Pakistan Journal of Pharmaceutical Sciences
Vol.15, No.2, July 2002, pp.41-50

IN VITRO ACTIVITY OF CEFAZOLINE AND CEFUROXIME IN PRESENCE OF ESSENTIAL AND TRACE ELEMENTS

NAJMA SULTANA AND M. SAEED ARAYNE*
Department of Pharmaceutical Chemistry, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Pakistan
**Department of Chemistry, University of Karachi, Pakistan.*

ABSTRACT

Present research focuses to evaluate changes in antimicrobial activity of cefazolin sodium and cefuroxime after interactions with essential and trace elements. The minimum inhibitory concentration (MIC) was observed and subsequently compared with the standard MIC's of the respective drug by agar dilution method against a variety of Gram positive and Gram negative organisms.

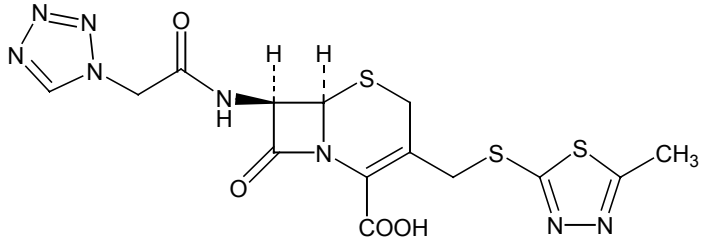
It was observed that these metal elements are essential for our body, either present within the body or co-administered with vitamins or otherwise, markedly influence the MIC's of antibiotics by producing synergism or antagonism.

INTRODUCTION

Cefazolin

Cefazolin (6 R-trans)-3[[(5-Methyl-1,3,4-thiadi azol-2-yl)thio]methyl]-8-oxo-7-[(1 H-tetrazol-1-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid or 7-(1-(1H)-tetrazolyl acetamido)-3-[2-(5-methyl-1,3,4-thiadiazolyl)thiomethyl]-Delta-3-cephem-4-carboxylic acid (structure I), having molecular formula: $C_{14}H_{14}N_8O_4S_3$ with molecular weight 454, semi-synthetic antibiotic derived from 7-aminocephalosporanic acid, (Merck Index 1999) lies in first generation cephalosporins (Anders 1974; Fong *et al.,* 1976). It was prepared by Koniwhi *et al.* (1970), synthesis and properties were given by Kariyone *et al.* (1970), activity and clinical studies were performed by Nishida *et al.* (1970 & 1970[a]) & Shibata, Fujii (1970), metabolic studies by Kozatani *et al.* (1972) and toxicology by Birkhead *et al.* (1973). Its comprehensive description was given by Zappala *et al.* (1975) and reviewed by Nakano (1977).

It has been used satisfactorily to treat infections of the respiratory tract, urinary tract, skin, soft tissues, joints, bones, endocorditis and septicimias. Its longer half-life has made it a drug of choice for surgical prophylaxia. Adverse effects include hypersensitivity reaction, thrombo-cytopenia, neutropenia, leucopenia and eosinophilia; high doses may cause convulsions in patients with impaired renal failure (Barton & Sammes 1971; Moellering & Swartz 1976).



(I)

Cefazolin appears as needles from aqueous acetone, m.p., 198-200°(d) and is easily soluble in DMF, pyridine, soluble in aqueous dioxane, aqueous ethanol, slightly soluble in methanol and practically insoluble in chloroform, benzene & ether. Its sodium salt is white to yellowish-white, odorless crystalline powder with a bitter, salty taste. It crystallizes in α, β, and γ forms and is easily soluble in water, slightly soluble in methanol, ethanol and practically insoluble in benzene, acetone, chloroform and ether (Fong *et al.,* 1976). Its reconstituted products are stable for 24 hours at room temperature and for 10 days if stored under refrigerator at 2°C to 8°C (Alfonso 1985).

