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ORIGINAL ARTICLE

Reviving the role of mecillinam against extended spectrum beta-lactamase producing enterobacterales

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ABSTRACT

Background and Objectives: This study was designed to determine the *in vitro* efficacy of mecillinam against extended spectrum beta lactamse producing Enterobacterales.

Materials and Methods: After proper permission from Ethical Review Committee of the Institute, all samples yielding growth of ESBL producing Enterobacterales were part of the study and were processed according to routine microbiological procedures. Routine antibiotic sensitivity testing was done on Muller Hinton Agar by Modified Kirby Bauer Method. All Gram negative isolates were subjected to concomitant detection of ESBL production by double disc synergy method. All ESBL producers were then subjected to the mecillinam Minimum Inhibitory Concentration (MIC) determination by E test. The results were interpreted as per CLSI Guidelines.

Results: A total of 120 ESBL producing Enterobacterales isolates were included in the study. The mean age of patients with ESBL infection was 45 ± 18.7 years. There were 44% male and 55% female patients. Majority of the ESBL producing Enterobacterales were isolated from urine samples (56%), followed by pus. Among the isolated organisms, *Escherichia coli* (45%) was the most frequently isolated organism followed by *Klebsiella* spp. (22%). Overall 83% of the isolates turned out to be sensitive to mecillinam. MIC50 of mecillinam against ESBL producing Gram negative rods (GNR) turned out to be 1 ug/ml and MIC90 turned out to be 2 ug/ml.

Conclusion: Mecillinam shows good *in vitro* efficacy against ESBL producing Enterobacterales in our study. Further studies with more sample size and from diverse areas across the country should be done to evaluate its efficacy.

Keywords: Extended spectrum β-lactamase; *In vitro* susceptibility

INTRODUCTION

The current menace human beings are facing is increasing incidence of infectious diseases. Among infectious diseases the most threatening and fear causing one are caused by emerging super bugs. These super bugs make the most effective and broad spectrum antimicrobials ineffective leading to treatment failures, high morbidity and mortality rates. These super bugs can be multi drug resistant (MDR), extremely drug resistant (XDR) and Pan drug resistant (PDR) (1, 2). These resistance challenges are increasing day by day. Few of the current challenges which mankind is facing these days is extended spectrum beta lactamase (ESBL) producing Gram negative bacteria (1, 3).

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The term ESBLs refers to all those Gram negative rods which produce beta (β) lactamase enzymes (2). These β lactamases confer resistance against many broad spectrum antimicrobials. All those antimicrobials which have β lactam ring in their structure are destroyed by β lactamase enzymes and hence all these antimicrobials are rendered ineffective (3). B lactam containing antimicrobials are penicillins, cepahlosporins, cephamycins and carbapenems. Whenever an ESBL producing organism is isolated, it should be reported as resistant to all penicillins, cepahlosporins and aztreonam, even if it is tested to be susceptible in "*in vitro*" settings (4).

One of the more important concern regarding ESBLs is the associated resistance of these isolates to aminoglycosides and trimethoprim/ sulfamethoxazole and high frequency of coexisting of resistance to fluoroquinolones (3, 4). Beta lactamase inhibitor compounds like clavulanate, tazobactam and sulbactam exhibit in vitro efficacy against these isolates but clinically we get mixed reports for their therapeutic effects of beta lactam/beta lactamase inhibitor combination antimicrobials (5).

A Sharpe increase was reported in incidence of nosocomial infections by ESBL producing organism in late 1990s and year 2000. Worrisome figures were shared regarding incidence of ESBL producers from Middle Eastern countries as well (5, 6). The situation in Asia especially, South Asia is also not promising. China and India reported very high incidence of ESBL producing organisms since 1990s. Different studies from different parts of Pakistan have reported frequency of ESBLS to be between 45-70%, which is quiet high (6).

