CHAPTER 2

REVIEW OF LITERATURES

A. CEFTRIAXONE

History :[26]

In 1945, Brotzu discovered that moulds of the species of Cephalosporium acremonium possesed a remarkable antibacterial activity. The most striking properties of this antibiotic was its stability in the presence of beta-lactamase produced by Staphylococcus aureus. Abraham and Newton described the chemical structure of this penicillinase-stable compound named Cephalosporin C, in 1961.[26]

The basic structure of this new antibiotic was the 7-amino cephalosporinic acid (7 ACA). A great number of semi-synthetic derivatives with substitution in position 3 or 7 of the beta-lactam ring (see figure 1 page 4) led in the 1960s to some much more active cephalosporin derivatives, such as cephalothin and cephaloridin. Derivatives with a broader spectrum, ie. covering a wide range of Gram-positive and Gram-negative pathogens, were developed in the early 1970s, among them, cefazolin, cefuroxime, cefamandole and cefoxitin. These are the second generation drugs after the first mentioned above. Between 1976 and 1978, a series of more potent derivatives, with a greater stability in the presence of beta-lactamases of Gram-negative rods than preyious compounds, were characterized by the aminothiazol-methoxyimino-acyl side chain on position 7 : cefotaxime, cefmenoxime, ceftizoxime, ceftazidime

and ceftriaxone, the third generation cephalosporins (already mentioned in page 6 chapter 1)

Ceftriaxone was synthesized by Riener in 1978. This new cephalosporin has a 6-hydroxy-2-methyl-5-oxo-as-triazin-3-thiomethyl side chain in position 3 (see figure 2 page 13) which adds to the properties mentioned above a greater potency than any pre-existing antibiotics against pathogens such as Neisseria sp., Haemophilus sp. and Proteus mirabilis. This cephalosporin has a very prolonged persistence of the unchanged active antibiotic in the human organism, especially at the site of infection.

Ceftriaxone had been tested both in-vitro and in vivo for its activity, its efficacy and safety to be used.[27] There are more than 400 publications of ceftriaxone up to the end of 1982.[27] It was proved to be a safe and highly active antibiotic. Currents of clinical studies of ceftriaxone in therapeutic use were reported. The followings are the details of ceftriaxone in every view.

Chemistry of Ceftriaxone

Ceftriaxone is a new semisynthetic cephalosporin with specific side chains. It is the new aminothiazolyl-oxyimino-acetamido-cephalosporin which is different from other cephalosporins in the nature of the substituent at position 3 of the nucleus (see figure 2).

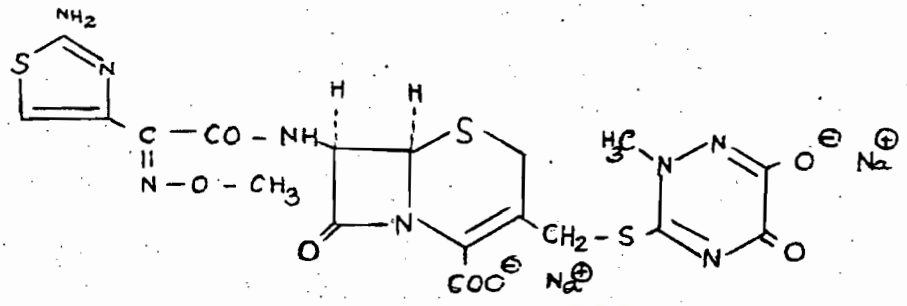


Figure 2[28] Structure of Ceftriaxone Sodium (Ro B-9904)

Ceftriaxone's chemical name,

(±)-(6R, 7R)-7- [2-amino-4- thiazolyl)-2-(methoxyimino) acetamido] -3- { [(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-triazin-3-yl) thio] methyl } -8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid disodium salt.[29]

Molecular formula[30] : $C_{18} H_{16} N_8 Na_3 O_7 S_3$

Molecular weight[30] : 598.5

Ceftriaxone Sodium is a faintly coloured crystalline compound. The solubility is excellent in water, fairly good in methanol and poor in ethanol. In a 12 % solution, pH ranges from 6.0 to 8.0 and a 10 % solution gives a pH range of 5.5 to 7.5.

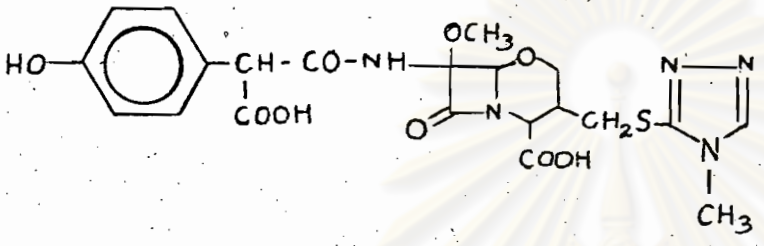
The three pKa values are between 2.0 and 4.5.[30,31,32]

Structure-Activity relationship :

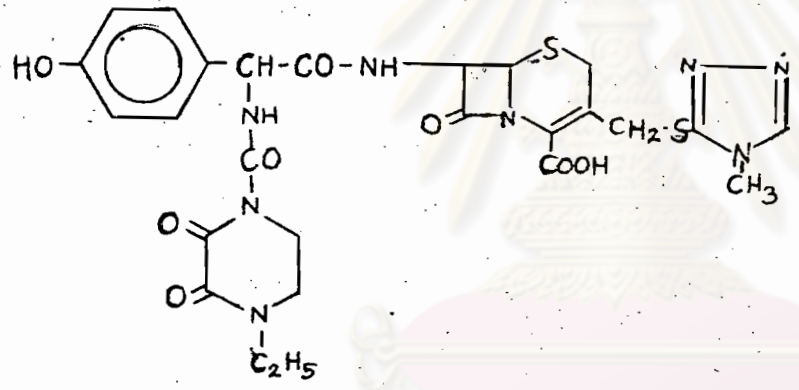
The aminothiazole-methoxymino-acyl side chain is responsible for the good beta-lactamase stability of this preparation. The absence of a methyl-tetrazol thiol side chain means that alcohol tolerance is unaffected. In this, ceftriaxone differs from lamoxactam, cefamandole,

cefoperazone and some of the newer cephalosporins, in which the presence of this side chain can cause an effect similar to disulfuram after administration of alcohol[30] (see figure 3).