Antimicrobial Activity

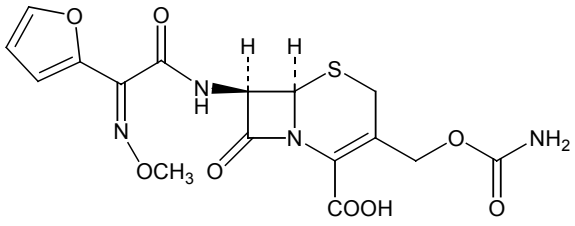
Cefazolin sodium shows activity against *Staphylococcus aureus* (including penicillinase producing strains), *Staphylococcus epidermidis, Streptococcus pneumoniae, Escherichia coli, Proteus mirabilis, Klebsiella* species and *Haemophilus influenzae*, while most strains as *Proteus vulgaris*, *Serratia* and *Pseudomonas* are resistant to cefazolin. Its broad spectrum of activity has made it suitable for the treatment of wide range of severe infections (David 1997; Sabath *et al.,* 1973).

Cefuroxime

Cefuroxime [6R-[6α,7β(Z)]]-3-[[(aminocarbonyl)oxy]methyl]-7-[2-furanyl(methoxyimino) acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid or (6R, 7R)-3-carbamoyloxymethyl-7-[2-(2-furyl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid or (6R, 7R)-3-carbamoyloxymethyl-7-[2-(2-furyl)-2-(methoxyimino) acetamido]ceph-3-em-4-carboxylic acid (structure II) having molecular formula $C_{16}H_{16}N_4O_8S$ and molecular weight 424.2 (Dollery *et al.,* 1991; James *et al.,* 1996) (Merck Index 1999[a]) is a broad spectrum second generation antibiotic (Chen *et al.,* 1973).

It was prepared by Ayres *et al.* (1973) and *in vitro* studies were carried out by OprimeCallaghan *et al.,* (1976) & Jones *et al.,* (1977). Its *in vitro* antibacterial activity and human pharmacokinetics were given by OprimeCallaghan *et al.,* (1976[a]), pharmacology by Schulz *et al.,* (1978), pharmacokinetics by Gower (1977) & Daikos *et al.* (1977), while clinical studies by Fowler *et al.,* (1978) & Norrby *et al.* (1977). An extensive review of its antibacterial activity, pharmacology and therapeutic efficacy are given by Brogden *et al.* (1979) and comprehensive description by Wozniak & Hicks (1991).

It is very stable to β-lactamases and remarkably well tolerated, has been in widespread clinical use since 1979 (Brogden *et al.,* 1979). It is a semi-synthetic analogue of cephalosporin C and is creamy white powder.



(II)

It is white or creamy white solid, freely soluble in water and buffered solutions, soluble in methanol, sparingly soluble in alcohol, very slightly soluble in ethyl acetate, diethyl ether, octanol, benzene and chloroform while insoluble in acetone, chloroform, ethylacetate and in toulene

(James *et al.,* 1996). Its solutions are stable at room temp for 13 hrs; <10% decomposes in 48 hrs at 25° (Alfonso *et al.,* 1985; Merck Index 1999[a]).

Antimicrobial Activity

Cefuroxime sodium has the ability to penetrate into cerebrospinal fluid which makes it specific for the treatment of meningitis caused by *Haemophilus influenzae.* It has also provided satisfactory results in the treatment of gonorrhea, urinary tract infections, cutaneous infections and septicemias ((Alfonso *et al.,* 1985; Brotzu *et al.,* 1962). It is primarily eliminated by the kidneys, 33% drug is recovered with elimination half life of 1.5 hours. It may cause haemolysis, aplastic anemia, agranulocytosis, elevation in blood urea, serum creatinine level and liver enzymes (Joel *et al.,* 1996). It has comparable activity with cefamandole and has increased activity than cefoxitin against Gram-positive organisms and *Enterobacter specie* (Brogden *et al.,* 1979). It has strong activity against β-lactamase producing strains of *Haemophilus influenzae* and *Neisseria gonorrhea* (Lossick *et al.,* 1982), but *Pseudomonas aeruginosa, Streptococcus faecalis*, many strains of *Serratia specie*, indole-positive *Proteus, Acinetobacter specie* and many isolates of *Staphlococcus epidermidis* are resistant to it. It also works against a wide range of Gram-positive and Gram-negative bacteria including *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria specie, Haemophilus influenzae* and *Klebsiella species* (O'Callaghan *et al.,* 1976; Ryan *et al.,* 1976; Eykyn *et al.,* 1976; Neu & Feu 1978; Knothe & Dette 1983; Davies & Dyas 1985).

