In vitro Temocillin efficacy against extended spectrum β-lactamase producing multidrug resistant gram negative bacterial isolates from Nepal

Kandel BP,[1,2] Bhatta DR,[1] Shakya G,[3] Upadhayay BP,[3] Dumre SP,[3,4] Poudyal S,[1] Adhikari N[5], Acharya D[5]

[1]Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal and [2]Janapriya Multiple Campus, Pokhara, Nepal; [3]National Public Health Laboratory, Kathmandu, Nepal; [4]Faculty of Allied Health Sciences, Thammasat University, Thailand; [5]Department of Microbiology, Kantipur College of Medical Science, Kathmandu, Nepal

Corresponding Author: Bhuwaneswor Prasad Kandel, Janapriya Multiple Campus, Pokhara, Nepal; e-mail: voidbhuwan@gmail.com

ABSTRACT

Temocillin is relatively more stable against most β-lactamases and requires re-evaluation to include it in common clinical practice as a therapeutic alternative. At the National Reference Laboratory of Nepal, we evaluated multidrug resistance (MDR) and extended spectrum β-lactamase (ESBL) phenotypes among 292 gram-negative clinical bacterial isolates of 18 different genera during 2009/2010 by Kirby-Bauer disc diffusion method following CLSI guidelines. ESBL screen positive isolates were tested for Temocillin efficacy by disc diffusion method following British Society of Antimicrobial Chemotherapy (BSAC) guidelines and other antibiotics following Clinical and Laboratory Standards Institute (CLSI) guidelines. Of the 292 isolates, 75.0% isolates were MDR, among which 61.6% were primarily screened positive for ESBL production but only 38.8% were confirmed as ESBL producers. We report relatively lower Temocillin resistance of 28.9% and 15.6% among MDR and ESBL positive populations, respectively. Among ESBL positive isolates, no *Proteus mirabilis*, 19.7% *Escherichia coli* and 33.3% *Klebsiella oxytoca* showed resistance to Temocillin, although such resistance was higher among *Acinetobacter* spp. (66.7%) and *K. pneumoniae* 50.0%. Among ESBL negative isolates, none of the *K. oxytoca* and few (13.3%) *Acinetobacter* spp. were resistant to Temocillin, while all *Citrobacter freundii*, *Pseudomonas aeruginosa* (85.7%) and *K. pneumoniae* (66.7%) showed Temocillin resistance. Only 14.8% and 3.0% of total MDR isolates were resistant to Imipenem and Meropenem, respectively. However, Imipenem resistance was remarkably high (86.7%) among ESBL negative *Acinetobacter* spp. than Meropenem (13.3%). Temocillin showed comparable efficacy against MDR and ESBL producing bacterial isolates and could be a next therapeutic option.

Keywords: Temocillin, ESBL, multidrug resistance, Nepal

INTRODUCTION

Temocillin, a narrow spectrum 6-α-methoxy derivative of Ticarcillin, is stable both *in vivo* and *in vitro* against hydrolysis by most β-lactamases produced by various gram-negative bacteria and has very consistent activity against *Enterobacteriaceae*.[1,2] Range of β-lactamases that do not hydrolyze Temocillin include classical and extended-spectrum β-lactamases (ESBLs), AmpC-type β-lactamases (both hyperproduced or acquired), hyperproduced K1 enzyme by *Klebsiella oxytoca*, KPC-β-lactamases and carbapenemases by resistant *K. pneumoniae* and *Escherichia coli*.[3-7] It is also relatively stable to some acquired metalloenzymes.[8] Temocillin, however, lacks the activity against gram-positive bacteria, anaerobes, some non-fermenting gram-negative bacteria and bacteria with altered penicillin-binding proteins.[7,9]

Being a relatively older drug not so frequently evaluated for its efficacy than other drugs in many parts of the world including Nepal, literatures regarding temocillin efficacy are still sparse. Temocillin with relative stability against most β-lactamases may serve as a therapeutic alternative in this clinical era worsened by emergence and spread of ESBL producing pathogens. Temocillin also has convenient dosage options and can be a potential alternative and reserve drug in treating serious infections by ESBL producers and other cephalosporin-resistant strains.[1,4,6,10-12]

In the context of emerging antimicrobial resistance and availability of limited number of therapeutically useful antibiotics, we aimed to determine the Temocillin efficacy against multidrug resistant (MDR) clinical bacterial isolates coupled with ESBL detection at a national reference laboratory of Nepal which may prove an important step in the antimicrobial chemotherapy to meet the present challenges.