Figure 3 The newer cephalosporins with a methyl-tetrazolthiol side chains.



moxalactam
(lamoxactam)



cefoperazone

The hydroxyl group in the triazine remnant, which forms and enolate, enhances the penetrating ability of the substance, particularly against gram-negative micro organisms and is responsible for its long elimination half life.[30,31,32]

Composition and stability

Ceftriaxone is presented in the form of the disodium salt. The contents of the vials of Rocephin® are indicated as the amount of the

active ingredient, ceftriaxone, present in the form of free acid.[33] The shelf-life of the dry substance is two years when kept below 25° C.[33]

After reconstitution, the solution remains stable for 24 hours at 5° C and for at least 6 hours at room temperature without loss of activity.[33]

Antibacterial Properties of Ceftriaxone

Ceftriaxone is a broad-spectrum antibiotic with remarkable activity against most Gram-negative bacteria, including those resistant to cefuroxime, cefamandole, cefoxitin, and cefazolin.[33] The antibacterial properties of ceftriaxone were investigated both in vitro and in vivo. It showed a good activity against Enterobacteriaceae and Gram-positive cocci including

Streptococcus pneumoniae,

S. pyogenes, except *S. faecalis*,[4]

and while *Haemophilus influenzae*

Neiseria gonorrheae and

N. meningitidis were extremely

susceptible to ceftriaxone (also see table 6p. 19)

Bactericidal properties of ceftriaxone were comparable to those of moxalactam[34] and cefotaxime,[28] the new third generation cephalosporins. These agents are active against the following organisms :-

Escherichia coli ,

Klebsiella pneumoniae ,

Enterobacter aerogenes ,

Citrobacter species ,

P. inconstans ,

Salmonella Species , and

Shigella Species while ceftriaxone is the most active compound against Proteus mirabilis (see table 6p 19).

The majority of Enterobacter cloacae ,

Morganella spp. and

Proteus vulgaris were inhibited but a few were resistant because of β-lactamase hydrolysis.[34] The comparative studies of ceftriaxone and other antibiotics in vitro and in yivo were done by Angehrn[28] and Neu, et al[35] and many other investigators.[36,37,38]

Ceftriaxone showed better therapeutic efficacy in mice against Pseudomonas aeruginosa than cefotaxime, cefoperazone, azlocillin and piperacillin[28] although it has just only moderate activity against the microorganisms[28] (see table 6,8,9)

Ceftriaxone is slightly less active than other cephalosporins against Staphyllococcus aureus but was extremely active against pneumococci and beta-hemolytic group A and B Streptococci, which were inhibitted at 0.12 µg/ml and 0.06 µg/ml respectively.[37] In the 39 gentamicin resistant strains, in Spain, ceftriaxone was somewhat less active than cefotaxime.[37] (see table 5p,18.).

The followings are the laboratory results of the in yitro and in yivo activities of ceftriaxone obtained from various investigation[23,28,36,37,38] and some more datae of the drug effects on the Gram-negative bacilli were shown in table 12., 14 and 15

Table 4[37] (in vitro)

Susceptibility of different clinical isolates to ceftriaxone

Organism (number of strains)	Cumulative percentage inhibited at concentration (µg/ml) of :												
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>
Salmonella spp.(85)	69.4%		97.6%	100%									
Serratia marcescens(44)		31.8%		61.3%	90%	94.5%		100%					
E. coli(19)	63.1%		100%										
Ps. aeruginosa(19)								18.1%	26.3%	52.6%	68.4%	84.1%	100%
H. influenzae(22)	95.6%	100%											
Enterobacter cloacae(2)	100%												
S. aureus(47)			4.2%			14.9%	72.3%	85.7%				98%	100%
Pneumococcus(17)	76.4%	100%											
Beta-hymolytic group A and B streptococci(9)	100%												
Viridans streptococci(1)						100%							

Table 5[37] (in vitro)

Susceptibility of 39 gentamicin resistant strains to different antimicrobials :-

Drug	Cumulative percentage inhibited at concentration (µg/ml) of :											
	≤0.25	0.5	1	2	4	8	16	32	64	128	256	>
Cefoxitin					10.2%	25.6%			35.8%		38.4%	100%
Cefotaxime	18.7%	34.3%	71.7%	81.1%	84.2%		87.3%	93.4%		100%		
(Rocephin)	41%	56.3%	71.7%	74.2%	79.3%	82%		87%		89.5%		100%
Amikacin		5.2%	48.6%	58.9%	76.8%			79.6%	89.6%	100%		

Table 6[28]

In vitro activity of Ro 13-9904 and other β-lactam antibiotics (MIC, µg/ml) from Angehrn/Probst.

Organism	Ro 13-9904	cefotaxime	cefoperazone	cefazolin	piperacillin
E.cloacae 47	1.6	1.6	6.3	>100	12.5
E.coli 1346	0.003	0.003	0.05	0.8	0.4
E.coli 25922	0.05	0.05	0.2	1.6	1.6
H.influenzae 11	0.003	0.012	0.05	1.6	6.3
H.influenzae 4347	0.006	0.012	0.05	6.3	0.025
K.pneumoniae 418	0.1	0.05	0.8	3.1	12.5
N.gonorrhoeae 5	0.0008	0.003	0.08	1.6	0.04
N.meningitidis 13077	0.0016	0.003	0.04	0.4	0.02
P.mirabilis 2117	0.006	0.025	3.1	12.5	>100
P.vulgaris 1028	3.1	3.1	12.5	>100	25
P.aeruginosa BA 34	6.3	12.5	6.3	>100	3.1
P.aeruginosa 143724	12.5	25	6.3	>100	6.3
P.aeruginosa 143811	6.3	6.3	6.3	>100	6.3
P.aeruginosa PA 791	3.1	6.3	1.6	>100	1.6
S.marcescens70147	0.4	0.4	3.1	>100	>100
S.aureus Schoch	1.6	0.8	3.1	0.2	0.4
S.aureus 887	3.1	1.6	3.1	0.4	100
S.faecalis 6	>100	>100	>100	50	3.1
S.pncumoniae BA	0.05	0.05	0.4	0.2	0.1
S.pyogenes 15	0.05	0.05	0.8	0.2	0.2

Table 7[36] (in vitro)

The effect of increasing the inoculum size on the minimum inhibitory concentration (mg. per Litre)

Microorganism	Characteristic	ceftriaxone		ceftazidime		cefoperazone		cefotaxime	
		10^5	10^7	10^5	10^7	10^5	10^7	10^5	10^7
E.coli	β-lactamase negative	1	1	1	1	1	2	1	2
E.coli	β-lactamase positive	1	1	1	1	2	512	2	4
K.pneumoniae	β-lactamase positive	4	4	2	8	1	64	2	4
S.aureus	β-lactamase negative	1	1	2	2	1	1	1	1
S.aureus	β-lactamase positive	2	2	1	2	2	4	1	1

Table 8[28] (in vivo)

Protective activity of Ro 13-9904 and other β-lactams antibiotics against systemic infections of the mouse. (ED_{50}, mg/kg sub. cut. x 2)

Organism	Ro 13-9904	cefotaxime	cefazolin	piperacillin
E.coli 1346	0.002	0.003	2.5	0.07
K.pneumoniae 418	0.14	0.18	8.4	8.6
P.mirabilis 2117	0.01	0.04	12	15
S.vulgaris 1028	1.5	1.5	>50	25
S.marcescens 70147	0.18	0.28	>50	>50
S.aureus Schoch	4.7	2.1	0.55	1.8
S.pneumoniae BA	<0.10	0.20	NT	NT
S.pyogenes 15	0.05	0.03	0.22	0.22

Compounds were administered 1 and 3 h after infection (except against S.pneumoniae BA : 1 and 5 h after infection), NT = not tested.