The present work comprises of the study of alterations in antimicrobial activity of cefazolin sodium and cefuroxime, due to the presence of salts of essential and trace elements like magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium. Different dilutions of drug and essential and trace element salts solutions were prepared in concentrations ranging from 256, 128, 64, 32, 16, 8, 4 and 2µg/ml. Interaction of the drug and metal salts was conducted in the ratio of 3:1 and the reaction was carried out at 37^{o}C and 60^{o}C concordant to *in vivo* conditions and accelerated temperature studies. The agar dilution method was adopted and the MIC was observed against various Gram positive and Gram negative organisms and compared with the standard MIC's of respective drugs.

EXPERIMENTAL

Materials

Cefazolin sodium and cefuroxime were gifted by Barret Hodgson Ltd., Karachi and GlaxoWellcome, Karachi respectively. The essential and trace element used were in the form of their hydrated or anhydrous salts as magnesium chloride, calcium chloride, chromium chloride, manganese chloride, ferric chloride, cobalt chloride, nickel chloride, copper chloride, zinc chloride and cadmium chloride.

Various Gram-positive and Gram-negative organisms used were *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhii*, *Proteus vulgaris* and *Staphylococcus epidermidis.*

Methods

Preparation of Solutions

Stock solutions of cefazolin sodium and cefuroxime as well as salts of essential and trace elements were prepared in sterilized buffer of pH 5 (Barry 1976; Washington 1985) in a concentration of 1000 µg/ml. Buffer of pH 5 was prepared by adding 0.1M HCl to 0.1M sodium

citrate until the pH was attained (Washington 1985). The amount of each cephalosporin required was calculated from its potency. These were stored in tightly sealed container in a refrigerator (Lorian 1986; Lorian 1991). According to the reported MIC's of both the drugs, different dilutions were prepared in concentrations of 128 to 2 μg/ml (Thomas 1988; Jaime & William 1991). Same procedure was adopted for preparing dilutions of metal salts i.e., from 128 to 2μg/ml (Ales 1997).

Preparation of Mueller–Hinton agar (MHA), nutrient broth, inoculums, agar dilution susceptibility test and preparation and inoculation of antimicrobial plates has already been reported earlier (Arayne *et al.,* 2001).

Antibiotic assay

The drug was incorporated in a liquefied agar medium (45 - 50^{o}C), which after mixing, was then poured into a petri dish and allowed to solidify. A series of petri dishes were prepared with increasing concentration of the drug [Samson *et al.,* 1985] and with the aid of a inoculating device, six different species of microorganisms were spotted and inoculated one by one on each plate. After overnight incubation at 37^{o}C, the MIC end point was read as the lowest concentration that completely inhibits growth (Hamilton-Miller 1970).

Interaction of antibiotic with essential and trace element salts

Different dilutions of cefazolin and cefuroxime, as well as essential and trace element salts ranging in concentration from 128 to 2 μg /ml were prepared. The ratio of each antibiotic and metal/element salts solution was 3:1. For this interaction, stock solution of each antibiotic was prepared in double concentration i.e., 100mg/50ml in order to have the same proportion of each antibiotic in respective antibiotic-trace element salt complexes. Accordingly same concentration was used in reference standards of each antibiotic solution. The two solutions of metal salt and antibiotic were mixed in two different sets and each heated separately at 37 and 60°C for 30 minutes (on water bath) and incorporated in a liquefied agar medium (45–50°C), which was then after mixing, poured into a petri dish and allowed to solidify. A series of petri dishes were prepared with increasing drug concentration (Joel *et al.,* 1996) and with the aid of a inoculating device, 6 different species of microorganisms were spotted and inoculated one by one on to each plate. After overnight incubation at 37°C, the MIC end point was read as the lowest concentration that completely inhibited growth (Washington 1985). This was compared with respective reference standard of each antibiotic by observing the change in MIC.

