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

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ABSTRACT

Tamocillin 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 Tamocillin 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 Tamocillin 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 Tamocillin, 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%). Tamocillin showed comparable efficacy against MDR and ESBL producing bacterial isolates and could be a next therapeutic option.

Keywords: Tamocillin, ESBL, multidrug resistance, Nepal

INTRODUCTION

Tamocillin, 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 Tamocillin 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 Tamocillin, 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 tamocillin efficacy are still sparse. Tamocillin 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. Tamocillin 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 Tamocillin 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. 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. The susceptibility to Amoxycillin (20 μg), Ticarcillin (75 μg), Ceftazidime (30 μg), Cefotaxime (30 μg) and Cefoxitin (30 μg), Imipenem (10 μg) and Meropenem (10 μg) was determined for ESBL screen positive isolates and used for comparison of Temocillin susceptibility. Zones of inhibition were measured, categorized 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 (30 μg), Ceftriaxone (30 μg), Cefpodoxime (10 μg), Ceftazidime (30 μg) and Cefotaxime (30 μg) (Mast Diagnostics, UK). The MDR isolates showing reduced susceptibility to one or all of the screening agents with zone of inhibit diameter for Cefpodoxime ≤17 mm, Ceftazidime ≤22 mm, Aztreonam ≤27 mm, Cefotaxime ≤27 mm, and Ceftriaxone ≤25 mm 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 MASTDISC™ ID ESBL detection discs (D52C) and MASTDISCS™ ID Cefepime ESBL ID disc set (D63C). The former kit consisted of Cefazidime (30 μg), Cefotaxime (30 μg) and Cefpodoxime (30 μg) alone and each in combination with Clavulanic Acid (10 μg). The later consisted of Cefepime (30 μg) alone and in combination with Clavulanic Acid (10 μg). 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 ≥5 mm 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)
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 MIC90 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 Enterobacteriaceae isolates with MIC50 and MIC90 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 MIC90 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

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<th>Table 1: ESBL production and resistance to penicillins, cephalosporins and carbapenems among ESBL screen positive multidrug resistant isolates (n=135)</th>
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<td>Organisms (number of total tested isolates)</td>
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<td>Acinetobacter spp. (18)</td>
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<td>C. freundii (6)</td>
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<td>P. aeruginosa (7)</td>
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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 Cefazidime 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 Cefazidime. 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 Cefotaxin and Cefazidime 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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REFERENCES