Table 9[28] (invivo)

Protective activity against pseudomonal septicemia of the mouse (ED_{50}, mg/Kg s.c. x 3)

Strain	Ro 13-9904	cefotaxime	SCE-1365	cefoperazone	azlocillin	piperacillin
P.aeruginosa						
BA 34	28	74	141	71	114	50
143724	38	117	141	113	117	74
143811	14	35	55	41	28	23
PA 791	8.5	38	85	28	44	34

The pathogens which are moderately sensitive and resistant to ceftriaxone in vitro were shown in table 10 and 11

Table 10[33]

Pathogens which are moderately sensitive tc ceftriaxone

Pathogen	MIC 50 µg/ml	MIC 90 µg/ml
S.epidermidis	31	25
S.faecalis (group D, Streptococci)	128.0	>128.0
Listeria monocytogenes	12.5	25.0
Ps.aeruginosa	16.0	64.0
Acinetobacter spp.	16.0	64.0
Chlamydia spp.	4.0	16.0
Bacteroides spp.	4.0	32.0

Table 11[33]

Microorganisms resistant to Ceftriaxone

Ureaplasma urealyticum
Mycoplasma spp.
Mycobacterium spp.
Fungi

Effects of ceftriaxone on Shigella

There are some useful reports of the drug's effect on Shigella, these were obtained by the following investigators ;-

Shelton et al had reported the in vitro susceptibility of 94 strains of Shigella and various Gram-negative bacilli which were isolated from paediatric patients in Dallas, Texas, USA[39]. The MICs of antibiotics tested against them were shown in table 12 and 13

Ceftriaxone, in comparison with other third generation cephalosporins available in Thailand, as well as cefotaxime showed the best in vitro activity against Shigella spp. (figure 9[23])

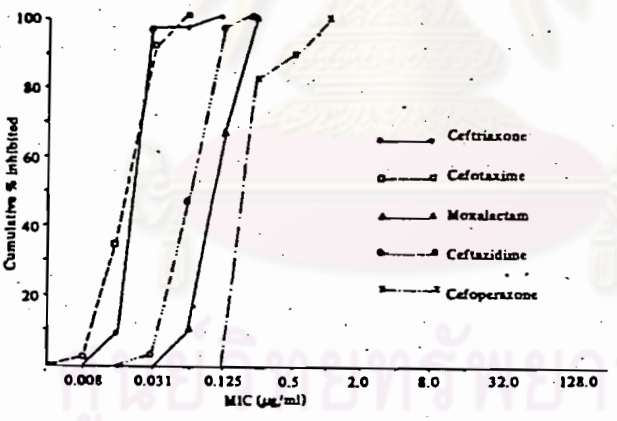


Fig. 9[23] Comparative activities versus *Shigella spp.* (40 strains).

Table 12[39]

MICs of antibiotics tested against various Gram-negative bacilli, Shelton et al, Dallas, Texas, USA

Bacteria	No.of strains tested	Modal MIC (µg/ml)										
		LY	Ro	CTX	CFT	CFM	CFX	GEN	KAN	AMI	NET	AMP
Salmonella spp.	49	0.12	0.06	0,06	2,5	0.62	10	1,25	5	2,5-5	0,62	0.62
Shigella spp.(ampicillin-resistant)	44	0.06	0.015	0,03	2.5	2.5-5	5	1,25	5	5	1,25	>20
Shigella spp.(ampicillin-susceptible)	50	0.06	0.015	0,03	2.5	0,31	2,5	1,25	5	5	0,62	5
Haemophilus spp.(β-lactamase-negative)	33	0.03	≤0.004	0,08	1,25	0.31	0,31	NT	NT	NT	NT	0.31
Haemophilus spp.(β-lactamase-positive)	27	0,015	≤0.004	≤0,04	0.62	0,31	0,31	NT	NT	NT	NT	10 0,31
Gonococci(β-lactamase-negative)	25	0.008-0.015	0.008	≤0.08	0.31	NT	0,015	NT	NT	NT	NT	NT
P.aeruginosa	31	8-16	16	16	NT	NT	NT	NT	NT	NT	NT	NT
Coliform bacilli (aminoglycoside-resistant)	57	0,06	0.03	0.06	2,5	1,25-10	2,5	>10	>20	2,5	>10	NT
Coliform bacilli (meningitis strains)	116	0.06	0,03	0,06	2.5	0,62	5	1,25	5	25	0,31	2.5

LY, Moxalactam; Ro. Ro13-9904; CTX cefotaxime; CFT cefoxitin; CFM cefamandole; CFX cefuroxime; GEN gentamicin; KAN kanamycin; AMI amikacin; NET netilmicin; AMP ampicillin; NT not tested.

Table 13[39]

MIC for 90 % of strains (Shelton et al)

Bacteria	No.of strains tested	MIC (µg/ml)*										
		LY	Ro	CTX	CFT	CFM	CFX	GEN	KAN	AMI	NET	AMP
Salmonella spp.	49	0.12	0.06	0.08	5	2,5	10	5	5	5	1.25	2,5
Shigella spp.(ampicillin-resistant)	44	0.25	0.03	0.08	5	10	5	1.25	10	10	1,25	>20
Shigella spp.(ampicillin-susceptible)	50	0,25	0,03	0.08	5	0,31	5	1.25	10	5	1,25	5
Haemophilus spp.(β-lactamase-negative)	33	0,03	≤0,004	0.08	2.5	0,62	0,62	NT	NT	NT	NT	0,31
Haemophilus spp.(β-lactamase-postive)	27	0.03	≤0,004	0,08	2,5	0,62	0,62	NT	NT	NT	NT	10
Gonococci(β-lactamase-negative)	25	0.06	≤0,008	≤0.08	0,31	NT	0,16	NT	NT	NT	NT	NT
P.aeruginosa	31	>16	>16	>16	NT	NT	NT	NT	NT	NT	NT	NT
Coliform bacilli (aminoglycoside-resistant)	57	0.12	0.12	0.1	10	>20	20	>10	>20	5	>10	NT
Coliform bacilli (meningitis strains)	116	0.25	0,06	0.1	10	>20	>20	2.5	20	5	0,62	>20

* See Table 12's footnote.

The other reports of ceftriaxone activity against 10 strains of shigellae were done by Angehrn et al[25] and Lolekha et al[23] as shown in table 14, 15

Table 14[25]

The susceptibity of Shigella and some other Gram-negative bacilli to ceftriaxone as reported by Angehrn et al.[14]

Organism(no.of strains)	Compound	MIC range (µg/ml)	50 % MIC (µg/ml)	90 % MIC (µg/ml)
P.vulgaris(10)	Ro 13-9904	0.012-12.5	0.05	1.6
	Cefotaxime	0.025-12.5	0.1	1.6
	Cefuroxime	1.6->100	12.5	>100
	Cefoxitin	1.6-25	6.3	12.5
	Cefamandole	0.8->100	6.3	100
	Cefazolin	3.1->100	100	>100
Salmonella sp. (14)	Ro 13-9904	≤0.012-0.4	0.05	0.2
	Cefotaxime	≤0.012-0.2	0.05	0.2
	Cefuroxime	0.1-6.3	1.6	3.1
	Cefoxitin	0.8-6.3	1.6	1.6
	Cefamandole	0.1-1.6	0.4	0.8
	Cefazolin	0.8-1.6	1.6	1.6
Shigella sp. (10)	Ro 13-9904	≤0.012-0.2	0.05	0.05
	Cefotaxime	≤0.012-0.2	0.05	0.05
	Cefuroxime	0.4-3.1	1.6	3.1
	Cefoxitin	1.6-12.5	3.1	6.3
	Cefamandole	0.1-6.3	0.4	1.6
	Cefazolin	1.6-12.5	1.6	6.3

