Appraisal of multifarious brands of Cephradine for their *in vitro* antibacterial activity against varied microorganisms

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Abstract: The astounding and exceptional growth of generic pharmaceutical Industry in Pakistan has raised certain questions for drug regulatory authorities contemplating their efficacy and quality. The current study focuses on assessing the *in-vitro* antimicrobial activity of 24 brands of Cephradine 500mg capsules against 4 different strains by employing standardized methods. Disk diffusion method was performed on all brands to look into the susceptibility and resistance patterns. Standard disk of 5µg Cephradine powder were used during evaluation. The zones of inhibitions were ranged from 24-40mm against *S. aureus*, 24-40mm against *E. coli*, 20-25mm *against K. pneumonia* and 19-23mm *P. mirabilis.* On the basis of mean value, the multinational brands were found to have better zone of inhibitions and were better than local Pharmaceutical companies but ANOVA cooperative study showed that all brands of Cephradine showed similar comparable results. Further investigations by employing MIC method, quality of raw material with special emphasis on the shelf-life, excepients and method of manufacturing will be needed to obtain more authenticated results. The price of National and Multinational brands ranges from Rs.156.00-212.00 for 10 capsules. It is concluded that Public health is at risk because of noticeable growing widespread curse of the manufacture and trade of sub-standard or below par pharmaceuticals. The pecuniary accountability of management of pharmaceutical agents is additionally apparent. The results of the study need to be made public to boost the confidence of medical profession about the quality of locally manufactured pharmaceuticals. It will succor the foreign exchange being incurred on the trade in of medicines.

Keywords: Cephradine, antimicrobial activity, zone of inhibition, sub-standard drugs.

INTRODUCTION

In establishing countries, most fake drugs are of life-saving medicines such as antibiotics, antimalarials, anti-tuberculosis and antiretroviral drugs. It is estimated that up to 25% of the medicines used in establishing countries are below par or substandard and Public health is at ever-increasing threat because of noticeable mounting widespread curse of this manufacture and trade (WHO, 2003 Fact sheet). Antibiotics are one of our utmost imperative armaments in struggling bacterial contagions and have significantly profited the health-related eminence of human life since their start. This accomplishment was nevertheless, overshadowed by the hurried counterattack by the microbes ensuing in "unyielding and steadfast ascend of antimicrobial resistance (Bhatia and Narian, 2010). Cephalosporins are remarkable antibiotic group which is usually favored in case of penicillin confrontation. It is second foremost antibiotic group that is most operative and has been categorized, first as publicizing stratagem and consequently for expediency, into altered compeers reliant upon their commotion against gram positive and gram negative organisms. There has been boost in public understanding of the subsistence of bogus and poor quality drugs which have been progressively more reported in developing countries where drug set of laws are unproductive (Kelesidis *et al.*, 2007). There is mounting trade in inferior and substandard drugs including antibiotics around the world (Parry, 2005). The pervasiveness of such substandard drugs in markets has been worrisome to both regulatory agencies and all fretful. The retention of drugs exclusively antibiotics in open market have led to indiscriminate use and may underwrite to high prevalence of antibiotic confrontation strains. Safety, efficacy and quality are the three most important benchmarks that have customarily been used by regulatory authorities worldwide to safeguard that populations originate the paramount benefit from pharmaceuticals (Waller, 2001). The amount of antimicrobial drugs disbursed in a public is directly related to the amount of antimicrobial drug resistance found in the community (Albrich *et al.* 2004). Studies had shown that two third of all antibiotics are sold without prescription, though under synchronized private sectors. A contemporary review of the adverse events in the USA (Hussain *et al.* 2001) shows these to be the fourth to sixth graded cause of death in the country, with economic costs of between US$ 30 to US$ 130 billion per year. Agreeing to a WHO report, India hints the Asian countries by fabricating 35% of Asia's counterfeit/substandard drugs. Whatever be the actual fig, it is a fact that in India there is