RESULTS AND DISCUSSION

The antibacterial activities of cefazolin sodium as well as cefuroxime of reference and standard against various Gram-positive and Gram-negative organisms are shown in table 1. They are also compared with the reported values. All the metal salts were found to be inactive or resistant, whereas MIC's of the drug after interaction with metals at 37 and 60^{o}C is reported in tables 2 - 5. In the current research work the MIC's of cefazolin and cefuroxime were found to increase against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Staphylococcus epidermidis* up to 64 & 32 μg/ml because of increased resistance of organisms as we used isolated strains from blood and urine. Different synergetic and antagonistic effects of drug metal complexation were observed and compared with standard drug after the interaction.

Cefazolin sodium

The MIC of cefazolin was found to be high against *S. aureus, E. coli* and *S. typhii* (Table 1). When cefazolin was treated with magnesium chloride at 37^{o}C, the antibacterial activity showed no

alteration in the efficacy of standard drug against *S. aureus* while antagonism was found against *E. coli* and *S. typhii* and synergism was observed against *S. faecalis, E. coli* and *S. epidermidis*. By carrying out the above reaction at 60^{o}C the MIC of drug against *Proteus vulgaris* was increased.

When the drug was reacted with calcium chloride at room temperature, antagonistic effect was observed against *S. aureus, E. coli, S. typhii* and *S. epidermidis* but *S. faecalis* was inhibited at the lower concentration thus leading to synergism. At 60^{o}C the MIC against *E. coli* was observed unchanged.

In the interaction between chromium chloride and drug at 37^{o}C and 60^{o}C antagonistic effect was observed against all the organisms. When the drug was interacted with manganese and iron chloride at 37^{o}C and 60^{o}C all the mentioned microbes except *S. faecalis*, were inhibited at the higher concentrations but synergism was noted against *S. faecalis.*

In the interaction of cefazolin with cobalt chloride at 37^{o}C and 60^{o}C, antagonism was observed against *S. aureus*, *E. coli, S. typhii and S. epidermidis* whereas synergism *against S. faecalis* and *Pr. vulgaris* was observed.

The drug after interaction with nickel chloride at 37^{o}C showed bactericidal effect at higher concentrations against *S. aureus*, *E. coli* and *S. typhii* and synergism was recorded against *S. faecalis.* At 60^{o}C *Proteus vulgaris* was inhibited at higher concentration.

The MIC of drug after interaction with copper chloride at 37^{o}C and 60^{o}C showed antagonism against *S. aureus*, *E. coli, S. typhii* and *S. epidermidis* but synergism was observed against *S. faecalis* and *Pr. vulgaris*. Similarly, the interaction of zinc chloride with drug at 37^{o}C has increased the MIC's of drug against *S. aureus* and *S. typhii* thus leading to antagonism, while synergetic behavior was observed against *S. faecalis* and *Pr. Vulgaris* .At 60^{o}C, the MIC against *S. faecalis* and *Pr. vulgaris* were found to decrease thus causing synergism.

The interaction of drug with cadmium chloride led to increase in MIC at 37^{o}C and 60^{o}C against *S. aureus, E. coli* and *S. epidermidis* and so causing antagonism and synergism against *S. faecalis* and *Pr. vulgaris*. All these results at 37^{o}C and 60^{o}C are shown in tables 2 & 3.

Cefuroxime

The bactericidal actions of the cefuroxime against isolated strains of *S. aureus, E. coli, S. typhii* and *Pr. vulgaris* were found at the higher concentrations than the reported MIC's (table 3).

Antibacterial studies of cefuroxime after interaction with magnesium chloride at 37 and 60^{o}C showed alteration in susceptibility of drug as the metal has caused great synergetic effect against *S. aureus, Pr. vulgaris* and *S. epidermidis* by decreasing the MIC values from 64 to 16μg/ml, 32 to 16 μg/ml and 32 to 2μg/ml respectively where as *E. coli* and *S. typhii* were inhibited at higher concentration so leading towards antagonism.

When the interaction of calcium was carried at 37^{o}C, synergetic effect against *S. aureus* but antagonism was observed against *Escherichia coli* and *S. typhii. A*t 60^{o}C against *S. epidermidis* antagonism was observed.