Table 15

The susceptibility of bacteria to ceftriaxone, reported by Lolekha, S. et. al[23], Ramathibodi Hospital Thailand,

Organisms (no. strains tested)	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Escherichia coli (60)	0.008-0.125	0.029	0.057
Klebsiella spp. (50)	0.016-8.0	0.043	1.58
Enterobacter spp. (27)	0.008-4.0	0.035	1.58
Proteus mirabilis (26)	<0.008-0.008	0.008	0.008
Indole-positive *Proteus* (23)	0.008-0.062	0.008	0.02
Shigella spp. (40)	0.016-0.125	0.022	0.03
Salmonella spp. (24)	0.031-0.5	0.046	0.288
Aeromonas hydrophila (29)	0.016-4.0	0.034	0.814
Acinetobacter spp. (19)	2.0-32.0	11.62	24.34
Pseudomonas aeruginosa (45)	1.0-128	13.99	30.8
Staphylococcus aureus (55)	2.0-32.0	2.84	3.85

Mechanism of action of Ceftriaxone

Ceftriaxone, like other more recent cephalosporins, shows a strong affinity for the penicillin-binding proteins (PBPs),[40,41] Blockage of these substances causes filament formation and eventually lysis of bacteria.[41] Ceftriaxone induces filamentous transformation of bacteria at subinhibitory concentrations and exerts bactericidal activity at levels that are not significantly above the MIC.[41]

Synergism and antagonism[33]

The combination of ceftriaxone and an amino glycoside has a marked synergistic effect against strains such as Pseudomonas aeruginosa and Streptococcus faecalis.[33] When ceftriaxone is combined with gentamicin, netilmicin, tobramycin or amikacin, the MIC is reduced from $\geq$ 16 μg/ml to $\leq$ 8 μg/ml against 14 of the 21 strains of P. aeruginosa.[33]

The antagonistic reactions of cefoxitin on ceftriaxone and other cephalosporins were noted, particularly, it completely inhibits the action of ceftriaxone against P. aeruginosa and Enterobacter cloacae.

Stability to β-lactamases[14]

Ceftriaxone is very stable to various type of β-lactamases as shown in table 15-1[14]

Table 15-1[14]

β-Lactamase stability of ‹Rocephin› compared with other cephalosporins and penicillin G

Species	β-Lactamase type	Relative rate of hydrolysis						References
		‹Rocephin›	penicillin	cefaloridine	cephalothin	cefuroxime	cefotaxime	
Citrobacter freundii 43	I	0	100		910	12	0	16
Ps. aeruginosa 143738	I	0	100		526	0	0	
E. cloacae 908	I_A	0	100		800	0	0	
P. vulgaris 1028	I_C	920	100		1,000	2,000	450	
Ps. aeruginosa SH	I_D	0	100		500	<1	0	
E. coli R^-_{TEM}	TEM	<1	100		21	1	<1	16
K. pneumoniae NCTC 418	II	0	100		3	0	0	
St. aureus 887	Penicillinase	0	100		0	0	0	
B. fragilis	Cephalosporinase	128		100	140		76	11
S. marcescens	Cephalosporinase	8		100	125		0	

Antibiotic sensitivity testing[33]

From the basis of the MIC values determined for 61 clinical isolates and comparison of plasma and connective tissues concentration after administration of standard doses of 1-2 g ceftriaxone, the

bacterial sensitivity was classified as follows :-

MIC ≤ 16 µg per ml ; sensitive

MIC 17 - 63 µg per ml : moderately sensitive

MIC ≥ 64 µg per ml ; resistant

The ceftriaxone discs containing 30 µg per disc are available in the agar diffusion test. The diameter of the inhibition zones obtained with these discs can be correlated with the minimum inhibitory concentrations (MIC) determined. The relationship is illustrated by the regression curve shown in figure 4,

On the basis of the above-mentioned limit values for the ranges of sensitivity, the pathogens tested using the discs can be rated as follows :-

inhibition zone diameter (mm.)	interpretation
≥ 16	sensitive
13 - 15	moderately sensitive
≤ 12	resistant

The limit values of 16 and 64 µg per ml, determined by various criteria and methods, are higher than the usual values obtained for the other cephalosporins. The reason for this is to be found in the exceptional pharmacokinetics of ceftriaxone. Twenty-four hours after administration of the standard doses of 1 or 2 grams ceftriaxone

(IV or IM), concentrations of 20 and 40 µg respectively of the unchanged substance per ml can still be measured in plasma or interstitial fluid.[33]

The long duration of such high concentrations means that pathogens only slightly sensitive in vitro (with higher MIC values), for example P. aeruginosa and Acetobacter, can also be inhibited. The differences in range of bacterial sensitivity between ceftriaxone and other cephalosporins make it inadmissible to substitute sensitivity testing of other cephalosporins by the diffusion and dilution.[33]

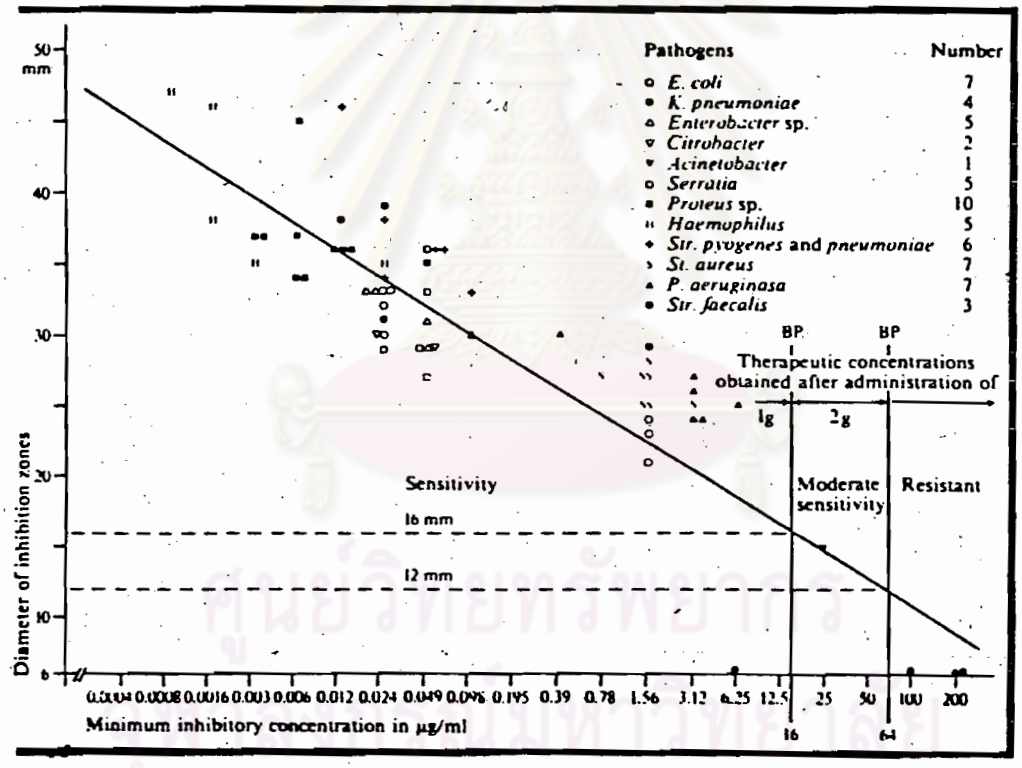


Figure 4 Regression curve for (Rocephin) (30 µg disk) on DST agar. Breakpoints (BP) determined on the basis of (Rocephin) concentrations in interstitial fluid after administration of 1 or 2 g i.v.