MATERIAL AND METHODS

Laboratory setting and bacterial isolates: The study was conducted prospectively at National Public Health Laboratory (NPHL), Kathmandu, Nepal. A total of 292

gram-negative bacteria were isolated from different clinical specimens during 2009/2010 and screened for MDR phenotype by the commonly used antibiotics. Re-confirmation of bacterial isolates was done by standard microbiological techniques. Isolates showing combined resistance to two or more different antibiotic classes were considered as MDR.[13] MDR isolates were further tested for ESBL production.

Antibiotic susceptibility testing: Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) for all other antibiotics and according to BSAC guidelines for Temocillin.[14,15] The susceptibility to Amoxycillin (20µg), Ticarcillin (75µg), Ceftazidime (30µg), Cefoxitin (30µg), Imipenem (10µg) and Meropenem (10µg) was determined for ESBL screen positive isolates and used for comparison of Temocillin susceptibility. Zones of inhibition were measured, categotized and reported accordingly using the standard chart. Isolates showing intermediate susceptibility were interpreted as resistant. Any aberrant result obtained during the experiment was confirmed by repeating the test twice and processed accordingly.

ESBL detection: The MDR isolates were screened for ESBL production by using all CLSI recommended screening agents, viz. Aztreonam (30µg), Ceftriaxone (30µg), Cefpodoxime (10µg), Ceftazidime (30µg) and Cefotaxime (30µg) (Mast Diagnostics, UK). The MDR isolates showing reduced susceptibility to one or all of the screening agents with zone of inhibiton diameter for Cefpodoxime ≤17mm, Ceftazidime ≤22mm, Aztreonam ≤27mm, Cefotaxime ≤27mm, and Ceftriaxone ≤25mm were considered as the possible ESBL producers. The suspected ESBL producers were subjected to combined disk (CD) test for phenotypic confirmation of ESBL production using MASTDISCS™ ID ESβL detection discs (D52C) and MASTDISCS™ ID Cefepime ESβL ID disc set (D63C). The former kit consisted of Ceftazidime (30µg), Cefotaxime (30µg) and Cefpodoxime (30µg) alone and each in combination with Clavulanic Acid (10µg). The later consisted of Cefepime (30µg) alone and in combination with Clavulanic Acid (10µg). The zone of inhibition for the Ceftazidime, Cefotaxime, Cefpodoxime and Cefepime disks alone was compared with that of respective disks containing Clavulanic Acid and an increase in zone diameter by ≥5mm in the presence of Clavulanic Acid for any one or all of the sets was concluded as confirmed ESBL producers. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive (ESBL producer) and negative (ESBL non-producer) controls, respectively. Temocillin efficacy among ESBL-producing and non-producing bacterial isolates was compared with that of other penicillins, cephalosporins and carbapenems.

RESULTS

Altogether, 292 gram negative isolates of 18 different genera were isolated from different clinical specimens and 75.0% of them were found to be MDR. Of these MDR isolates, 61.6% of them were ESBL screen positive and 38.8% were confirmed as ESBL producers. Also, 77.6% of *E. coli*, 75.0% of *K. oxytoca*, 66.7% of *K. pneumoniae*, 50.0% of *Citrobacter freundii* and 16.7% of *Acinetobacter* spp. tested ESBL positive. One isolate of *Proteus mirabilis* also produced ESBL. No ESBL production was detected in *Providencia* spp. and *Pseudomonas aeruginosa*. (Table-1)