Interaction of chromium with the drug at 37^{o}C has produced no change in its behavior against *S. aureus* but has caused antagonism against other remaining microbes. At 60^{o}C, synergetic effect was observed instead of antagonism against *Pr. vulgaris* at MIC 16μg/ml.

Reaction of manganese chloride with drug at 37°C is responsible to increase in MIC value from 2μg/ml to 32μg/ml and 8μg/ml against *S. faecalis* and *S. typhii* respectively and thus causing antagonism. By increasing the temperature of the above reaction to 60°C antagonism was noted against *Escherichia coli* and *S. epidermidis* due to rise in MIC value.

The complexation of iron chloride with drug at 37°C was responsible for the antagonistic effect against *S. aureus*, *E. coli* and *S. typhii* by raising the MIC values while inhibition of *Pr. vulgaris* and *S. epidermidis* was noted at the lower range of MIC values.

The interaction of cobalt with cefuroxime at room temperature has promoted the antagonism for drug against *S. aureus*, *E. coli* and *S. typhii* while synergetic effect of cobalt was observed against *Pr. Vulgaris*. At 60°C antagonism was noted against *E. coli* and *S. typhii* whereas synergism against *Pr. vulgaris* and *S. epidermidis* was observed.

Cefuroxime when reacted with nickel at 37°C, MIC was found to increase against *S. faecalis*, *E. coli*, *S. typhii* and *S. epidermidis* up to 64 μg/ml hence exhibiting antagonism but *Pr. vulgaris* was inhibited at lower concentration i.e. 16μg/ml and so synergetic effect was observed.

When interaction between the drug and copper chloride was carried out at 37°C antagonism was recorded against *E. coli, S. typhii,* and *Pr. vulgaris* whereas at 60°C the inhibiting concentration was found unchanged only against *S. faecalis* while remaining all the microbes exhibited antagonism.

Complexation of zinc chloride at 37°C with the drug has caused antagonism against *S. aureus, E. coli, S. typhii* and *S. epidermidis* due to increase in MIC. Antibacterial activity at 60°C has showed antagonism only against *S. typhii* and *S. epidermidis*.

At 37°C the presence of cadmium has caused a decrease in MIC of drug against *S. aureus* and *Pr. vulgaris* leading to synergism while antagonism was observed against *E. coli, S. typhii* and *S. epidermidis. A*t 60°C the effects were found to be same as that at room temperature against all microbes except *Pr. vulgaris.* These results are shown in tables 4 and 5.

It is concluded that metal elements vital for our body functions, either present within the body or coadministered with vitamins or otherwise, markedly influence the MIC's of antibiotics by producing synergism or antagonism.

Table 1
MIC's of cefazolin and cefuroxime against various microorganisms

Organisms	Reference		Standard	
	Cefazolin	Cefuroxime	Cefazolin	Cefuroxime
Staphylococcus aureus	0.25-05	1-4	8	64
Streptococcus faecalis	-	-	8	2
Escherichia coli	0.5-4	1-4	16	32
Salmonella typhii	1-2	1-4	8	8
Proteus vulgaris	R	R-4	32	32
Staphylococcus epidermidis	-	-	8	32

Table 2
MIC's of cefazolin sodium when reacted with essential and trace elements at 37°C against various microorganisms

↓Organisms	MIC (μg/ml)									
Metals→	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	8	64	64	64	64	64	64	128	32	16
Streptococcus faecalis	2	2	64	2	2	2	2	2	2	2
Escherichia coli	32	32	64	64	64	32	32	128	16	16
Salmonella typhii	16	16	128	64	128	128	64	128	32	16
Proteus vulgaris	8	32	128	64	128	16	32	16	8	16
Staphylococcus epidermidis	2	16	128	64	32	16	8	128	8	64

Table 3
MIC's of cefazolin sodium when reacted with essential and trace elements at 60°C against various microorganisms

↓Organisms	MIC (μg/ml)									
Metals→	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	8	16	32	128	128	64	64	128	8	16
Streptococcus faecalis	2	2	16	2	2	2	2	2	2	2
Escherichia coli	32	16	128	32	128	64	32	64	16	32
Salmonella typhii	16	16	128	32	128	64	32	128	8	16
Proteus vulgaris	32	32	128	64	64	16	64	16	8	32
Staphylococcus epidermidis	2	16	128	64	64	64	8	64	8	16