Clinical efficacy and tolerance of Ceftriaxone : L. Hayas et al have analyzed the literature of the clinical efficacy and tolerance of ceftriaxone in 3,961 cases.[27]

Eighty-one papers deal with clinical findings obtained in 3,961 patients in total serve as the basis of literature survey on the efficacy and tolerance of ceftriaxone. Ceftriaxone was investigated in non comparative and open comparative trials performed in 71 trial centers in 21 countries. The distribution of the trial centers are shown in table 15-2[27]

Table 15-2[27]

Distribution of ceftriaxone trial centers.

Country	No. of trial centers
1. Italy	14
2. USA	13
3. Switzerland	8
4. France	6
5. West Germany	5
6. Austria	4
7. Belgium	3
8. Argentina	2
9. Brazil	2
10. Finland	2
11. Grece	2
12. other 10 countries	10
total	71

Of the 3,961 cases reported,

2,832 patients were treated with ceftriaxone and

1,129 patients received reference drugs.

Approximately 20 % of the patient received ceftriaxone for severe, life threatening infections such as septicaemia and septic conditions or meningitis; one-third for severe urinary tract or respiratory tract infections, including pneumonia; 10 % for pre-and post-surgical infections; and more than one quarter for gonorrhea see fig 5.[27]

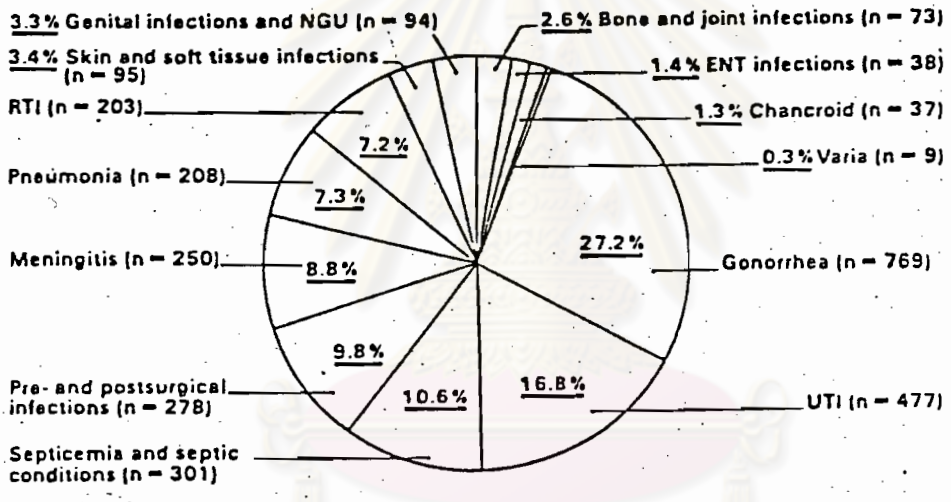


Fig. 5[27] Analysis of the main indications and numbers of patients treated with ceftriaxone (n = 2,832).

The therapeutic efficacy of ceftriaxone was assessable in 2,715 patients who received the antibiotic for 21 different infectious diseases[27] (Table 16). Most of the patients were treated with ceftriaxone alone; some others suffering from severe septic conditions received a combi-

TABLE 16 [27]

Patients treated with ceftriaxone, grouped according to indication (compiled from 81 publications and presentations)

Indication	No. of papers	No. of:		Results					Success rate (very good and good results)
		treated patients	evaluable patients	++	+	(+)	–	?	
1) ENT infections	3	38	38	28	9	–	1	–	97%
2) Respiratory tract infections	15	203	202	128	56	7	11	1	91%
3) Pneumonia	20	208	194	138	41	2	13	14	92%
4) Urinary tract infections	30	477	475	378	58	25	14	2	92%
5) Gonorrhea	11	769	690	679	2	–	9	79	99%
6) Urethritis (nongonorrheal)	2	74	74	63	–	–	11	–	85%
7) Chancroid	2	37	37	36	–	–	1	–	97%
8) Prostatitis, orchiepididymitis	2	4	4	3	1	–	–	–	
9) Genital infections	3	16	15	14	1	–	–	1	
10) Pre- and postsurgical inf. (surg./urol./gyn.)	10	206	204	172	13	–	19	2	91%
11) Bone and joint infections	9	73	73	59	5	–	9	–	88%
12) Peritonitis (local and gen.)	5	43	41	33	4	3	1	2	90%
13) Abdominal abscess, empyema	4	18	18	8	6	–	4	–	
14) Biliary tract infections	5	11	11	11	–	–	–	–	
15) Enteritis, diverticulitis	2	2	2	1	1	–	–	–	
16) Skin and soft tissue infections	14	95	95	68	16	–	11	–	88%
17) Septicemia and septic conditions	21	289	278	245	14	5	14	11	93%
18) Endocarditis, pericarditis	1	2	1	1	–	–	–	1	
19) Typhoid fever	5	10	10	8	–	–	2	–	
20) Meningitis	15	250	250	170	23	–	57	–	77%
21) Varia	2	7	3	3	–	–	–	4	
Total		2,832	2,715						

nation of ceftriaxone plus aminoglycosides, In gonorrhea, ceftriaxone was adminiatered in a dose of 32-500 mg as one-shot therapy. In the other indications the daily dosage varied between 250 mg and 4,000 mg given mostly once or twice daily by IM. or IV. injection or in IV. infusion. The duration of therapy varied between one and 182 days, in most cases lasting 1-2 weeks.[27]

The criterial for successes (very good or good results) were disappearance of or improvement in signs and symptoms of the infection with or without reinfection during the post-therapy follow up period,[27] The result was considered moderate if the initial pathogens were eliminated during therapy but recurred during the follow up period. 'Failure' denotes an unsatisfactory response to therapy.

