinical cultures

Cefadroxil brands and standard	Isolates of *Escherichia coli*					
	E. coli 1	*E. coli* 2	*E. coli* 3	*E. coli* 4	*E. coli* 5	*E. coli* STD*
CFD 1	32	128	128	128	64	32
CFD 2	128	8	128	128	64	16
CFD 3	128	128	128	128	64	64
CFD 4	8	64	128	128	16	16
CFD 5	128	64	128	128	64	8
CFD Ref. Std.	128	8	128	32	16	16

* *E. coli* standard culture (ATCC 25922), Ref. Std. = reference standard.

Table-3: MICs of cefadroxil (reference standard) and its five brands against *Staphylococcus aureus* standard and clinical cultures

Cefadroxil brands and standard	**Isolates of *Staphylococcus aureus***					
	Staph 1	*Staph* 2	*Staph* 3	*Staph* 4	*Staph* 5	*Staph* STD*
CFD 1	32	32	32	32	16	16
CFD 2	32	64	8	32	64	8
CFD 3	32	32	16	32	32	16
CFD 4	32	32	16	32	32	4
CFD 5	64	64	32	64	64	8
CFD Ref. Std.	16	8	8	2	32	4

**Staph. aureus*, standard culture (ATCC 25923), Ref. Std. = reference standard.

Table-4: Results of statistical analysis to compare cefadroxil reference standard and its brands for their activity against *E. coli* and *Staphylococcus aureus*

Source of variation		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Clinical isolates of *E. coli*	Between Groups	10173.867	5	2034.773	0.868	0.517
	Within Groups	56268.800	24	2344.533		
	Total	66442.667	29			
Clinical isolates of *Staphylococcus aureus*	Between Groups	5495.067	5	1099.013	6.165	0.001*
	Within Groups	4278.400	24	178.267		
	Total	9773.467	29			

*$p<0.05$ was considered statistically significant