In this Era of rapid development of new drugs, most of the pharmaceutical companies are focused on development of novel antimicrobials for treatment of superbugs but to our bad luck all recently developed compounds are focused against multi drug resistant Gram positive organisms. The impending menace of pan drug resistant Gram negative bacteria can only be dealt with if there is rapid development of new antimicrobials against MDR, XDR and PDR Gram negative bacteria (7, 8). The pace of development of novel antimicrobials is not keeping up with developing resistance, hence it is necessary to reevaluate and revive forgotten and older antimicrobials. Fosfomycin, colistin, rifampicin and polymyxin B are some of older revived antimicrobials which are used successfully these days for treatment of super bugs.

Mecillinam is an amido penicillin with selective and good activity against Gram negative isolates. Many European and Scandinavian countries have recently included mecillinam in their empirical treatment guidelines, especially for treatment of community based urinary tract Infections (UTI) (9). Many studies from Belgium, United Kingdom, France and Norway have reported good *in vitro* as well as In vivo activity of mecillinam against extended spectrum beta lactamase producing Gram negative isolates. The important prospective benefits of mecillinam are that it is available as oral prodrug, it has convenient twice daily dosage regimen, it spares carbapenems and its use is less associated with high risk of *Clostridium difficile* associated diarrhea (10).

Research data from all over the world prompts us to evaluate the *in vitro* efficacy of this older forgotten antimicrobial against ESBL producing Enterobacterales.

Objective of this study was to evaluate the *in vitro* efficacy of mecillinam against ESBL producing Enterobacterales.

MATERIALS AND METHODS

It was descriptive and cross sectional study, carried out at Department of Pathology, Foundation University Medical College, FUI and Department of Microbiology, Fauji Foundation Hospital Rawalpindi. Duration of study was 6 months (Jan 2020 - June 2020). After proper permission from Ethical review Committee (Letter No.FF/ FUMC/215Phy/19) of the institute, all samples received in hospital lab from various patients of all ages and both genders reporting to different departments yielding growth of ESBL producing Enterobacterales were part of the study. All duplicate samples and samples of patients already receiving antibiotics were excluded from the study. It was consecutive probability sampling. Sample size was calculated according to W.H.O Criteria by taking the expected proportion of ESBLs sensitive as 90% and precision as 5% and 95% confidence interval.

All clinical samples received in the Fauji Foundation Hospital (FFH) microbiology laboratory for culture and sensitivity were processed by direct staining and inoculating culture on appropriate culture media and incubated for 18-24 hours at 37°C. Microorganisms were identified by Gram staining reaction, colony morphology, catalase test, cytochrome oxidase

test, motility testing, routine biochemical tests and using API20E (Biomerieux) tests where required. Routine antimicrobial susceptibility testing was performed on Muller Hinton agar by Modified Kirby Bauer Method. Antimicrobials included in the routine susceptibility testing were amoxicillin/clavulanate (20/10 µg), ceftriaxone (30 µg), aztreonam (30 µg), cefixime (5 µg), cefepime (30 µg), gentamicin/ amikacin (30 µg), aztreonam (30 µg), cefoxitin (30 µg) imipenem (10 µg) ceftazidime (30 µg) gentamicin (10 µg), piperacillin/tazobactam (100/10 µg), ciprofloxacin (5 µg), cefoperazone + sulbactam (75 µg + 30 µg), nitrofurantoin (300 µg), mecillinam (10 µg), doxycycline (30 µg), and trimethoprim + sulfamethoxazole (1.25 µg + 23.75 µg). The inhibition zone of each antimicrobial was interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines (11). All Gram negative isolated organisms were subjected to concomitant detection of ESBL production by double disc synergy method of Jarlier et al. (11). *Escherichia coli* ATCC 25922 ESBL strain was used as control strain.

All ESBL producing Enterobacterales were subjected to determination of minimum inhibitory concentration (MIC) of mecillinam by E test (LiofilChem).

The results were interpreted as per CLSI Guidelines (11). Isolate was termed as Resistant to mecillinam if MIC ≥32 ug/ml, Intermediate if MIC was 16ug/ml and Sensitive if MIC was ≤8 ug/ml (11).