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a hefty amount of such drugs existent in the market. The main reasons which have been put frontward for this are insufficiencies in drug testing, unembellished scarcity of regulatory inspectors, corruption, and lack of law enforceement (Mashelkar Committee, 2003). In the current past, the WHO has introverted the AIDS drugs contrived by an Indian pharmaceutical company from the list when they found that the Contract Research Organization (CRO) that conceded out the clinical study was not accurately regulated as per the WHO standards (Fleck, 2004). In Pak. like other establishing countries, overall increase in antibiotic resistance has been testified (Khoharo and Shaikh, 2011). There is mounting trade in substandard and counterfeit drugs take in antibiotics around the world. The disproportionate use of cephalosporin has led to an increase in confrontation among *Staphylococcus aureus.* In many progressing countries use of antibiotics are poorly restrained which results in a high rate of happenstance. Physicians and clinician are exasperating to interpret the delinquent of growing drug resistance by using amalgamation of antibiotics and advising patients about the deleterious effects of over and abuse of antibiotics (Lin *et al.*, 2011). The present study was conducted to appraise antimicrobial activity of diverse brands of Cephradine in contrast to innumerable microrganisms by retaining standardize pharmacopoeal methods.

MATERIALS AND METHODS

Chemicals and Medias

Mueller Hinton Agar (Oxide USA), Nutrient Agar (Oxide USA), Nutrient broth (Oxide USA), Commercial antibiotics discs (Oxoid, England), Barium Sulphate (Merck, Germany) and Sulfuric Acid (BDH)

Bacterial strains

Staphylococcus aureus (ATCC.25923), *Escherichia coli* (ATCC.25922), *Klebsiella pneumoniae* (ATCC.700603), *Proteus mirabilis* (ATCC.12453)

Inoculum preparation

Inoculums were prepared by transferring a large number of bacterial cells from bacterial cell culture to test tube devouring 10ml nutrient broth and by incubating them for 24 hours at 37ºC. The tubes were dazed intermittently to hasten growth (Panthi and Chaudhary, 2006).

Preparation of filter paper discs

Disks (6mm diameter) were punched out from Whatman's filter paper, placed in Petri dishes allowing a distance of 2-4 mm between each disk and sterilized in hot air oven at 160 centigrade for 1hr. 500mg capsule of Cephradine open and drug added to solvent to liquefy stirrer until dissolve in 10 ml sterile distilled water and diluted for use in two dilutions concentrations thus Direct=500mg 1:2= 250mgAn aliquot of 0.02 ml of each concentration was pipette onto separate disk incubated at 37 centigrade for 1 hr placed in labeled air tight container until use (Baron *et al*. 1990).

Disk diffusion method (Kirby-Bauer method)

The antimicrobial assay for strains was conducted by Disk diffusion method (Bauer *et al.*, 1966).

RESULTS

We have steered a study to conclude sensitivity or susceptibility of diverse microorganisms against 20 dissimilar Cephradine brands (500mg) of different companies (table1). Disk diffusion (Kirby-Bauer method) is used for susceptibility testing. We have made dilution of 250mg for different brands of Cephradine. Different zones of inhibition were observed and measured in our study for different brands of Cephradine. According to table 2, among the 20 brands of Cephradine of 500mg, the zone of inhibitions were ranged from (24mm-40mm) against *Staphylococcus aureus*, PPE (PME) was found to have largest zone of inhibition as compared to other brands while Cephradine (PMA) has least zone of inhibition. The zones of inhibitions were ranged from (24mm-40mm) against *E. coli*, PPE (PME) has largest zone of inhibition, Cefradine (PMA) has shown small zone of inhibition. PPL (PML) has greater zone of inhibition (25 mm) and PPS (PMS) has smaller inhibition zone (20 mm) against *Klebsiella pneumonia*. *Protius mirabilis* has swarming pattern of movement show greater resistance to antibiotics. It is also used as testing organism. PPL (PML) show high degree of inhibition (23mm) and PPN (PMN) show least inhibition (19 mm) which is observed during experiment. According to table3, among 20 brands of Cephradine of 250 mg dilution, the zone of inhibitions were ranged from (20mm-35mm) against *Staphylococcus aureus*, PPL (PML) was found to have largest zone of inhibition as compared to other brands while PPI (PMI) has least zone of inhibition. The zones of inhibitions were ranged from (20mm-40mm) against *E. coli*, PPE (PME) has largest zone of inhibition, PPH (PMH) has shown small zone of inhibition. PPL (PML) has greater zone of inhibition (20 mm) and Sinocef (safina) has smaller inhibition zone (17mm) against *Klebsiella pneumonia*. *Protius mirabilis* has swarming pattern of movement show greater resistance to antibiotics. It is also used as testing organism. PPQ (PMQ) brand of Cephradine show high degree of inhibition (21mm) and PPK (PMK) show least inhibition (17 mm) which is observed during experiment. In table 4, we have conducted a comparative susceptibility study amidst national and multinational brands. In table 5 depicts multiple responses test organisms to different brands of cephradine. ANOVA was used to test the statistically significance of treatments (Zar, 1999). The results were found highly non significant at P=1.00.

