Original Article

Activity of Mecillinam and Clavulanic Acid on ESBL Producing and Non- ESBL Producing *Escherichia Coli* Isolated From UTI Cases

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Abstract

Mecillinam is one of the very few oral antibacterial agents used against extended spectrum β-lactamase (ESBL) producing *Escherichia coli (E. coli)* causing urinary tract infection (UTI)). It is reported that, resistance to mecillinam can be reversed to some extent by adding beta lactamase inhibitor like clavulanic acid. The present study was aimed to determine *in-vitro* activity of mecillinam and mecillinam-clavulanic acid combination on the susceptibility of ESBL producing and non-ESBL producing *E. coli*. Total 124 *E. coli* (78 ESBL positive and 46 ESBL negative) isolates from urine samples of patients with UTI were included in the study. Organisms were isolated from patients attending BIRDEM General Hospital from July 2012 to December 2012. ESBL production was tested by double disc synergy test. Minimum inhibitory concentration (MIC) of mecillinam and clavulanic acid against *E. coli* was determined by agar dilution method. Of the total *E. coli* isolates, 62.9% was ESBL positive and 37.1% was negative for ESBL. Out of ESBL positive isolates, 75.6% was sensitive to mecillinam while ESBL negative isolates showed the sensitivity as 67.4%. The sensitivity to mecillinam of ESBL positive and negative isolates increased to 85.9% and 86.9% respectively by addition of clavulanic acid with mecillinam. The MIC values of intermediate and resistant isolates converted to sensitive MIC range after addition of clavulanic acid with mecillinam. Conversion of resistance of ESBL producing isolates by adding clavulanic acid was also evident by the reduction of MIC_{50} and MIC_{90} from 4μg/ml to ≤1 μg/ml and from 128 μg/ml to 64 μg/ml respectively. Similar trend of reduction of MICs was also observed in non-ESBLs.

In conclusion, both ESBL positive and negative *E. coli* demonstrated considerable sensitivity to mecillinam and the sensitivity increased significantly ($p < 0.05$) by adding clavulanic acid with mecillinam.

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Introduction

Escherichia coli (E. coli) remains an important cause of urinary tract infections (UTIs). UTI by extended-spectrum â-lactamase (ESBL) producing strains of *E. coli* is difficult to treat. Concomitant resistance to other non â-lactam antibiotics like aminoglycosides and fluoroquinolones has further complicated the situation and left very limited treatment options, especially per oral regimes.[1] Mecillinam is an oral amidinopenicillin which acts by binding with penicillin binding protein-2 (PBP-2). It is relatively stable to â-lactamase enzymes and reaches very high concentration in urine.[2] Thus mecillinam is proven to be a suitable antimicrobial agent against ESBL producing uropathogens like *E. coli* and *Klebsiella* spp. But, chromosomal mutation and few beta-lactamase induced mechanism results in mecillinam resistance in clinical isolates.[3-5] Though mecillinam resistance has been reported from various part of the world it is less

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pronounced than other beta-lactams.[6,7] In vitro studies have shown that mecillinam resistance conferred by higher inoculum can be reversed by addition of beta-lactamase inhibitors like clavulanic acid.[8] Clavulanic acid is a beta lactam compound that has weak intrinsic antibacterial activity. When used in combination with other â-lactam drugs it exerts synergistic effect by inhibiting beta lactamase enzymes.[9,10]

The aim of the present study was to assess in vitro activity of mecillinam alone and in combination with clavulanic acid against ESBL producing and ESBL non-producing uropathogenic *E. coli*.

Material and Method

Bacterial strains

Total 124 *E. coli* strains isolated from urine samples were included in the study. Urine was collected from both indoor and outdoor patients attending BIRDEM General Hospital during the period of July 2012 to December 2012. Isolation and identification of the species was done according to standard laboratory methods.[11]

Detection of ESBL

Double disc synergy test was employed to detect ESBL production.[12] Bacterial suspension of 0.5 McFarland standard was plated in Muller-Hinton agar with Amoxycillin-clavulanic acid (30 μg) disc in between and 20 mm apart from Ceftazidime (30 μg) and Ceftriaxone (30 μg) discs. Expansion of the zone of inhibition around Ceftriaxone and/or ceftazidime disc towards the amoxycillin-clavulanic acid disc was considered ESBL production.