Data was entered and analyzed in Microsoft Excel 2020. *In vitro* efficacy of mecillinam was calculated as frequency and percentage of resistant and sensitive microorganisms according to MIC of mecillinam against the isolates. MIC50 and MIC90 were calculated for mecillinam.

RESULTS

A total of 120 ESBL producing Enterobacterales were part of the study. Mean age of the patients with ESBL infections was 45 ± 18.7 years. Gender distribution of patients in terms of frequency was also calculated. There were 44% males and 55% female participants who were involved in the study according to mentioned inclusion criterion. Most of the ESBL producing Enterobacterales were isolated from urine samples (56%), followed by Pus and other samples (blood, high vaginal swab, catheter tips, tissue and fluid) (Table 1). Outpatient department (OPD) (25%) contributed to most of the samples followed by urology (22.5%) ward and gynecology/ obstetrics (16%) (Table 2). Among these isolates, *Escherichia coli* (45%) was the most frequently isolated organism followed by *Klebsiella* spp. (22%) (Table 3). *In vitro* antimicrobial susceptibility profile to commonly used antimicrobials is depicted in Fig. 1. MIC values of these organisms against mecillinam are indicated in Table 4.

MIC50 of mecillinam against ESBL producing GNR turned out to be 1 ug/ml and MIC90 turned out to be 2 ug/ml.

DISCUSSION

The injudicious use of antimicrobials to treat infections is leading to development of ESBLs, Amp C producers and metallo beta lactamase producers (12). We are heading towards era of XDR and PDR bacteria. Keeping in view the current increasing trend of resistance this study was planned to evaluate the current susceptibility pattern as well as *in vitro* efficacy of mecillinam against ESBL producing Enterobacterales in our setup (13). The isolation frequency of ESBL producing Gram negative bacteria is quiet high in our set up. Studies from different parts of Pakistan have quoted the incidence of ESBL producing Gram negative bacilli between 45-72%. Ibrar et al. reported the overall ESBL proportion from Pakistan as 40% (5). Saboor et al. indicated 72% incidence of ESBL production from Islamabad, Pakistan (6). Studies from china also indicate incidence between 46-70 percent (13,14). The reported incidence of ESBL isolation in developed countries like America, Germany is quiet less than reported from Asian and Middle Eastern countries (14). In our study 45% of the isolated ESBL producing Enterobacterales were *E. coli*. A study carried out in Islamabad in year 2014 reported 49% of the ESBL producing organisms to be *E. coli* (5). The major concern about these extended spectrum beta lactamase producing bacteria is their potential to cause outbreaks. In our study majority of the ESBL producing were isolated from the urine samples which is in accordance with the studies carried out in USA (2015), Germany (2012), United Kingdom (2017) and Norway (2016) (15-18). ESBL isolates showed good activity against beta lactam and beta lactamase combinations like amoxicillin/clavulanic acid (57%), cefoperazone/sulbac-

Table 1. Frequency of ESBL isolates from different samples

Serial number	Clinical Samples	No. of isolated ESBL Enterobacterales isolates	Percentage of Isolated ESBL Enterobacterales
1	Urine	67	56%
2	Blood	24	20%
3	Pus	19	16%
4	Catheter tips	5	4%
5	High Vaginal Swabs	3	2.5%
6	Tissue	2	1.5%
	Total	120	100%

Table 2. Frequecy of samples from out patient department or different wards

Sr. No.	Department/ Wards	No. Of isolated ESBL Enterobacterales isolates	Percentage of Isolated ESBL Enterobacterales
1	Outpatient Department	30	25 %
2	Urology Ward	27	22.5%
3	Gynecology	19	16%
4	Medicine/ICU/HDU	17	14.5%
5	Surgery	12	10%
6	Pediatrics	4	3%
7	Others	11	9%
	Total	120	100%