Comparative susceptibility study of national and multinational brands

On the basis of mean value, the multinational brands were found to have better zone of inhibitions and was better than local pharmaceutical companies but ANOVA cooperative study showed that all brands of Cephradine showed similar comparable results.

Table 1: Details of Different brands of Cephradine

S No.	Brands	Pharma Company	Dosage Forms
1	PPA	PMA	Capsule (500mg)
2	PPB	PMB	Capsule (500mg)
3	PPC	PMC	Capsule (500mg)
4	PPD	PMD	Capsule (500mg)
5	PPE	PME	Capsule (500mg)
6	PPF	PMF	Capsule (500mg)
7	PPG	PMG	Capsule (500mg)
8	PPH	PMH	Capsule (500mg)
9	PPI	PMI	Capsule (500mg)
10	PPJ	PMJ	Capsule (500mg)
11	PPK	PMK	Capsule (500mg)
12	PPL	PML	Capsule (500mg)
13	PPM	PMM	Capsule (500mg)
14	PPN	PMN	Capsule (500mg)
15	PPO	PMO	Capsule (500mg)
16	PPP	PMP	Capsule (500mg)
17	PPQ	PMQ	Capsule (500mg)
18	PPR	PMR	Capsule (500mg)
19	PPS	PMS	Capsule (500mg)
20	PPT	PM	Capsule (500mg)

DISCUSSION

During the last decades, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Protius mirabilis* are developed as significant nosocomial pathogens which are an important cause of morbidity and mortality among the patients. Understanding the collaboration of patients (patient immune system, organism and environment) comprising use of antibiotics and of medical expedients is indispensable in preclusion and control.

The existent study was piloted on these rampant microorganisms to conclude the susceptibility or resistance configurations of gram positive and gram negative organisms in contradiction of different brands of Cephradine existing at the time of study, to expedite the drug authorities to select a cheap and efficacious brand amidst various brands available in Pakistan and to create awareness amongst public nearby the quality of antimicrobial drugs accessible in the country. Cephradine bustle is similar to that of Cephalexin with negligible alterations (Moellering and Swartz *et al.,* 1976). Cephradine is less vigorous against most bacterial species equaled to cephalothin and cephaloridine, just like Cephalexin. *Staphylococcus aureus*, comprising supreme penicillin sturdy are typically sensitive, but not the methicillin resistant strains is sensitive (Lambert *et al*. 1992). Gram positive cocci such as *Staphylococcus epidermidis, Streptococcus pyogenes, Strep-Pneumoniae* and *Streptococcus viridians* are vulnerable to Cephradine, streptococcus fecalis is resistant (Hamilton Miller, 1974). *Peptococcus* and *Peptostreptococcus* spp. are subtle and preponderance of strains that recuperated from airway concomitant infections are sensitive, but other strains are less (Busch *et al*., 1976).

Cephradine has analogous inhibitory achievement to Cephalexin in contrast to gram- positive bacilli. Gram negative bacteria like *E. coli, Protius mirabilis* and *Klebsiella* species are susceptible (McGowan *et al*., 1974; Bill *et al*., 1977; Wise *et al*. 1979). It is moderately active against *Neisseria gonorrhea* (Phillips *et al*., 1976), active against beta lactamases producing strains (Selwyn and Bakhtiar, 1977). It is inactive against H. influenza, many strains of which are completely resistant to this drug (Sinai *et al*., 1978; Watanakunakorn and Glotzbecker, 1977). Zone diameters were determined for 2 strains of bacteria to inaugurate the regression curves (Sirot *et al*., 1982). Antibacterial activity of 1st second and third generation cephalosporin's were tested in vitro by disk diffusion method against 1920 strains of gram positive and gram negative bacteria. The staphylococcus lamoxactam was utmost effective against the gram negative bacteria (Messmer *et al*., 1983). The assortment and spread of resilient organism in mounting countries, which can frequently be traced to complex, such as abuse of antibiotics by health professionals, self-medication, unskilled practitioners, laypersons, and poor drug quality are one of the origins for spread of resistant bacteria. The inadequate surveillance in one of the reasons for drug resistance as well.