The success rate for patients treated with ceftriaxone ranged in the main indications from 77 % (meningitis) to 99 % (gonorrhea) and these are shown in table.[27]

TABLE 17[27]

Success rate (very good and good results) of ceftriaxone therapy in the main indications

Indication	Success rate	No. evaluable patients
Meningitis	77%	250
Urethritis (nongonorrheal)	85%	74
Bone and joint infections	88%	73
Skin and soft tissue infections	88%	95
Peritonitis (local and gen.)	90%	41
Respiratory tract infections	91%	202
Pre- and postsurgical infections	91%	204
Pneumonia	92%	194
Urinary tract infections	92%	475
Septicemia and septic conditions	93%	278
ENT-infections	97%	38
Chancroid	97%	37
Gonorrhea	99%	690

TABLE 18[27]

Open comparative trials: ceftriaxone versus drugs for comparison. Clinical results from individual papers

Indication	Class of antibiotics	Author(s)	Ceftriaxone group			Group with drugs for comparison			
			No. of evaluable cases	Therap. result: success*	Success* rate	Generic names of antibiotics	No. of evaluable cases	Therap. result: success*	Success* rate
Meningitis	aminopenicillins	Cadoz et al.[11]	163	111	68%	amoxicillin	137	77	56%
Pre- and postsurgical infections	chloramphenicol	Hauri et al.[27]	47	40	85%	thiamphenicol	48	34	71%
	cephalosporins	Henning et al.[28]	28	27	96%	cefazolin	30	24	80%
		Kamlot[29]	20	20	100%	cephalotin	19	16	84%
		Sequeira et al.[30]	20	19	95%	cephalotin	20	17	85%
	placebo	Childs et al.[31]	43	41	95%	placebo	49	34	69%
Pneumonia	tetracycline	Pichler et al.[32]	23	19	83%	doxycycline	22	17	77%
Bronchitis (chronic)	aminopenicillins	Seysen[33]	20	14	70%	amoxicillin	15	7	47%
	aminopenicillin or cephalosporin	Chibante et al.[34]	31	29	94%	ampicillin or cephalexin	10 10	15	75%
	cephalosporins	Montanari et al.[35]	20	19	95%	cefotaxime	20	17	85%
ENT infections	aminoglycosides	Pesavento et al.[36]	30	29	97%	gentamicin	30	28	93%

(continued)

TABLE 18(continued)

Indication	Class of antibiotics	Author(s)	Ceftriaxone group			Group with drugs for comparison			
			No. of evaluable cases	Therap. result: success*	Success* rate	Generic names of antibiotics	No. of evaluable cases	Therap. result: success*	Success* rate
UTI (chronic)	aminoglycosides	Bernstein-Hahn et al.[37, 38]	43	39	91%	gentamicin	37	22	59%
		Lentini et al.[39]	20	14	70%	"	20	9	45%
		Zattoni et al.[40]	20	18	90%	"	20	17	85%
		Seller et al.[41]	22	22	100%	tobramycin	23	12	52%
	cephalosporins	Childs et al.[42]	43	39	91%	cefazolin	42	32	76%
Gonorrhea	penicillin + probenecid	Lutz et al.[43]	21	20	95%	procaine penicillin G + probenecid	27	25	93%
	cephalosporin + probenecid	Zajdowicz et al.[44]	36	36	100%	cefoxitin + probenecid	45	44	98%
	aminoglycosides	Rufli et al.[45]	45	45	100%	spectinomycin	39	38	97%
		Rajan et al.[20]	188	185	98%	kanamycin	185	170	92%

*success = very good and good results

Tolerance of ceftriaxone[27]

The local and systematic tobrance of ceftriaxone could be analyzed in 2,832 treated cases. The incidence of adverse reactions observed during treatment of ceftriaxone (but without proof of a causal relationship between the adverse reaction and the medication) was : hematological abnormalities, 1.8 % ; skin reactions, 1.1 % ; gastro-intestinal disturbances, 1.8 % ; various, 3.2 %.[27]

The main hematological abnormalities, from a total of 52, were eosinophilia (25 cases) and thrombocytosis (15 cases), these side-effects were laboratory findings without clinical symptoms.[27]

Of 33 cases of skin reactions, most of them, 29, were skin rashes or exanthema.

Of the other side-effects (92 cases), pathological liver function (increased enzyme activity) accounted for 40 cases. The others were phlebitis (22), drug fever (12), serum creatinine or blood urea nitrogen elevation (6), local pain after injection (5) and one case each of cumarin resistance, tachycardia, chills, dizziness, numbness of the lips, sensation of rigor in the back and sterile abscess,[27]

The majority of the side-effects mentioned were slight, and disappeared within a few days, mostly during the course of treatment. Interruption of treatment was necessary in 28 cases (1 %), mostly due to skin rash, leuconeutropenia and, more rarely, gastrointestinal disturbances.[27]

Summary of clinical efficacy and tolerance[27]

The therapeutic results of 3,961 patients, described in 81 clinical papers, indicate that ceftriaxone is a safe and highly active antibiotic.[27] The once-a-day administration is proved to be the optimal dosage regimen from pharmacokinetic point of view. It may be used and may give a very good result on treatments of severe septic conditions, meningitis , venereal disease as a one-shot therapy or as a singledose prophylaxis of infection in surgery and other indicated infections.

Clinical Pharmacology[33]

The main defference between ceftriaxone and other beta-lactam antibiotics lies in its pharmacological properties. The pharmacokinetics of the other major β-lactam antibiotics is characterized by the elimination by the renal path, mostly. Then, their rate of elimination is dependent to a large extent of kidney function, and because of the high clearance rates, the elimination half life is relatively short (0.5 - 2.7 hours).[28,42] Ceftriaxone is different, its elimination half life is about 8 hours,[6] a substantial portion of it is eliminated via the bile.[33] The plasma concentration of ceftriaxone after 1 hour of 1 gm IV injection is about 120 µg/ml.[33]

In children, plasma concentrations of 227 µg per ml. were obtained 15 minutes after IV injections of 50 mg per Kg bodyweight (average value for 10 children aged from 7 months to 2 years), after sixteen hours the level had fallen to 22 µg per ml, with an elimination half life of about 6.5 hours.[33]

Protein binding[33]:

The extent of binding of ceftriaxone to plasma proteins-particularly albumin- at therapeutic concentration of up to 650 mg per ml is between 58 and 95 %.[15,43] It is a non-linear function of plasma concentration, decreasing as plasma concentration rises, at first slowly and then more rapidly.[43]

Distribution in the body[33]

After intravenous or intramuscular administration, ceftriaxone passes rapidly into the interstitial fluid and reaches to the site of numerous infections in bactericidal concentrations.

In cerebrospinal fluids of 24 patients with purulent meningitis, the peak CSF concentrations of 4,8 $\pm$ 1.7 µg/ml were measured after 6 hours after administration of a mean dose of 46 mg/Kg body weight of ceftriaxone. And after 24 hours, the concentration was still 0.7 $\pm$ 0.3 µg/ml.[44,45]

The drug can pass across the placental barrier of pregnant women.[46] After given 1.5 gm. of the drug in half hour infusion every 24 hours, the concentration in the amniotic fluid remained fairly constant for 10 hours at 2.2 to 8.2 µg/ml.[46]

Ceftriaxone gives a good therapeutic concentration in the bone tissues. (> 32 µg/ml after 30-120 minutes of 2 gram IV injection)[47]

Metabolism :

Ceftriaxone is excreted unchanged in the urine and in the bile. The portion eliminated in the bile is broken down in the feces by the

intestinal flora into inactiye compounds.[33]

Elimination

Ceftriaxone is eliminated as follows :-

a) two-thirds via the kidneys[33]

b) one-third via the gall bladder*[33]

concentration in the urine : After administration of 1,5 gm of ceftriaxone,the urine concentration is 1,044 ± 716 µg/ml[48] from the 6th to the 8th hour and 160 µg/ml in that collected from the 12th to the 24th hour.[33]

Ceftriaxone is not excreted by tubular secretion, this is demonstrated by the fact that probenecid has no effect on its elimination half-life. The drug is excreted almost entirely by glomerular filtration.