Table 3. Frequecy of different organisms among ESBL producing Enterobacterales

Sr. No.	Organism	No. and percentage of isolated ESBL producing Enterobacterales
1	*Escherichia coli*	54 (45%)
2	*Klebsiella* Spp.	27 (22%)
3	*Serratia marcenencs*	11 (9%)
4	*Enterobacter* spp.	8 (7%)
5	*Proteus* spp.	8 (7%)
6	*Citrobacter* spp.	6 (5%)
7	*Morganella* spp.	6 (5%)
	Total	120 (100%)

tam (77%), piperacillin/tazobactam (79%). A study carried out in Cork University Hospital, Ireland in year 2017 also reported similar *in vitro* results for beta lactam/beta lactamase inhibitor combinations (19). The high portion of co resistance against different antimicrobials among ESBLs limits the available treatment options, especially the oral options (19). The menace of resistance to antimicrobials is still not a major problem in some of the developed countries, the yearly surveillance data suggests a steady increasing trend to majority of the antimicrobial agents. Amoxicillin/clavulanic acid was inactive in more than around 50% of the isolated organisms in a study carried out in Norway, and it suggests the presence of additional beta lactam resistance mechanisms (20). Amoxicillin/clavulanic acid resistance co-related with increased prevalence of resistance to gentamicin, tobramycin and trimethoprim/ sulfamethoxazole (19-21). In our study we also found high resistance frequency against gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole. Tigecycline showed very good *in vitro* efficacy in our study that is 86% against these ESBL producing Enterobacterales. A similar study conducted in Pakistan in year 2016 showed 97% sensitivity of ESBL producers against tigecycline (22, 23). In one of the another studies conducted in year 2006 in Spain , Morosini et al. found that tigecycline (MIC 50, 0.5 µg/ml; MIC 90,1 µg /ml) had up to 256 times better activity than doxycycline and minocycline (23). In our study, considerable numbers of ESBL producing Enterobacterales isolates depicted high sensitivity to mecillinam (88%). The high sensitivity rate of ESBL producing

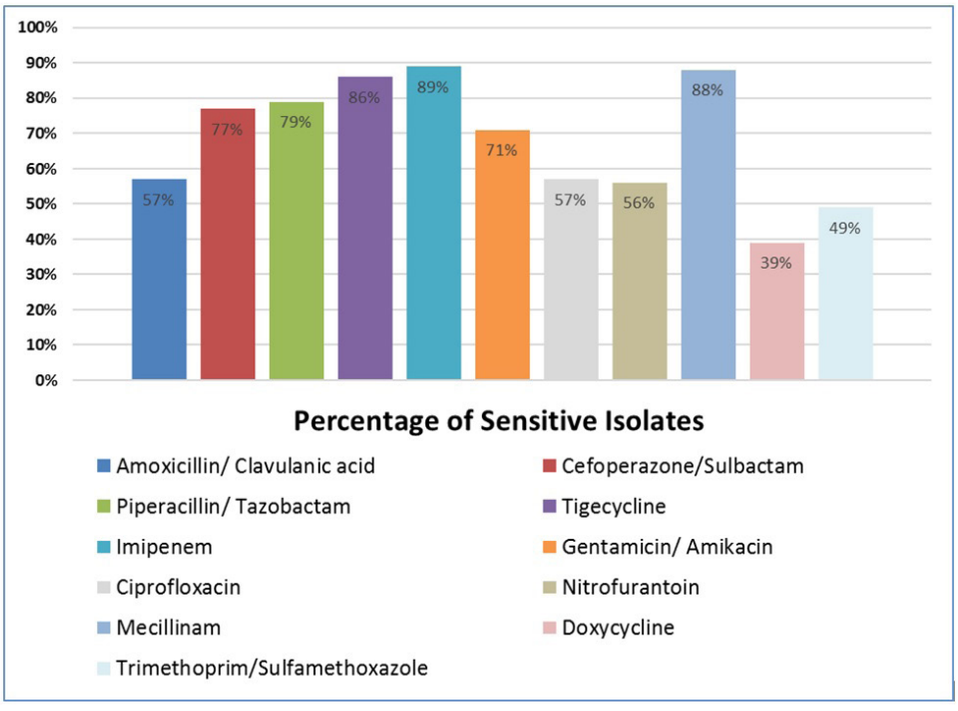

Fig. 1. Sensitivity profile of ESBL Producing organisms against different antimicrobials