Almost all isolates tested were resistant to Amoxycillin (97.8%) and Ticarcillin (89.6%). Among 85 ESBL positive isolates, maximum resistance (97.6%) was observed against Amoxycillin followed by Ticarcillin (94.1%). On the flip side, 86.0% ESBL negative isolates were also resistant to Amoxycillin and 71.9% were resistant to Ticarcillin. Altogether, Temocillin resistance was observed among 28.9% of all MDR gram negative isolates tested. Regarding ESBL production, 24.7% ESBL positive and 36.0% ESBL negative isolates were resistant to Temocillin. Among ESBL positive isolates, only 33.3% *K. oxytoca* and 19.7% *E. coli* showed resistance to Temocillin while 66.7% *Acinetobacter* spp., 50.0% *K. pneumoniae*, and 33.3% of *C. freundii* also showed resistance to Temocillin. One isolate of *P. mirabilis* tested was also susceptible to Temocillin. Among ESBL negative isolates, all isolates of *C. freundii*, 85.7% of *P. aeruginosa* and 66.7% of *K. pneumoniae* showed resistance to Temocillin. No isolate of ESBL negative *K. oxytoca* and few isolates (13.3%) of ESBL negative *Acinetobacter* spp. were resistant to Temocillin. (Table-1)

Moreover, resistance to Ceftazidime was 62.7% as a whole but no isolates of *P. aeruginosa* and only 20.0% of ESBL negative isolates of *Acinetobacter* spp. were resistant to Ceftazidime. Altogether, Cefoxitin resistance was 52.6% with 43.5% resistance for ESBL positive isolates and 59.6% for ESBL negative isolates. Moreover, 14.8% and 3.0% of total MDR isolates were resistant to Imipenem and Meropenem, respectively. Resistance to Meropenem and Imipenem was 0.0% and 2.3%, respectively, for ESBL positive isolates. Imipenem resistance was remarkably high among ESBL negative *Acinetobacter* spp. (86.7%) than Meropenem (13.3%). Carbapenem resistance was also observed among *C. freundii*, *E. coli*, *K. pneumoniae*, *Providencia* spp. and *P. aeruginosa*. Higher degree of Imipenem resistance (31.6%) but less (7.0%) for Meropenem, was seen among ESBL negative bacteria. (Table-1)

Table 1: ESBL production and resistance to penicillins, cephalosporins and carbapenems among ESBL screen positive multidrug resistant isolates (n=135)

Organisms (number of total tested isolates)	ESBL production (Number of isolates)	Antibiotics and no of resistant isolates						
		A	TC	TEM	CAZ	FOX	IMI	MEM
***Acinetobacter* spp. (18)**	Positive (3)	3	1	2	2	2	1	0
	Negative (15)	15	15	2	3	15	13	2
***C. freundii* (6)**	Positive (3)	3	3	1	2	3	0	0
	Negative (3)	3	2	3	2	3	1	1
***E. coli* (85)**	Positive (66)	65	64	13	55	26	1	0
	Negative (19)	18	16	4	3	5	1	0
***K. oxytoca* (8)**	Positive (6)	6	6	2	6	4	0	0
	Negative (2)	2	1	0	0	0	0	0
***K. pneumoniae* (9)**	Positive (6)	5	5	3	5	2	0	0
	Negative (3)	3	2	2	2	3	1	0
***P. mirabilis* (1)**	Positive (1)	1	1	0	1	0	0	0
	Negative (0)	0	0	0	0	0	0	0
***Providencia* spp. (1)**	Positive (0)	0	0	0	0	0	0	0
	Negative (1)	1	1	1	1	1	1	1
***P. aeruginosa* (7)**	Positive (0)	0	0	0	0	0	0	0
	Negative (7)	7	4	6	0	7	1	0
Total (135)	Positive (85)	83	80	21	71	37	2	0
	Negative (50)	49	41	18	11	34	18	4

A, Amoxycillin (20µg); FOX, Cefoxitin (30µg); CAZ, Ceftazidime (30µg); IMI, Imipenem (10µg); MEM, meropenem (10µg); TC, Ticarcillin (75µg); TEM, Temocillin (30µg)

DISCUSSION

The present study may probably be the first study in Nepal with the aim to determine in vitro efficacy of Temocillin against gram-negative MDR clinical bacterial isolates with reference to ESBL production. We observed relatively higher resistance to Amoxycillin and Ticarcillin which makes them now clinically ineffective in most infections and such resistance arises due to the similar mechanisms mainly by β-lactamase production. Higher Amoxicillin resistance might reflect the rampant and empirical use as it remains the most frequently sold antibiotic in Nepal.[16]