Concentration in the bile : The mean drug concentration in the bile collected between the 12th and 24th hour after 2 gm administration was 400 µg/ml.

Total Clearance[49]

Figure 6[49] shows the total clearance of ceftriaxone comparing to that of other cephalosporins, the ceftriaxone has lowest total clearance of all cephalosporins; this explains its long plasma half-life.

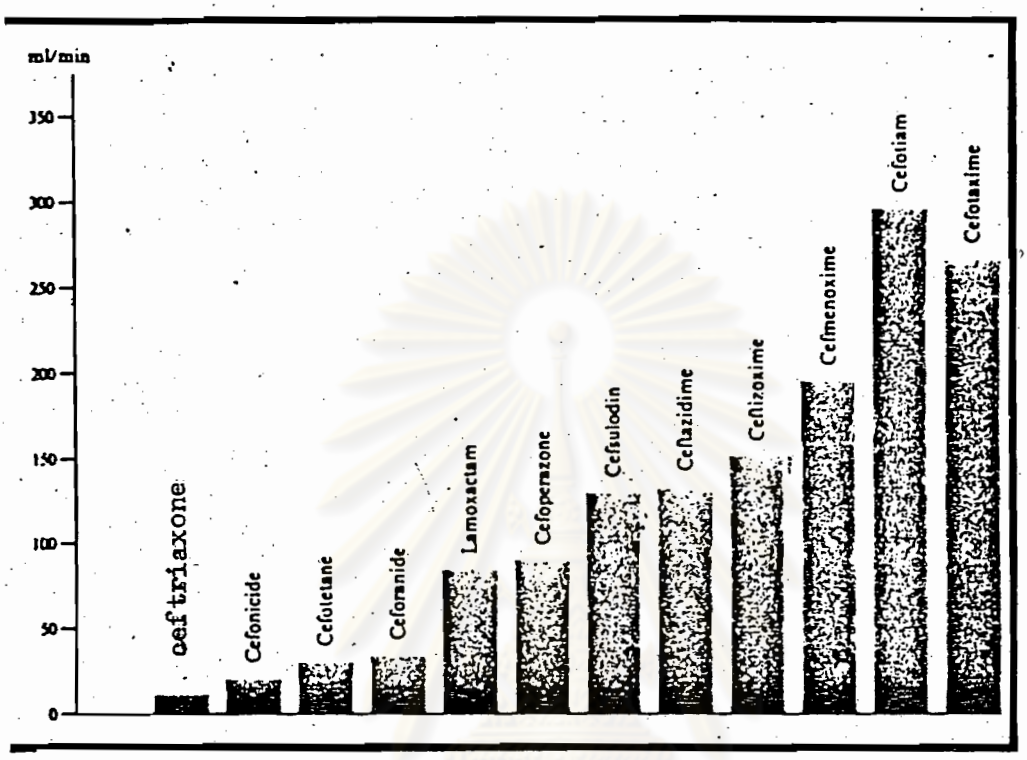


Figure 6 [49,33] Total clearance of various cephalosporins.

El... ... fficiency

a. Renal insufficiency

Findings in patients with anuria suggest that renal insufficiency does not necessitate an adjustment of the dosage as long as it does not exceed 2 gm daily. Assuming normal hepatic function, the reduced excretion via the kidneys is compensated by increased extrarenal (ie. biliary) clearance.[50,33]

b. Hepatic insufficiency[50]

The elimination half-life of patients suffering from severe parenchymal damage is not changed because the kidneys compensate this short fall. No dosage adjustment is necessary in such patients either. But, in the event of simultaneous severe renal and hepatic function disorders, the surveillance of the plasma concentration of the drug is indicated, the dosage may be reduced.

B. Shigellosis (Bacillary Dysentery)

- Definition[1] : Shigellosis is an acute bacterial disease of the intestinal tract with predominant involvement of the lower colon, sigmoid, and rectum characterized by diarrhea, abdominal cramps, tenesmus, and occasionally fever and vomiting. In severe cases the stool may contain blood mucus, and pus.

- History : Dysentery has long been a scourge of military populations and of groups living under crowded and unsanitary conditions. In the American Civil War from 1861 to 1865 a total of 1,739,135 soldiers[51] suffered from dysentery, and 44,558 died, There are two kinds of dysentery, amoebic and bacillary dysentery, and their real causes were just been clearly distinguished some years after the war. The Entamoeba histolytica was first isolated from amoebic dysentery by Lesch in 1875 and the causative organisms of bacillary dysentery were isolated by Shiga in 1898, and by Flexner, Kruse and Strong in 1900.[51]

In bacillary dysentery, Shigella dysenteriae, type 1 (Shiga's bacillus), was the most common cause in the early part of this century. Since then S. sonnei and S. flexneri, type 2, have emerged as the most commonly isolated Shigellae throughout the world.[1,51]

Bacteriology :-

Shigella is a genus of bacteria in the family of Enterobacteriaceae,

the organisms in this genus are always called the dysentery bacilli or the shigellae. Shigellae are non-motile Gram-negative bacilli which do not produce spores, capsules or gas. There are currently 34 serotypes[16,52] divided into four main groups, group A, S. dysenteriae; group B, S. flexneri; group C, S. boydii, and group D, S, sonnei. Each group is subdivided into numbered serotypes as shown in table 19.

Table 19[16,52]

The different groups and types of shigellae

Group	Organisms	Results in the tests of			No, of serotypes
		Lactose *	Manitol *	Indole	
A	S.dysenteriae	-	-	v	1-10
B	S.flexneri	-	+	v	1-6, x, y
C	S.boydii	-	+	v	1-15
D	S.sonnei	+	+	-	1

NB. * positive test means yielding acid from fermentation, V = variable

Cultures of Shigella

The organisms are usually present in large numbers in the intestinal mucous or the feces in the early stage of the disease (first 3 days[16]). At high atmospheric temperatures they survive less than do salmonellae, and may be out grown by acid-producing fecal organisms ; when feces are kept alkaline, shigellae may survive for days, but in

acid stools they die in a few hours. If there is likely to be much delay between the bedside and the laboratory, it may be desirable to collect the feces into a buffered 30 percent glycerol saline solution[51] or the proper transport medium such as the Stuart's medium.[16] The Stuart's transport medium consists of buffered semisolid agar devoid of nutrients and containing sodium thioglycollate as a reducing agent and is used in conjunction with cotton swabs, this medium maintains a favorable pH and prevents both dehydration of secretions during transport as well as oxidation and enzymatic self-destruction of the pathogen present.[16] The medium had been proved effective in preserving the viability of pathogenic agents in clinical materials.[16] When stool specimens are not readily obtainable in patients, rectal swabs afford the most practical[16] and satisfactory[51] method for securing material for culture although they should not be relied on for maximum recovery.[16]

Urine cultures and blood cultures are almost invariably negative or positive only rarely.[51]

The shigellae cultures are similar to most other enterobacteria except that on Mac Conkey or DCA (Desoxycholate Citrate Agar) or on SS agar medium (Salmonella-Shigella agar medium) they are lactose-non-fermenting after incubation for 18 to 24 hours; thus on such differential media colonies are pale and similar to Salmonella. By simple tests, shigellae can be differentiated from salmonellae since the latter almost invariably produce gas as well as acid when grown in sugar solutions and are motile. Tests for indole production will reveal that members of the genus Salmonella are persistently negative whereas some species of Shigella

have this ability; conversely H_2S production is common in salmonellae but does not occur in shigellae,[53]

Identification of shigella isolate is made with group specific antisera; members of group A (S. dysenteriae) can be subdivided into 10 specific serotypes, S. flexneri into 8 serotypes and S. boydii into 15 serotypes.[52] On the other hand, members of group D, are serologically homogeneous and for epidemiological purposes a useful marker is the ability of such strains to produce different colicines, which allows subdivision into 17 types.[53] The further details of identification of Shigella would be described in chapter 3 page 67.