Table 4. Number and percentage of isolates showing respective MIC value of Mecillinam (n=67)

Sr. No.	MIC Value (µg/ml)	Number and percentage of Isolates having this MIC (n=67)
1	0.125 µg/ml	2 (3%)
2	0.25 µg/ml	3 (4%)
3	0.5 µg/ml	13 (20%)
4	1 µg/ml	20 (30%)
5	2 µg/ml	17 (26%)
6	4 µg/ml	1 (1%)
7	8 µg/ml	1 (1%)
8	16 µg/ml	2 (3%)
9	32 µg/ml	2 (3%)
10	64 µg/ml	6 (9%)

E. coli to mecillinam as determined by MIC method in our study is comparable to the findings of others, who found 94% and 85% sensitivity respectively (24-26). A study carried out in Bangladesh in year 2009 reported *E. coli* sensitivity against mecillinam as 43-67% only. This deviation may be due to the fact that in that study Both ESBL and non ESBL producing *E. coli* were included in the study and sensitivity was checked by disc diffusion method only (27).

Mecillinam resistance is generally low among *E. coli* isolated from studies carried out in Scandinavian countries (27, 28). Among the 880 *E. coli* isolates studied, 4.8% (n = 42) were mecillinam resistant and 78.5% of the resistant isolates expressed high-level expression penicillinase (HEP) phenotype, although this molecule is thought to be resistant to hydrolysis by most of the beta lactamases. TEM 1 is prevalent worldwide and almost around 50% of *E. coli* harbor this betalactamase (29). Single gene mutation in the P3 promoter gene can transform it in to the Pa/Pb promoter sequence, thus conferring high level expression of TEM penicillinase and it can cause Mecillinam resistance in vitro settings. This particular mutation is not the only mechanism to induce HEP phenotype, but it was frequently observed in clinical isolated resistant to Mecillinam in studies carried out in developed countries (30).

MIC50 of mecillinam against ESBL producing GNR turned out to be 1 ug/ml and MIC90 turned out to be 2 ug/ml in our study. A study carried out in University Hospital Cologne, Germany in year 2021 concluded mecillinam MIC50 to be 8 ug/ml against MDR enterobacterales (31). The MICs of mecillinam ranged from 0.125 to 8 mg/L, with an MIC50 of 0.5 mg/L and an MIC90 of 4 mg/L in a study carried out in year 2010 at University Hospital of Wales (32). Mecillinam exhibited MIC50 and MIC90 values of

0.25 and 4 µg/ml against ESBL producing bacteria in a study carried out in USA in year 2014 (27). Unfortunately there are no studies published on this matter from countries which have high burden of these super bugs.

The oral derivative of mecillinam is already being widely used in Scandinavian Countries and there are reports of good clinical outcome and great safety profile especially against acute uncomplicated urinary tract infections (27, 28, 32). The added benefit is that this older forgotten drug has high safety profile in pregnancy as well. Data from these countries have shown that the resistance to mecillinam has not increased in these countries despite its widespread use for more than 20 years (29, 31, 32).

This is the time when we should also start investigating about the possible use of this older forgotten antimicrobial in our setup.

CONCLUSION

Mecillinam shows good *in vitro* efficacy against ESBL producing Enterobacterales in our study. Further studies with larger sample size should be done to evaluate its efficacy so that it can be introduced in clinical practice as empirical therapeutic options in community acquired urinary tract infections in our set up.

Limitations of the study: Minimum inhibitory reference criteria is only available for urinary isolates in both CLSI and EUCAST guidelines so non urinary isolates cannot be interpreted according to that criterion so they have been excluded for MIC of mecillinam.

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