In-vitro Activity of Three Brands of Ceftriaxone against different Clinical Isolates

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ABSTRACT

Introduction: Ceftriaxone is an extended spectrum cephalosporin that has been used for decades for combating infections caused by wide array of pathogens and prescription of antibiotic is in favor of specific brands without realizing the potency of the brand. The in-vitro activity of three brands of ceftriaxone and various antibiotics against clinical isolates is studied.

Methods: This comparative study was carried in Kathmandu Medical College Teaching Hospital (KMCTH), Basic Science Complex, Duwakot, Bhaktapur in 2009. Thirteen clinical isolates isolated from the specimens processed in the Microbiology Laboratory at KMCTH were used. Minimum inhibitory concentration (MIC) of three brands of ceftriaxone (A, B, and C) was determined by agar dilution and antibiotic resistance patterns were deduced by disc diffusion test.

Results: MIC for three brands of ceftriaxone ranged from <0.25 to >256 mg/L. The MIC for brands A and C of ceftriaxone were consistent for all isolates while for brand B, MIC was higher by at least two fold suggesting this brand had less in-vitro activity against isolates tested. All isolates were multi-drug resistant. Ceftriaxone resistant isolates (n=6) were simultaneously resistant to various antibiotics tested. Imipenem and amino glycosides were sensitive to the isolates studied.

Conclusions: Different brands of ceftriaxone are available in Nepal and imprudent use of less potent antibiotics should be controlled to stop the emergence and spread of multi-drug resistant pathogens and to prevent undue economic burden to the patient and the country.

Keywords: agar dilution, ceftriaxone brands, multi-drug resistant

INTRODUCTION

Ceftriaxone is an injectable antibiotic, 90% protein bound, has a prolong half life of 5.8 hours, good penetration to cerebro spinal fluid (CSF) and has been used for treating meningitis.¹ It has also been used for treating typhoid and combating various infections, bacteremia and septicemia in Nepal and abroad.²⁻⁵ It is also commonly used in intensive care units of Nepal.⁵ Despite its success, ceftriaxone resistant strains of *Enterobacteriaceae* and *Staphylococcus aureus* have emerged.⁵⁻⁷ Ceftriaxone is imported in Nepal and the success of the treatment relies on correct identification of the pathogen, appropriate antibiotic and dose, duration, and most importantly potency. Different brands of ceftriaxone are available in the market and prescription of one brand primarily depends upon the favor of the physician involved in the treatment process giving little attention to the potency. Hence, we have determined

Correspondence: Dr. Badri Thapa Department of Microbiology Kathmandu Medical College, Kathmandu, Nepal. E-mail: badri_bishal@yahoo.com Phone: 977-9841220108 the in-vitro antimicrobial activity of three brands of ceftriaxone against the clinical isolates and studied their antibiotic resistance patter.

METHODS

A comparative study was carried out in Department of Microbiology, KMCTH, Duwakot from October to December 2009. Thirteen clinical isolates consisting of *Escherichia coli* (n=8), *Klebsiella* spp. (n=3), *Proteus* spp. (n=1), and *S. aureus* (n=1) were isolated using standard procedures from different specimens (urine, pus, sputum, and endotracheal tube aspirate) at Microbiology Laboratory, KMCTH, Sinamangal, Nepal.

Three brands of ceftriaxone powder for injection was purchased from a commercial source and were blinded as A, B, and C. MIC (mg/L) to three brands of ceftriaxone was determined by agar dilution as recommended by CLSI.8 Briefly, the stock concentrations of each brand of ceftriaxone were prepared as previously described.9 Ceftriaxone solution was added to the molten Muller Hinton agar (Himedia, India) to provide two fold serial dilutions ranging from 0.25 to 256 mg/L. Few pinpoint colonies of overnight grown bacteria were suspended in peptone water to reach the turbidity of 0.5 McFarland standard (BaSO, turbidity standard).9 The 0.001 ml inoculating loop was used to deliver 10⁴ colony forming unit (cfu)/spot of bacteria into the agar plate containing the ceftriaxone. The plates were incubated at 35°C for 16 to 20 hours. The growth of a single colony or a haze was not considered. The lowest concentration

of antibiotic that showed no bacterial growth was considered as MIC. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as a quality control strains.

Antibiotic susceptibility test for the isolates was performed following CLSI guidelines.⁸ The antibiotic discs (μ g/disc) used were; Amoxycillin (10), Amoxycillin-Clavulenic acid (20/10), Piperacillin (100), Trimethoprim-Sulphamethoxazole (25), Ciprofloxacin (5), Norfloxacin (10), Gentamicin (30), Netilmicin (30), Kanamicin (30), Cefoxitin (30), Ceftriaxone (30), Ceftazidime (30), Cefuroxime (30), Cephazolin (30), Ceftpodoxime (10), and Imipenem (10). All antibiotic discs were purchased from Himedia Pvt. Ltd, India except cefoxitin and cephzolin which were purchased from Oxoid, UK.

E. coli ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control strains.

RESULTS

The in-vitro activity of three brands of commonly used ceftriaxone was studied by determining MIC by agar dilution. The result showed that two brands (A and C) had consistent in-vitro activity against all the isolates. MIC for these two brands ranged from <0.25 to >256 mg/L while brand B was relatively less active and the MIC was higher by at least 2 fold for some of the isolates (Table 1). MIC obtained for the control strains were within the acceptable limits and all brands had MIC less than 0.25 mg/L. The zone of inhibition obtained by disc diffusion test correlated well with the MIC obtained by agar dilution.