Determination of MIC

Antimicrobial susceptibility of *E. coli* strains was tested by agar dilution method as described in CLSI guideline.[11] Minimum inhibitory concentrations (MICs) of mecillinam was determined in Muller-Hinton agar plate containing two fold (Log_2) serial dilutions of mecillinam (from 1024 μg/ml to 1 μg/ml) using standard inoculum of $1x10^4$ cfu/spot. MICs of mecillinam were also determined in presence of clavulanic acid at a concentration of 0.04μg/ml in each plate. MIC values (μg/ml) were interpreted as: sensitive: ≤ 8, Intermediate: 16 and Resistant: ≥ 32. *E. coli* ATCC 25922 was used as negative control and a mecillinam resistant *E. coli* isolates having MIC of >1024 μg/ml (pre-determined) was used as positive control. Pivmecillinam and potassium clavulanate were obtained from General Pharmaceuticals Ltd. Bangladesh, and Incepta Pharmaceuticals Ltd. Bangladesh.

Results

Table-1 shows the susceptibility pattern of ESBL positive and negative *E. coli* isolates to mecillinam and mecillinam+clavulanic acid according to MIC values. Mecillinam sensitivity was demonstrated in 75.6% ESBL positive and 67.4% ESBL negative isolates which was augmented to 85.9% and 86.9% respectively after addition of clavulanic acid. In ESBL positive and negative strains 5.2% and 8.7% isolates were intermediately sensitive to mecillinam, whereas none of the isolates were intermediate sensitive to mecillinam+ clavulanic acid combination. Resistance to mecillinam was found in 19.2% and 23.9% ESBL positive and negative isolates that were reduced to

Table-1: *Susceptibility pattern of ESBL positive and ESBL negative E. coli to mecillinam and mecillinam+ clavulanic acid according to MIC values (N=124)*

Susceptibility pattern	MIC (μg/ml)	No (%) of isolates inhibited			
		ESBL Positive (n=78)		ESBL Negative (n=46)	
		MEC	MEC+CLA	MEC	MEC+CLA
Sensitive	≤1-8	59 (75.6%)	67 (85.9%)	31 (67.4%)	40(86.9%)
Intermediate	16	04 (5.2%)	0 (0.0%)	04 (8.7%)	0 (0.0%)
Resistant	≥32	15 (19.2%)	11 (14.1%)	11 (23.9%)	6 (13.1%)

Note: MEC=mecillinam, CLA=clavulanic acid

Table-2: *Change of susceptibility in relation to MIC values of intermediate sensitive and resistant isolates after adding clavulanic acid*

MIC mecillinam μg/ml	Intermediate N sensitive & resistant isolates to MEC N	Isolates converted sensitive to MEC adding CLA N (%)
16	8	8 (100%)
32	9	9 (100%)
64	5	2 (40%)
≥128	12	0 (0%)
Total	34	19 (55.9%)

Note: *MEC=mecillinam, CLA=clavulanic acid*

14.1% and 13.1% after adding clavulanic acid with mecillinam respectively.

All the isolates having 16 μg/ml and 32 μg/ml MIC of mecillinam converted to sensitive after addition of clavulanic acid. Out of 5 isolates having MIC of 64μg/ml, 2 (40%) were converted to sensitive. But the isolates having MIC of ≥ 128 demonstrated no change in susceptibility. Total 55.9% intermediately sensitive and resistant *E. coli* became sensitive to mecillinam by adding clavulanic acid (Table-2).

The MIC of mecillinam against ESBL producing *E. coli* ranged from ≤1-≥1024 μg/ml. MIC_{50} and MIC_{90} were 4 and 128 μg/ml respectively. After adding clavulanic acid with mecillinam MIC_{50} and MIC_{90} reduced to ≤1 μg and 64 μg/ml respectively. In non-ESBL producing *E. coli* isolates MIC_{50} and MIC_{90} were 4 and 64 μg/ml respectively with mecillinam and ≤1and 32 with mecillinam+clavulanic acid (Table-3).

Table-3: *Change of MIC_{50} and MIC_{90} values of ESBL producing and non-ESBL producing E. coli isolates by addition of clavulinic acid with mecillinam*

E. coli isolates	Compound	MIC (μg/ml)		
		MIC_{50}	MIC_{90}	Range
ESBL	MEC	4	128	≤1– ≥1024
producer	MEC+CLA	≤1	64	≤1– ≥1024
Non-ESBL	MEC	4	64	≤1– ≥1024
producer	MEC+CLA	≤1	32	≤1– 512

Note: *MEC=mecillinam, CLA=clavulanic acid*

Discussion

ESBL producing *E. coli* is isolated in very high frequency in nosocomial as well as community acquired urinary tract infections.[8,13-15] In the present study, about 69% inpatient and 57% outpatient *E. coli* isolates were found ESBL producer. This proportion of ESBL isolation is similar to those described in several studies in home and abroad.[8,13,14] A considerable numbers of isolates irrespective of ESBL production showed sensitivity to mecillinam. The high susceptibility rate of ESBL producing *E. coli* to mecillinam as determined by MIC method in this study is comparable to the findings of others who found 94% and 85% sensitivity respectively.[13,14] But in the context of Bangladesh, mecillinam sensitivity of *E. coli* was reported as 43-67% in 2009.[14,15] This divergence may be due to the fact that, in those studies uropathogenic *E. coli* irrespective of ESBL production was considered and disc diffusion method was used to determine the sensitivity instead of MIC method.

