

## Efflux Pump Mediated Carbapenem and Fluroquinolone Resistance among Nosocomial Isolates of *Escherichia coli*: A Study from North East India

Satabdi Bhattacharjee<sup>1</sup>, Debarati Choudhury<sup>2</sup>, Anand Maurya<sup>1</sup>,  
Debadatta Dhar(Chanda)<sup>3</sup> and Amitabha Bhattacharjee<sup>1\*</sup>

<sup>1</sup>Department of Microbiology, Assam University, Silchar, Assam - 788011, India.

<sup>2</sup>Department of Life Science & Bioinformatics, Assam University, Silchar, Assam - 788011, India.

<sup>3</sup>Silchar Medical College and Hospital, Silchar - 788014, India.

(Received: 23 February 2014; accepted: 10 April 2014)

Emergence of antibiotic resistance in pathogenic variants of *Escherichia coli* is a major concern worldwide. Efflux pump overexpression was reported to play major role in conferring multi drug resistant phenotype in *E. coli*. The aim of this study was to investigate the role of efflux pump in carbapenem and fluroquinolone resistance in nosocomial isolates of *E. coli*. A total 105 non duplicate, consecutive clinical isolates of *E. coli* were obtained from a tertiary referral hospital of northeast India. An efflux pump inhibitor (CCCP) based method was used for determination of efflux pump activity. Multiplex PCR was performed for molecular characterization of efflux pump and to determine the presence of plasmid mediated fluroquinolone resistant determinants. ERIC PCR was performed to determine clonal relatedness of the isolates. A total number of 20 (19%) demonstrated efflux pump mediated carbapenem resistance and 18 (17%) demonstrated efflux pump mediated fluroquinolone resistance. AcrA-TolC efflux system was predominant type. Coexistence of qnr-B gene was also observed in few isolates. All the isolates were clonally nonrelated. This is the first report emphasizing role of efflux pump in carbapenem and fluroquinolone resistance from this part of the country and the emergence of these mutants call for proper therapeutic options and diagnostic interventions.

**Key words:** *Escherichia coli*, AcrAB-TolC, Carbapenem, Fluroquinolone.

---

Pathogenic variants of *Escherichia coli*, the most common member of the family *Enterobacteriaceae* associated with human infectious diseases have been reported to develop resistance against a wide range of antimicrobial agents including amoxicillin, amoxicillin-clavulanic acid, cephalosporis, fluoroquinolones, and trimethoprim-sulfamethoxazole, carbapenems etc.<sup>1</sup> Efflux pump overexpression confers multi drug

resistant phenotype in *E. coli*. Efflux pump in Gram negative bacteria generally belong to resistance nodulation cell division family (RND) and in *E. coli*, seven homologue of RND type have been reported. The clinically significant pump in *E. coli*, the tripartite efflux pump, AcrAB-TolC complex, containing a fusion protein AcrA, a cytoplasmic membrane transporter protein AcrB and an outer membrane channel TolC is often associated with multi drug resistant (MDR) phenotype of *E. coli*.<sup>2</sup> Overexpression of regulatory gene *marA*, leads to increases increased expression of AcrAB which ultimately results into resistance to a large number of antimicrobial agents<sup>3</sup>.

---

\* To whom all correspondence should be addressed.  
Mob.: +91-8471939088;  
E-mail: ab0404@gmail.com

The aim of this study was to examine the contribution of efflux pump in fluoroquinolone and carbapenem resistance in clinical isolates of *Escherichia coli* collected from north east India by an inhibitor based method followed by confirmation of efflux pump genes by polymerase chain reaction.

## MATERIALS AND METHODS

### Bacterial strains

A total 105 non duplicate, consecutive clinical isolates of *E. coli* were obtained from the patients who were admitted or attended OPDs of Silchar medical college and hospital from March, 2012 to February 2013.

### Phenotypic detection of efflux pump activity

Efflux pump activity of the strains were phenotypically detected by double disc synergy test using Ciprofloxacin (10 mg, Himedia, Mumbai), Imipenem (10 mg, Himedia, Mumbai) and CCCP (carbonyl cyanide m-chlorophenylhydrazone, 100mM, Himedia, Mumbai)<sup>4</sup>.

### Molecular characterization of efflux pump system

The presence of the RND efflux system was determined using PCR. The primers, PCR conditions and reaction mixtures used were as described previously<sup>5</sup>.