Table 4
MICs of cefuroxime when reacted with essential and trace elements at 37°C against various microorganisms

↓Organisms	MIC (μg/ml)									
Metals→	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	16	32	64	64	128	128	64	64	128	8
Streptococcus faecalis	2	2	64	*32*	2	2	64	2	2	2
Escherichia coli	128	64	64	32	128	64	64	128	64	128
Salmonella typhii	32	32	64	32	64	64	64	128	16	32
Proteus vulgaris	16	32	64	32	8	16	16	64	32	16
Staphylococcus epidermidis	2	32	64	32	16	32	64	32	128	64

Table 5
MIC's of cefuroxime when reacted with essential and trace elements at 60°C against various microorganisms

↓Organisms	MIC (μg/ml)									
Metals→	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	4	16	64	64	128	64	64	128	64	16
Streptococcus faecalis	2	2	16	64	2	2	32	2	2	2
Escherichia coli	128	128	64	64	128	64	64	128	32	64
Salmonella typhii	32	64	16	64	64	32	32	128	64	64
Proteus vulgaris	4	32	16	32	16	16	32	64	32	32
Staphylococcus epidermidis	2	64	64	64	32	16	64	64	64	64

REFERENCES

Ales, G. (1997) *Introduction to Medicinal Chemistry, How Drugs Act and Why*, Wiley-VCH, Inc., 216-220.

Alfonso, R.G., Grafllan, D.C., Melvin, R.G., Stewart, C.H., Robert, E.K., Thomas, M., Ewart, A.S. and Gilbert, L.Z. (1985). *Remington's Pharmaceutical Sciences*, Mack Publishing Co., 17th Ed., 1185.

Andres, G. (1974). *Medical Pharmacology, Principles and Concepts*. The C.V. Mosby Saint. Louis, 7th Ed, p.583.

Arayne, M.S., Sultana, N. and Rafiq, K. (2001). *In vitro* activity of ceftizoxime and ceftazidime in presence of essential and trace elements. *Pak. J. Pharm. Sci*. **14**(2): 57-64.

Ayres, B., Cook, M., Davis, B., Gregory, G., Newall, C., Pearce, D., Phillipps, G. and Weir, N. (1973). Novel 21-acyloxy-3α-hydroxy-5α-pregnone-11,20-diones – anesthetics. Patent Assignee(s): Glaxo Laboratories Ltd. US Patent No.3763195.

Barry, A.L. (1976). *The Antimicrobial Susceptibility Test*, Principles and Practices, Lea and Febiger, Philadelphia.

Barton, D.H. and Sammes, P.G. (1971). Chemical relationships between cephalosporins and penicillins. *Proc. R. Soc. Lond. B (Biol. Sci.)*, **179**(57): 345-355.

Birkhead, H.A., Briggs, G.B. and Saunders, L.Z. (1973). Toxicology of cefazolin in animals. *J. Infect. Dis*., **128**: S379-380.

Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S. (1979). Cefuroxime: a review of its antibacterial activity, pharmacological properties and therapeutic use. *Drugs,* **17**(4): 233-266.

Brotzu, G. (1948). Ric Nuovo Antibiot., Lav. Ist., Ig. Cagliari, ed. by Abraham, E.P. (1962). *Pharmacol. Rev*. **14**: 473.

Chen, C.H., Johnson, A.G., Kasai, N., Key, B.A., Levin, J. and Nowotny, A. (1973). Heterogeneity and biological activity of endotoxic glycolipid from *Salmonella minnesota* R595. *J. Infect. Dis*. **128**: S43-51.

Daikos, G.K., Kosmidis, J.C., Stathakis, C. and Giamarellou, H. (1977). Cefuroxime: antimicrobial activity, human pharmacokinetics and therapeutic efficacy. *J. Antimicrob. Chemother.* **3**(6): 555-562.

David, W.S. (1997). *Physician Desk Reference*, Medical Economics Co., Inc., 51 Ed, 1511-1513.

Davies, A.J. and Dyas, A. (1985). Antimicrobial susceptibility of *Staphylococcus epidermidis* and *Staph. aureus*. *J. Antimicrob. Chemother*. **15**(1): 127-128.