The present study also demonstrated a significant proportion of *E. coli* (27.4%) was mecillinam. Interestingly, we have observed higher rate of resistance in ESBL negative isolates. Arguably, ESBL positive strains should exhibit higher resistance than negative strains. This discrepancy could be due to small number of samples. However, though the percentage of resistance was more in ESBL negative isolates but the level of resistance was more pronounced in ESBL positive isolates as shown by the MIC_{50} and MIC_{90} values.

Mecillinam is one of the very few oral drug used in treating community acquired urinary tract infection. According to *in-vitro* findings, it is stable against beta lactamase enzymes produced by gram negative as well as gram positive bacteria. Extensive use of mecillinam because of its effectiveness in UTI has exerted selective pressure resulting in chromosomal mutation of the drug target and emergence of resistance. Further, few beta lactamases like type IIIa and IVc have activity against mecillinam causing hydrolysis of the agent.[10] These factors together results in mecillinam resistance in clinical practice. Addition of any compound that has inhibitory effect on these enzymes may improve the antibacterial activity of mecillinam. In this ground, activity of mecillinam in combination with a beta lactamase inhibitor, clavulanic acid, was also evaluated in the study. Using the agar

dilution method with standard inoculum of $1x10^4$ cfu/spot, there was a marked decrease in the MIC of mecillinam when combined with clavulanate. As a result, sensitivity of ESBLs producing *E. coli* improved from 75.6% to 85.9% and for non-ESBLs producers from 67.4% to 86.9%. The MICs of individual isolates markedly reduced after adding clavulonic acid, but overall range of MICs was not changed (ranging from ≤1 - ≥1024). Because MICs of some isolates were out of the test range it was not possible to determine whether there was a significant (≥8 fold) decrease in MIC or not. The lowest value of MIC in our assay was 1 μg/ml; so in those isolates where MIC reduced beyond the concentration of 1 μg/ml could not be determined. Similar things happened in some highly resistant isolates having the MIC of >1024 μg/ml which was the highest MIC value tested. But the additional inhibitory effect of clavulanic acid could be predicted from the reduction of MIC_{50} and MIC_{90} of mecillinam after adding clavulonic acid (Table-3). Using the standard inoculum $1x10^4$ cfu/spot, MIC_{50} of mecillinam was reduced from 4 μg/ml to ≤1 μg/ml and MIC_{90} from 128 μg/ml to 64 μg/ml. These findings were in accordance with previous studies.[8,17] In the present study similar trend of reduction of MICs was also observed among ESBL negative *E. coli*. The observed synergistic effect of mecillinam-clavulanic acid combination on ESBL negative isolates could be due to the presence of other broad spectrum â-lactamases which were inhibitable by clavulanic acid or due to the primary affinity of clavulanic acid for PBP-2 of *E. coli* like mecillinam.[18,9] Altogether, by adding clavulonic acid with mecillinam about 56% of resistant isolates became sensitive which were resistant or intermediately sensitive with mecillinam alone (Table-2). In the present study, we have further observed that all intermediate and resistant isolates having MIC of 16 μg/ml and 32 μg/ml became sensitive (MIC ≤1-8) when clavulanic acid was added with mecillinam. But, only 40% isolates having MIC of 64 μg/ml reverted to sensitive range and none of the isolates having MIC ≥128 μg/ml became sensitive by adding clavulanic acid. It suggests that there is chance of treatment response with mecillinam-clavulanic acid combination in infection with resistant *E. coli* having such range of MICs. However, this combination may not be useful in severe infection by ESBL producers due to the inoculum effect of the offending organism on the drugs.[7,8,19]

The results of our study suggest that clinical trial should be done to confirm the usefulness of the mecillinam-clavulanic acid combination therapy in UTI with intermediate and resistant strains of *E. coli* having such range of MICs.