Cephradine is used to treat a number of infections including: Otitis media, streptococcal pharyngitis, bone and joint infections, pneumonia, cellulites, and urinary tract infections. It may be used to avert bacterial endocarditis (Middlebrook, 2007). Cephalosporin group was used in previous antimicrobial therapy and was recommended by doctors, which comprises Cefaclor, Cephradine, Cefoperazone, Cefotaxime, and Ceftriaxone. Nevertheless, over the past few eras these health benefits are under menace as many frequently used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to advent of drug- resistant bacteria. It is indispensable to explore newer drugs with lesser conflict (Sarkar *et al*., 2003). The undue use of

Table 2: Sensitivity inhibition zones of Cephradine brands of potency 500mg on *S. aureus, E. coli, K. pneumonia* and *P. mirabilus*

Brands	Pharmaceutical Company	*S. aureus* (500 Mg) Zone (Mm)	*E. coli* (500 Mg) Zone (Mm)	*K. pneumoniae* (500mg) Zone (Mm)	*P. mirabilis* (500mg) Zone (Mm)
PPA	PMA	24	24	22	20
PPB	PMB	32	32	23	22
PPC	PMC	38	38	22	23
PPD	PMD	27	27	21	22
PPE	PME	40	40	22	21
PPF	PMF	30	30	24	22
PPG	PMG	24	24	23	23
PPH	PMH	30	30	22	22
PPI	PMI	29	29	22	22
PPJ	PMJ	29	29	23	22
PPK	PMK	34	34	20	20
PPL	PML	34	34	25	23
PPM	PMM	35	35	22	22
PPN	PMN	34	34	22	19
PPO	PMO	31	31	23	20
PPP	PMP	34	34	24	21
PPQ	PMQ	30	30	23	20
PPR	PMR	30	30	22	21
PPS	PMS	34	34	20	23
PPT	PM	24	24	21	19

Table 3: Sensitivity inhibition zones of 250mg dilution of Cephradine brands on *S. aureus, E. coli, K. pneumonia* and *P. mirabilus*

Brands	Pharmaceutical company	*S. aureus* (250 Mg)Zone(Mm)	*E. coli* (250 Mg) Zone (Mm)	*K. pneumoniae* (250 Mg) Zone (Mm)	*P. mirabilis* (250mg) Zone (Mm)
PPA	PMA	25	23	19	19
PPB	PMB	20	22	19	18
PPC	PMC	26	32	19	19
PPD	PMD	22	26	19	16
PPE	PME	20	40	19	20
PPF	PMF	20	40	19	20
PPG	PMG	22	26	19	19
PPH	PMH	24	20	19	19
PPI	PMI	20	28	19	20
PPJ	PMJ	20	22	20	20
PPK	PMK	24	28	18	17
PPL	PML	35	28	20	19
PPM	PMM	22	30	19	19
PPN	PMN	23	31	17	19
PPO	PMO	25	30	19	20
PPP	PMP	26	29	19	21
PPQ	PMQ	26	24	18	21

cephalosporin has led to an escalation in confrontation among *S. aureus*. In many emerging countries use of antibiotics are poorly controlled which marks in a high rate of conflict (Kunin, 1993). Physicians and clinician are trying to decipher the problem of growing drug resistance by using a mishmash of antibiotics and counseling patients about the venomous effects of over and misuse of antibiotics (Trakulsomboon *et al*., 2001). Although some preparations enclosed too much or too little of the active drug contents were acknowledged and manufacturers were from Belgium, China, Pakistan, Egypt, Germany, Switzerland, United Kingdom and Nigeria, is the menace of the sales of forged tainted and sub-standard drugs which has eaten deep into the fabric of our society like bad ulcer (Popoola, 2001). *Staphylococcus aureus* causes localized infection

distributing into blood stream (Espersen, 1995). *E. coli* has developed conflict to antimicrobial agent and incident is escalating both in outpatients and hospitalized patients (Akram *et al*., 2007; Garcia *et al*., 2007). The zone of inhibition revealed by these brands of antibiotics against the test organism indicates their potencies (Cheesbrough, 2006). The Product-related factors, such as: physical and chemical properties of the drug i.e. solubility, degree of ionization, crystalline forms, chemical form, isomers as well as variables related to manufacturing, formulation, or both i.e. coatings, compression force, particle size, presence or absence of excipients (Blacke, 1988; Riley, 1987; Henderson 1992).