Dollery, S.C., Alan, R.B., Denis, B., David, M.D., Donald, S.D. and Leon, I.G. (1991). *Therapeutic Drugs*. Churchill Livinstone, **1**: 133-148.

Eykyn, S., Jenkins, C., King, A. and Phillips, I. (1976). Antibacterial activity of cefuroxime, a new cephalosporin antibiotic, compared with that of cephaloridine, cephalothin, and cefamandole. Antimicrob. *Agents Chemother*. **9**(4): 690-695.

Fong, I.W., Engelking, E.R. and Kirby, W.M. (1976). Relative inactivation by *Staphylococcus aureus* of eight cephalosporin antibiotics. *Antimicrob. Agents Chemother*. **9**(6): 939-944.

Fowler, W., Rahim, G. and Brown, J.D. (1978). Clinical experience in the use of cefuroxime in gonorrhoea. *Br. J. Vener. Dis*. **54**(6): 400-402.

Gower, P.E. and Dash, C.H. (1977). The pharmacokinetics of cefuroxime after intravenous injection. *Eur. J. Clin. Pharmacol*. **12**(3): 221-227.

Hamilton-Miller, J.M., Newton, G.G. and Abraham, E.P. (1970). Products of aminolysis and enzymic hydrolysis of the cephalosporins. *Biochem J*. **116**(3): 371-384.

James, E.F., Anne, V.P. and Sean, C.S. (1996). *Martindale, The Extra Pharmacopoeia*, Royal Pharmaceutical Society, 31st Ed., p.197.

Jaime, N.D. and William, A.R. (1991). *Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry,* J. B. Lippincott Co., Philadelphia, 9th Ed, p.255-256.

Joel, G.H., Lee, E.L., Perry, B.M., Raymond, W.R., Alfred, G.G. (1996). *Goodman and Gillman's the Pharmacological Basis of Therapeutics*, The McGraw Hill Co., Inc., 9th Ed., p.1089.

Jones, R.N., Fuchs, P.C., Gavan, T.L., Gerlach, E.H., Barry, A.L. and Thornsberry, C. (1977). Cefuroxime, a new parenteral cephalosporin: collaborative *in vitro* susceptibility comparison with cephalothin against 5,887 clinical bacterial isolates. *Antimicrob. Agents Chemother*. **12**(1): 47-50.

Kariyone *et al.* (1970). Cefazolin, a new semisynthetic cephalosporin antibiotic. I. Synthesis and chemical properties of cefazolin. *J. Antibiot*. **23**(3): 131-136.

Knothe, H. and Dette, G.A. (1983). The current state of cephalosporin antibiotics: microbiological aspects. *Infection*, **11**(S1): S12-15.

Koniwhi, N., Kurita, M., Jp., Mera, M., Nakagawa, R., Nikaido, H., Takano, A. and Takano, T., Jp (1970). 3,7-Disubstituted Cephalosporin Compounds and Preparation thereof. Patent Assignee(s): Fujisawa Pharmaceutical Co. Ltd., Japan. US Patent No.3516997.

Kozatani, J., Okui, M., Noda, K., Ogino, T. and Noguchi, H. (1972). Cefazolin, a new semisynthetic cephalosporin antibiotic. V. Distribution of cefazolin- 14C in mice and rats after parenteral administration. *Chem. Pharm. Bull.* (Tokyo), **20**(6): 1105-1113.

Lorian, V. (1986). *Effect of Low Antibiotics Concentration, In Antibiotics In Laboratory Medicine*: Ed. 2, edited by Lorian V., Williams and Wilkins, Baltimore, pp.596-661.

Lorian, V. (1991). *Antibiotics In Laboratory Medicine*, Williams and Wilkins, U.S.A., 3rd Ed., pp.137-39.

Lossick, J.G., Thompson, S.E. and Smeltzer, M.P. (1982). Comparison of cefuroxime and penicillin in the treatment of uncomplicated gonorrhea. *Antimicrob. Agents Chemother*. **22**(3): 409-413.

Merck Index (1999). CD version. Hampden Data Services. Merck & Co. Inc. White House Station, NJ, USA. Published on CD-ROM by Chapman & Hall/CRC. Monograph number1967.