### Investigation of plasmid mediated quinolone resistance

The plasmid mediated FQ resistant determinants, *qnrA*, *qnrB*, *qnrS* and *qepA* were detected by PCR. The reaction condition and primers used were as described earlier<sup>6</sup>.

### Antibiotic susceptibility

Antibiotic susceptibility testing was performed on Mueller-Hilton agar (Himedia, Mumbai, India) plates by Kirby Bauer disc diffusion method and interpreted as per CLSI

recommendations<sup>7</sup>. The antibiotics tested were, Ciprofloxacin (5µg), Amikacin (30µg), Gentamycin (10µg), Tigicyclin (15µg), Ceftazidime (30 µg), Piperacillin-Tazobactam (100/10 µg), Cotrimoxazole (Himedia, Mumbai, India).

### Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined on Muller Hilton Agar plates by agar dilution method against Levofloxacin, Gatifloxacin, Ofloxacin, Ciprofloxacin, Imipenem, merpenem and ertapenem (Himedia, Mumbai, India)<sup>7</sup>.

### DNA fingerprinting by ERIC (Enterobacteriaceae Repetitive Intergenic Consensus sequence) PCR.

ERIC PCR was performed to determine clonal relatedness of the isolates. The primers and reaction condition used were as described previously<sup>8</sup>.

## RESULTS

Out of 105 isolates, 38 (36%) was positive for efflux pump activity out of which, 20 (19%) were found to be positive for efflux pump mediated carbapenem resistance and 18 (17%) were positive for efflux pump mediated fluoroquinolone resistance.

All the positive isolates were further subjected for molecular characterization by PCR assay and it was found AcrAB-TolC (n=24) was predominant type responsible for efflux pump activity. Coexistence of Plasmid mediated quinolone resistance was also observed in three efflux positive isolates which were found to possess *qnr-B*.

All the isolates efflux positive isolates were multidrug resistant phenotype. However, Ceftazidime was found to have moderate activity. Other group of antibiotics came up with very low efficacy (Table.1)

**Table 1.** Antibiogram of *E. coli* with increased efflux pump activity

Antibiotics	Total no. of isolates with increased efflux pump activity	No. of susceptible samples	%
Amikacin	38	22	57
Gentamycin	38	11	28
Piperacillin-Tazobactam	38	14	36
Cotrimoxazole	38	17	44
Ceftazidime	38	15	39
Tigicycline	38	11	28

**Table 2.** MIC<sub>50</sub> and MIC<sub>90</sub> of efflux mediated Carbapenem resistant *E.coli* isolates

Antibiotics	MIC <sub>50</sub>	MIC <sub>90</sub>
Imipenem	8 µg/ml	64 µg/ml
Meropenem	8 µg/ml	32 µg/ml
Ertapenem	8 µg/ml	128 µg/ml

**Table 3.** MIC<sub>50</sub> and MIC<sub>90</sub> of efflux mediated Fluroquinolone resistant *E.coli* isolates

Antibiotics	MIC <sub>50</sub>	MIC <sub>90</sub>
Levofloxacin	16 µg/ml	64 µg/ml
Ciprofloxacin	128 µg/ml	256 µg/ml
Ofloxacin	64 µg/ml	128 µg/ml
Gatifloxacin	16	64

The MIC<sub>50</sub> and MIC<sub>90</sub> of the isolates were showed in Table 2 and Table 3. It was found that majority of the isolates were above the breakpoint for all the carbapenem and fluroquinolone drug tested. ERIC PCR revealed that all the isolates were clonally non related.

### DISCUSSION

Multi drug resistant *E.coli* are emerging at an alarming rate, and the infections caused by them complicates the treatment procedure. It becomes essential to investigate the molecular mechanism responsible for conferring such resistant phenotype to stop further spread of resistant strains. Efflux pumps, one of the most important class bacterial membrane transporters can lead to multidrug resistance when expressed at elevated levels<sup>1</sup>. Plasmid mediated acquired resistance mechanism were also found to contribute to multi drug resistance<sup>6</sup>. In this study, it was found that AcrAB-TolC efflux pump system was mainly responsible for efflux pump mediated fluroquinolone and carbapenem resistance among clinical isolates of *E.coli* from a tertiary care hospital of North east India and those isolates also exhibited cross resistance to other group of antimicrobial agents as well.

Presence of plasmid mediated quinolone resistance in efflux positive strains further

complicates the scenario as the therapeutic options are being critically limited by presence of two different resistant mechanisms.