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References

1. Garau J. Other antimicrobials of interest in the era of extended spectrum beta-lactamases: fosfomycin, nitrofurantoin and tigecycline. *Clin Microbiol Infect* 2008; **14**(Suppl 1): 198–202.
2. Sougakoff W and Jarlier V. Comparative potency of mecillinam and other beta-lactam antibiotics against *Escherichia coli* strains producing different beta-lactamases. *J Antimicrob Chemother* 2000; **46**(Suppl 1): 9–14.
3. Rahman MM, Haq JA, Hossain MA *et al*. Prevalence of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in an urban hospital in Dhaka, Bangladesh. *Int J Antimicrob Agents* 2004; **24**(5): 508-10.
4. Abhilash KP, Veeraraghavan B and Abraham OC. Epidemiology and outcome of bacteremia caused by extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella spp*. in a tertiary care teaching hospital in south India. *J Assoc Physicians India* 2010; **58**: 13-7.
5. Hussain M, Hasan F, Shah AA *et al*. Prevalence of class A and AmpC beta lactamases in clinical *Escherichia coli* isolates from Pakistan Institute of Medical Science, Islamabad. Pakistan. *Jpn J Infect Dis* 2011; **64**(3): 249-52.
6. Kahlmeter G, Poulsen HO. Antimicrobial susceptibility of Escherichia coli from community-acquired urinary tract infections in Europe: the ECO.SENS study revisited. *Int J Antimicrob Agents* 2012; **39**: 45–51.
7. Thomas K, Weinbren MJ, Warner M, Woodford N, Livermore D. Activity of mecillinam against ESBL producers *in vitro*. *Journal of Antimicrobial Chemotherapy* 2006; **57**: 367-68.

8. Lampri N, Galani I, Poulakou G et al. Mecillinam/clavulanate combination: a possible option for the treatment of community-acquired uncomplicated urinary tract infections caused by extended spectrum â-lactamase producing *Escherichia coli*. *J Antimicrob Chemother* 2012; 1-5.

9. Moosdeen F, Williams JD, Yamabe S. Antibacterial characteristics of YTR 830, a sulfone â- lactamase inhibitor, compared with those of clavulanic acid and sulbactam. *Antimicrob Agents Chemother* 1988; **32**: 925–27.

10. Bongaerts GPA and Bruggeman-ogle KM. Effect of Beta-Lactamase and salt on mecillinam Susceptibility of Enterobacterial strains. *Antimicrob agents and chemotherapy* 1980; **18**(5): 680-86.

11. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Informational Supplement, CLSI Document M100-S20, Wayne, PA: Clinical and Laboratory Standards Institute, 2010.

12. Jarlier V, Nicolas MH, Fournier G and Philippon A. Extended spectrum beta lactamases conferring transferable resistance to newer beta lactam agents in *Enterobacteriaceae*- Hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; **10**: 867-78.

13. Søraas A, Sundsfjord A, Jørgensen SB, Liestøl K and Jenum PA. High rate of per oral mecillinam treatment failure in community acquired urinary tract infections caused by ESBL producing *Escherichia* coli. *PLoS ONE* 2014; **9**(1): e85889.

14. Saleh AA, Ahmed SS, Ahmed M et al. Changing trends in Uropathogens and their Antimicrobial sensitivity pattern. *Bangladesh J Med Microbiol* 2009; **3**(1): 9-12.

15. Rahman F, Chowdhury S, Rahman MM et al. Antimicrobial Resistance Pattern of Gram-negative Bacteria Causing Urinary Tract Infection. *S J Pharm Sci* 2009; **2**(1): 44-50.

16. Auer S, Wojna A and Hell M. ESBL producing *Escherichia coli* in uncomplicated urinary tract infections – oral treatment options? 49th ICCAC, San Fransisco CA. 2009

17. Lo´pez-Cerero L, Pico´n E, Morillo C et al. Comparative assessment of inoculum effects on the antimicrobial activity of amoxycillin–clavulanate and piperacillin–tazobactam with extended-spectrum â-lactamase-producing and extended-spectrum â-lactamase-non-producing *Escherichia coli* isolates. *Clin Microbiol Infect* 2010; **16**: 132–36.

18. Goldstein EJ, Citron DM and Cherubin CE. Comparison of the inoculum effects of members of the family *Enterobacteriaceae* on cefoxitin and other cephalosporins, â-lactamase inhibitor combinations, and the penicillin-derived component of these combinations. *Antimicrob Agents Chemother* 1991; **35**: 560-66.

19. Brenwald NP, Andrews J and Fraise AP. Activity of mecillinam against AmpC b-lactamase producing *Escherichia coli*. *J Antimicrob Chemother*. 2006; 58: 223–24.