Ibid. (1999a). Monograph number2002.

Moellering, R.C. and Swartz, M.N. (1976). Drug therapy: The newer cephalosporins. *N. Engl. J. Med.* **294**: 124-128.

Nakano, H. (1977). In: "Pharmacological and Biochemical Properties of Drug Substances", Vol. 1, M.E. Goldberg, Ed. (Am. Pharm. Assoc., Washington, DC, p.155-182.

Neu, H.C. and Fu, K.P. (1978). Cefuroxime, a beta-lactamase-resistant cephalosporin with a broad spectrum of gram-positive and -negative activity. *Antimicrob. Agents Chemother*. **13**(4): 657-664.

Nishida, M., Matsubara, T., Murakawa, T., Mine, Y. and Yokota, Y. (1970). Cefazolin, a new semisynthetic cephalosporin antibiotic. II. *In vitro* and *in vivo* antimicrobial activity. *J. Antibiot*. **23**: 137-148.

Nishida, M., Matsubara, T., Murakawa, T., Mine, Y., Yokota, Y., Goto, S. and Kuwahara, S. (1970[a]). Cefazolin, a new semisynthetic cephalosporin antibiotic. III. Absorption, excretion and tissue distribution in parenteral administration. *J. Antibiot.* **23**: 184-194.

Norrby, R., Foord, R.D. and Hedlund, P. (1977). Clinical and pharmacokinetic studies on cefuroxime. *J. Antimicrob. Chemother*. **3**(4): 355-362.

O'Callaghan, C.H, Sykes, R.B., Griffiths, A. and Thornton, J.E. (1976). Cefuroxime, a new cephalosporin antibiotic: activity *in vitro*. *Antimicrob. Agents Chemother*. **9**(3): 511-519.

O'Callaghan, C.H., Sykes, R.B., Ryan, D.M., Foord, R.D. and Muggleton, P.W. (1976[a]). Cefuroxime - a new cephalosporin antibiotic. *J Antibiot*. (Tokyo), **29**(1): 29-37.

Ryan, D.M., O'Callaghan, C. and Muggleton, P.W. (1976). Cefuroxime, a new cephalosporin antibiotic: activity *in vivo*. *Antimicrob. Agents Chemother*. **9**(3): 520-525.

Sabath, L.D., Wilcox, C., Garner, C. and Finland, M. (1973). *In vitro* Activity of cefazolin against recent clinical bacterial isolates. *J. Infect. Dis*., **128**: S320-326.

Samson, S.M., Belagaje, R., Blankenship, D.T., Chapman, J.L., Perry, D., Skatrud, P.L., VanFrank, R.M., Abraham, E.P., Baldwin, J.E. and Queener, S.W. (1985). Isolation, sequence determination and expression in *Escherichia coli* of the isopenicillin N synthetase gene from *Cephalosporium acremonium*. *Nature*, **318**(6042): 191-194.

Schulz, E., Sack, K., Freiesleben, H. and Henkel, W. (1978). Therapeutic studies on two different models of experimental pyelonephritis (author's transl). *Zentralbl. Bakteriol*. [Orig A], **242**(2): 206-215.

Shibata, K. and Fujii, M. (1970). Clinical Studies of Cefazolin in the Surgical Field. *Antimicrobial Agents Chemother.* **10**: 467-472.

Thomas, N. (1988). *Medicinal Chemistry, A. Biochemical Approach*, Oxford University Press, New York, 2nd Ed, p.361.

Washington, J.A. (1985). *Susceptibility Test: Agar Dilution, In manual of Clinical Microbiology*, Ed 4, Eddited by Lennette, E.H., Balows, A., Hausler, W.J. and Shadomy, H.J., American Society for Microbiology, Washington D.C., pp.967-971.

Wozniak, T.J. and Hicks, J.R. (1991). Cefuroxime. *Anal. Profiles Drug Subs*. **20**: 209-236.

Zappala, A.R., Holl, W.W. and Post, A. (1975). Cefazolin in *Anal. Profiles Drug Substances.* Vol. 4, Klaus Florey Ed., Academic Press, N.Y., p.1-20.