Efflux pump selected mutants are reported to exhibit four to eight fold higher MIC<sup>9</sup>. Present study is in agreement of this fact as it was observed that for fluroquinolone and carbapenem group of drugs, MIC of all the strains possessing efflux pump activity was above breakpoint range.

ERIC-PCR revealed different DNA banding pattern suggesting that the efflux mediated resistance was not caused by the spread of identical strain.

### CONCLUSION

In the present study, it has been observed that increased efflux plays crucial role in carbapenem and fluroquinolone resistance and presence of other adjuvant mechanism (production of carbapenemases) also contribute to multi drug resistant phenotype of strains belonging to Enterobacteriaceae family. To the best of our knowledge, this is the first report emphasizing role of efflux pump in carbapenem and fluroquinolone resistance from this part of the country.

The high correlation between expression of the efflux system and resistance to the antimicrobial agents may raise a serious concern about the use of some antimicrobial agents. This mechanism should now be taken fully into account for the development of new antibiotics as well as for the future of chemotherapy in the long term.

Efflux pump inhibitors (EPI) are widely used to experimentally determine and evaluate the efflux mechanisms of bacterial pathogens. However, EPIs are still not in clinical use which demonstrates need for further research that might lead to the development of new EPIs. Further, new genotypic tests should be developed to improve the characterization of efflux mechanisms in clinical isolates for diagnostic purpose as well as search for newer antimicrobial chemotherapy.

### Source of Funding

University grants commission (UGC-MRP) Government of India and Department of Biotechnology (DBT-NER, Twinning) Government of India.

## ACKNOWLEDGMENTS

The authors would like to acknowledge the help of HOD, Microbiology, Assam University for providing infrastructure. The authors sincerely acknowledge the financial support provided by University Grants Commissions (UGC-MRP) Government of India and Department of Biotechnology (DBT-NER twinning Scheme) to carry out the work. Authors also acknowledge the help from Assam University biotech hub for providing laboratory facility to complete this work.

## REFERENCES

1. Sotto, A., De Boever, C.M., Fabbro-Peray, P., Gouby, A., Sirot, D. and Jourdan, J. Risk Factors for Antibiotic-Resistant *Escherichia coli* Isolated from Hospitalized Patients with Urinary Tract Infections: a Prospective Study. *J. Clinis. Micro.*, 2001; **39**(2): 438-444.
2. Yasufuku, T., Shigemura, K., Shirakawa, T., Matsumoto, M., Nakano, Y., Tanaka, K., Arakawa, S., Kinoshita, S., Kawabata, M. and Fujisawa, M. Correlation of Overexpression of Efflux Pump Genes with Antibiotic Resistance in *Escherichia coli* Strains Clinically Isolated from Urinary Tract Infection Patients. *J. Clinis. Micro.*, 2011; **49**(1): 189-194.
3. Okusu, H., MA, D. and Nikaido, H. AcrAB Efflux Pump Plays a Major Role in the Antibiotic Resistance Phenotype of *Escherichia coli* Multiple- Antibiotic-Resistance (Mar) Mutants. *J. Bacterio.*, 1996; **178** (1): 306-308.
4. Quale J, Bratu S, Landman D, and Heddurshetti R. Molecular Epidemiology and Mechanisms of Carbapenem Resistance in *Acinetobacter baumannii* Endemic in New York City. *Clinical Inf Diseases* 2003; **37**: 214-220.
5. Mesaros N, Glupczynski Y, Avrain L, Caceres NE, Tulkens PM and Van Bambeke F. Combined phenotypic and genotypic method for the detection of Mex efflux pumps in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2007; **59**: 378-386.
6. Wu JJ, WC Ko, SH Tsai, and JJ Yan. Prevalence of Plasmid-Mediated Quinolone Resistance Determinants QnrA, QnrB, and QnrS among Clinical Isolates of *Enterobacter cloacae* in a Taiwanese Hospital. *Antimicrobial agents and chem* 2007; **51**:1223-1227.
7. Clinical Laboratory Standards Institute: Performance standard for antimicrobial disc susceptibility test. 21<sup>st</sup> informational supplement. M100-S21. CLSI Wayne PA: 2011.
8. Versalovic J, Koueth T, and Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acid Res* 1991; **19**: 6823-6831.
9. Zhang Li, Li XZ, Poole K. Fluroquinolone susceptibilities of efflux-mediated multidrug resistant *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Bulkholderia cepacia*. *J Antimicrob Chemother* 2001; **48**: 549-552.