Table 4: Comparative susceptibility study of national and multinational brands

Multinational brands		National brands	
PPL	39	PPQ	34
PPE	40	PPL	34
PPF	38	PPN	35
PPG	35	PPD	27

It is fulfilled that Public health is at escalating risk because of an apparent mounting global pandemic of the fabricate and trade of sub-standard pharmaceuticals. The monetary encumber of exploitation of pharmaceutical agents is evident. In addition to the direct costs on the purchaser, there are many indirect costs which ultimately the consumer has to bear, such as cost of undesirable side effects of drugs, delay in diagnosis leading to referral to higher facility or laboratory costs. There is need to make the domino effects of the study public to boost the confidence of the community and medical profession about the worth of locally manufactured. On the basis of mean value, the multinational brands were set up to have better zone of inhibitions and was better than local pharmaceutical companies. As the P-value is less than 1 (0.994) so result were insignificant (P>0.01), which shows all the 20 brands of different pharmaceutical companies shows approximately same equal effects against 4 microorganism *Staphylococcus Aureus*, *Escherichia coli*, *Klebsiella Pneumonia*, *Protius mirabilis*. Disk diffusion method was used to assess sensitivity and resistance and can be interrelated clinically; auxiliary investigations employing MIC method will be needed to obtain more unswerving results (Abu-Bakr, 2009). Safety, efficacy and quality are the three most imperative criteria that have customarily been used by regulatory authorities wide-reaching to ensure that populations derive the greatest benefit from

Table 5: Multiple response of test organisms to different brands of Cephradine

Groups	Count	Sum	Average	Variance
PPA	4	94	23.5	16.33333333
PPB	4	103	25.75	22.91666667
PPC	4	112	28	54
PPD	4	95	23.75	7.583333333
PPE	4	107	26.75	79.58333333
PPF	4	100	25	12
PPG	4	94	23.5	0.333333333
PPH	4	100	25	14.66666667
PPI	4	96	24	11.33333333
PPJ	4	98	24.5	9.666666667
PPK	4	94	23.5	49
PPL	4	102	25.5	36.33333333
PPM	4	103	25.75	38.91666667
PPN	4	110	27.5	67
PPO	4	96	24	23.33333333
PPP	4	102	25.5	33.66666667
PPQ	4	99	24.75	18.25
PPR	4	98	24.5	16.33333333
PPS	4	103	25.75	36.25
PPT	4	90	22.5	9.666666667

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	150.3	19	7.910526316	0.283955476	0.998210324	1.762547
Within Groups	1671.5	60	27.85833333			
Total	1821.8	79				

Table 6: Multiple responses of test organisms to different brands of Cephradine

ANOVA: Single factor				
Summary				
Groups	Count	Sum	Average	Variance
PPA	4	86	21.5	9
PPB	4	79	19.75	2.916667
PPC	4	96	24	39.33333
PPD	4	83	20.75	18.25
PPE	4	99	24.75	103.5833
PPF	4	99	24.75	103.5833
PPG	4	86	21.5	11
PPH	4	82	20.5	5.666667
PPI	4	87	21.75	17.58333
PPJ	4	82	20.5	1
PPK	4	87	21.75	26.91667
PPL	4	102	25.5	56.33333
PPM	4	90	22.5	27
PPN	4	90	22.5	38.33333
PPO	4	94	23.5	25.66667
PPP	4	95	23.75	20.91667
PPQ	4	89	22.25	12.25
PPR	4	94	23.5	25.66667
PPS	4	86	21.5	25.66667
PPT	4	89	22.25	12.25

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	190.9375	19	10.04934	0.340896	0.994254	1.762547
Within Groups	1768.75	60	29.47917			
Total	1959.688	79				

pharmaceuticals (Waller, 2001). Further investigations by employing MIC method, the quality of raw material with particular prominence on the shelf-life and method Of manufacturing will be needed to acquire more consistent and authenticated consequences.

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