Table 1. A	ntibiotic resistance	pattern and MIC	of three brands of	ceftriaxone against test isolates
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laglatag	Isolate no.	Posistant to DDT	Break point MIC	MIC (mg/L)		
Isolates				Α	В	С
E. coli	EC1010	A, PC, CU, CO	≤8S ≥64R	< 0.25	< 0.25	< 0.25
	EC1013	A,AC,PC,CI,CA,CU,KZ,CEP,FOX,CF,NX, K,NT,CO	≤8S ≥64R	128	256	128
	EC1019	A,AC,PC,CA,CU,CF,NX,G,K,CO	≤8S ≥64R	< 0.25	< 0.25	< 0.25
	EC1020	A,PC,CU,CO	≤8S ≥64R	< 0.25	1	< 0.25
	EC1032	A,PC,CI,CA,CU,KZ,CEP,NX	≤8S ≥64R	64	64	64
	EC10320	A,PC,CA,KZ,CO	≤8S ≥64R	< 0.25	< 0.25	< 0.25
	EC10165	A,AC,PC,CI,CA,CU,KZ,CEP,CF,NX,CO	≤8S ≥64R	64	128	64
	EC10159	A,PC,CU,NT	≤8S ≥64R	< 0.25	< 0.25	< 0.25
Klebsiella spp.	KS1026	A,AC,CI,CA,CU,KZ,CEP,FOX,I,CF,NX,G, K,NT,CO	≤8S ≥64R	>256	>256	>256
	KS1028	A,AC,PC,CI,CA,CU,KZ	≤8S ≥64R	< 0.25	< 0.25	< 0.25
	KS1035	A,AC,PC,CI,CA,CU,KZ,CEP,FOX,NX	≤8S ≥64R	>256	>256	>256
Proteus spp.	PS1070	PC,CI,CA,CU,KZ,CEP,CF,NX	≤8S ≥64R	64	128	64
S. aureus	SA1029	A,PC,CA,CO	≤8S ≥64R	< 0.25	2	< 0.25

DDT, Disc Diffusion Test; A, B and C, Three brands of ceftriaxone; S, Sentsitive; R, Resistant; A, Amoxycillin; AC, Amoxycillin + Clavulenic Acid; PC, Piperacillin; CI, Ceftriaxone; CA, ceftazidime, CU, Cefuroxime, KZ, Cephalothin; CEP, Cefpodoxime; FOX, Cefoxitin, I, Imipenem, CF, Ciprofloaxin; NX, Norfloxacin; G, Gentamicin; K, Kanamicin; NT, Netilmicin; and CO, Trimithoprim + Sulphamethoazole.

The disc diffusion test was performed on these isolates with 16 antibiotics representing different classes. All isolates were multi-drug resistant (Table 1). Ceftriaxone resistant isolates (n = 6) showed in-vitro resistance to numerous antibiotics (7 to 15) while most of them were susceptible to imipenem and amino glycosides.

DISCUSSION

Ceftriaxone, an extended spectrum cephalosporin is active against Gram positive and negative bacterial isolates and mostly for pathogens causing meningitis due to its high level in CSF.¹ On light of emergence of multidrug resistant pathogens to penicillins, quinolones, tetracyclines, and narrow spectrum cephalosporins the role of extended spectrum cephalosporins is even more crucial. The effective treatment relies on several factors and most importantly the correct antibiotic and potency of the active drug.

We determined the in-vitro activity of three different brands of commercially available cefriaxone. The brand B had lower in-vitro activity than its competitor brands A and C. The MIC obtained for this brand was at least two fold higher than other brands for some isolates while for others all brands had MIC either <0.25 mg/L or >256 mg/L. The ceftriaxone concentration tested ranged from 0.25 mg/L to 256 mg/L, the in-vitro activity on two extremes couldn't be known. The activity of brand B could have been known if ceftriaxone concentration <0.25 or >256 mg/L were tested. For an isolate EC1032, MIC was 64 mg/L for all brands and this couldn't be explained. Study comparing different

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brands of ceftriaxone is scare in literature and we could not compare our study with others.

Among 13 multidrug resistant clinical isolates studied 6 were resistant to ceftriaxone. Ceftriaxone resistant isolates were further resistant to numerous antibiotics leaving the choice for imipenem and aminoglycosides. Ceftriaxone resistant clinical strains are emerging worldwide. Although, ceftriaxone resistant *E. coli* and *Klebsiella* spp. are scarce in literaturers, ceftriaxone resistant *Salmonella* spp. has been rising in Nepal.^{7,10} The resistance to this antibiotic is also a global concern.⁶

Ceftriaxone resistance is conferred by the plasmid borne CTX-M, CMY-2, TEM and SHV extended spectrum -lactamase enzyme (ESBL) and plasmid and chromosomal borne AmpC ESBL and resistance can spread rapidly to the susceptible isolates through horizontal gene transfer.^{6,11,12} Molecular epidemiology of resistance genes conferring resistance to this antibiotic is mandatory to formulate treatment guidelines and to know the extent of resistance gene spread caused by indiscriminate use of less potent antibiotic.

CONCLUSIONS

Ceftriaxone is an antibiotic widely used in clinical practice and is imported in Nepal. Hence, concerned authority has to consider the cost implications to the patient, family and the country before endorsing less potent ceftriaxone brands in the country. Prudent and potent antibiotic use will help prevent emergence and spread of antibiotic resistant strains, which in turn will benefit the patient and Nation as a whole.

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