Toxin Production :-

1. The exotoxin of Shigella dysenteriae 1 (S. shiga) was discovered as a heat-labile toxin which is present in bacteria-free filtrates which when injected intravenously into the rabbit, produces changes both in the central nervous system and in the bowel,[1] This toxin may be involved in causing the watery diarhea prior to dysentery, called the enterotoxin, but the mechanism is still unknown though it seems to stimulate adenyl cyclase enzyme's activity like those of Vibrio cholerae and Enterotoxigenic E. coli (ETEC),[22,54] Many other organisms in this genus give the similar toxin.[54]

2. The endotoxin . This is the somatic antigen of the shigellae which is released from the organisms that died and lysed,[55] It if capable of producing severe damage in the human body : it affects the small blood vessels, but its full clinical effects is shown by

haemorrhages, low blood pressure, leukopenia and hypoglycemia ; the adrenal glands are especially liable to damage.[1] This is not related to the pathogenicity of the organism and occurs rarely.[1]

Pathogenicity and pathogenesis :-

All Shigella species are pathogenic for man, causing typical dysentery.[51] After ingestion, the organisms are capable of surviving the deleterious effects of gastric acid and other nonspecific defense mechanisms such as the normal cleansing mechanism of the bowel and protection by normal enteric flora. Shigellae probably inhabit the small intestine only transiently. The distal colon and rectum are the primary sites of multiplication, invasion and inflammation. After multiplication in the lumen, shigellae must penetrate the epithelial cells of the large intestine intracellularly, then they multiply in the submucosa or lamina propria and induce an intense inflamatory response. Distortion of crypts occurs as clumps of cells are sloughed and causes blockage. The accumulation of inflamatory cells behind obstruction leads to the formation of micro abscesses. Through spread and coalescense larger abscesses form. Long segments of affected colon or sigmoid may be covered by fibrinous exudate containing large numbers of neutrophils. Bleeding occurs from superficial ulcerations that are about 5 mm. in diameter. The acute colitis leads to fever, abdominal pain and the production of bloody, mucous-containing diarrheal feces. Invasion of the blood stream is rare and perforation is not a complication because of the superficial localization of the infection. The disease is self-limited, spontaneous recovery 2-7 days after onset is the rule.[22]

Many patients infected with shigellae do not develop dysentery but have a watery diarrhea of short duration as is also common prior to the onset of dysentery.[22,54] The mechanism involved are unknown but may include an enterotoxin with biologic activity similar to that of Vibrio cholerae and Enterotoxigenic E. coli (ETEC).[9,22,54] It was found that many species of Shigella such as S. flexneri, S. sonnei secrete the enterotoxin which give the similar biological and immuno logical properties to the shiga toxin produced by the S. dysentery 1 (S. shiga).[54] This toxin was shown the enterotoxic activity by inducing the intestinal secretion from the ligated rabbit ileum besides its neurotoxic and cytotoxic activities.[54] But the mechanism is still under investigation.

Clinical manifestations :

Incubation period Usually, the symptoms accur about 2-3 days after ingestion of the organisms.[1] A low number such as 200 bacilli or more[22] may cause the disease.

Symptoms The initial symptoms are not specific; fever and cramping abdominal pain are prominent. Diarrhea usually appears after 48 hours, with frank dysentery supervening about 2 days later. In many cases the diarrhea may continue without the appearance of blood and mucous. Abdominal tenderness is usually general, with no rigidity of the abdominal wall.

Sigmoidoscope reveals intense hyperemia; multiple, small bleeding sites; loss of transverse mucosal folds; and thick, purulent

mucous secretions. In patients with these findings, tenesmus is present, and the feces are bloody, mucoid and of small volume,

Fluid and electrolyte loss may be significant, particularly in pediatric and geriatric patients. Infection with S. dysenteriae is occasionally associated with a peripheral neuritis,[22] Some patients have generalized pains, rigors and malaise.[51] In the more severe cases, the patients have a sharp rise of temperature to 101° to 103° F (38.3 to 39.4° C), and rapidly begin to look toxic and dehydrated. Their cheeks are flushed, they have flistened eyes, are anxious and restless. Their pulses are rapid and feeble, the skin is pinched, the tongues are coated. They suffer from great thirst, and cramps in the limbs and usually a violent diarrhea develops after a day or two, the stools consist of the typical dysenteric blood and mucous.[51]

In most patients, after 10 days, the symptoms gradually abate, and the patient enters a period of slow convalescence.[51] Shigellosis is a self-limited disease,

Treatment of Shigellosis

a. correction of dehydration

The first important thing is correction of dehydration and electrolyte abnormalities,[1] including the intravenous administration of fluids. Babies with a severe attack of diarrhea rapidly become dehydrated, and restoration of fluid balance is far more important than antibiotic therapy. There are two routes by which the fluids and electrolytes are fed, orally and intravenously. The main electrolytes

contents are glucose, sodium chloride, sodium bicarbonate and potassium chloride in various proportions, but the recommended WHO's oral rehydrating solution contents are 20, 4,4 4 and 1 gram (s) in 1 litre of water, respectively.[3]

b. Specific treatments[3,21] :-

Antimicrobial treatments depend on the susceptibility of causative organisms in each area. In Thailand, the routine specific treatments are as follows: -

1. Ampicillin

The usual oral dose is 50-100 mg/Kg body weight per day for 5 days.

2. Co-trimoxazole, the combination between Trimethoprim and Sulphamethoxazole in the ratio 1 : 5 or 80 mg : 400 mg in one tablet, It is given 2 tablets orally, two-times daily for 5 days.

3. Tetracycline capsules :-

The dose is 50 mg/Kg/day, for 5 days

4. Furazolidone, oral

The dose is 4-8 mg/Kg/day, for 5 days

5. Nalidixic acid tablet

The dose is 55 mg/Kg/day for 5 days

However, the world wide spread of Drug resistance mediated by resistance transfer factor[22] (RTF) has been the important problem in many Gram-negative infections treatment including shigellosis, The change in drug susceptibility of shigellae organisms was shown in table 1,2. Chapter 1 page 2,3.This is the problem we are facing, though it

is not very serious now, but, it needs some more attention to find another better treatment.