In our study, susceptibility of the isolates to Temocillin was remarkable (71.1%) as a whole in contrast to other penicillins. Only 24.7% ESBL positive isolates and 36.0% ESBL negative isolates were resistant to Temocillin. The higher susceptibilities of ESBL negative MDR *Acinetobacter* spp. isolates to Temocillin was worthy to note. We adopted the Temocillin susceptibility breakpoints according to the BSAC guidelines given for *Enterobacteriaceae* as diameter of inhibition zone of ≥20mm (corresponding MIC = 8µg/ml) for susceptibility and ≤19mm for resistance for systemic isolates while for urinary isolates inhibition zone diameters of ≥12mm (corresponding MIC = 32µg/ml) and ≤11mm were regarded as susceptible and resistant, respectively.[15] Temocillin breakpoints remain controversial having certain discrepancies with the disc diffusion method in multiresistant strains, however, a study suggests that 8 mg/L as more appropriate on the basis of inter-individual variabilities in serum drug levels despite its good *in vitro* activity against the majority of *Enterobacteriaceae* isolates with a modal MIC of 4µg/ml and a MIC_{90} of 16µg/ml.[5,10,17]

Temocillin showed good efficacy against most of the ESBL positive isolates. Among ESBL negative isolates, all isolates of *C. freundii* and most of *P. aeruginosa* and *K. pneumonia* isolates showed resistance to Temocillin. However, no isolate of ESBL negative *K. oxytoca* and few isolates of ESBL negative *Acinetobacter* spp. were resistant to Temocillin. Despite the limited clinical data on efficacy of Temocillin in the treatment of infections by ESBL-producers, some retrospective and multicentric Belgian studies demonstrated 79.0 - 100% efficacy against multiresistant and mostly ESBL producing clinical *Enterobacteriaceae* isolates with MIC_{50} and MIC_{90} values of 8 and 32µg/ml while a similar study in UK reported nearly 90.0% Temocillin susceptibility of the AmpC- and ESBL-producing *Enterobacteriaceae* isolates.[6,12,18,19] Furthermore, Ertapenem resistant and KPC-β-lactamase producing clinical isolates of *K. pneumoniae* and *E. coli* in United States showed MIC_{90} to Temocillin being 32µg/ml.[3]

Good pharmacokinetic properties and dosage convenience of Temocillin make it a potential alternative to cephalosporins and carbapenems in treatment of infections caused by the *Enterobacteriaceae* producing various broad-spectrum β-lactamases.[20,10] In contrary

to its primary use against *Enterobacteriaceae*, and in particular against strains producing extended spectrum β-lactamase or AmpC β-lactamase, the efficacy of Temocillin against ESBL-negative *Acinetobacter* spp. in our study was remarkable which suggests for its potential use in severe infections by such bacteria. However, efficacy of Temocillin against other resistant bacteria was not determined in this study.[4,6]

Regarding other cephalosporins, we found that Ceftazidime resistance was 62.7% as a whole. No isolate of *P. aeruginosa* and 20.0% of ESBL negative isolates of *Acinetobacter* spp. were resistant to Ceftazidime. Good efficacy of ceftazidime against *P. aeruginosa* makes it a potent antipseudomonal agent. Altogether, Cefoxitin resistance was also higher among both ESBL positive and ESBL negative isolates. Significant resistance to Cefoxitin and Ceftazidime may imply higher level of AmpC β-lactamase production.[21] In our study, 93.0-100% susceptibility of the isolates to Meropenem matches with the similar findings in Belgium with more than 99.0% Meropenem susceptible isolates regardless of the species or resistance mechanisms.[5]

We did not determine the MIC values of Temocillin against pathogens and various resistance mechanisms to Temocillin in the present study. However, acquired resistance to Temocillin usually arises via combination of several mechanisms including the presence of ESBLs, AmpC hyperproduction and impermeability of the drug to the cell (by altered penicillin-binding proteins or blockade by cell surface structures) or upregulated efflux of the drug from the cell.[7]

Temocillin retains good *in vitro* activity against most clinical isolates of *Enterobacteriaceae* and non-fermenters with or without ESBL production. Although carbapenems are still effective against ESBL producing clinical isolates, Temocillin could be a next potential therapeutic alternative for the treatment of severe infections caused by ESBL- and/or AmpC-producing bacterial strains when justified by reliable microbiological investigations. Prospective clinical studies are therefore warranted in order to confirm its therapeutic efficacy.

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