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DETECTION OF MULTIDRUG RESISTANT EFFLUX PUMPS IN CLINICAL ISOLATES OF E.COLI IN TERTIARY CARE HOSPITAL.

DR. C. R. SURENDRANATH[1], DR. B. V. RAMANA[2], DR. ABHIJIT CHAUDHURY[3]

1. Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati.
2. Asso. Prof, Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati.
3. Prof and Head, Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati.

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Abstract: Fluoroquinolones are widely used in both community and hospital settings. *E.coli* exhibits multiple resistant mechanisms; recent studies showed that multidrug resistant (MDR) efflux mechanism is most common. The effects of MDR inhibitors of the RND efflux pumps, Reserpine 25 µg/ml and MC 207, 110 (Phe-Arg-beta-naphthylamide (PaβN), Sigma) 25 µg/ml, on the MIC of fluoroquinolones for clinical isolates of MDR *E.coli* were studied. The MIC of *E.coli* collected during the period of two years is tested with fluoroquinolones in the presence and in the absence of inhibitors by using the CLSI broth dilution method. In the presence of PaβN, only one strain of *E.coli* (1.8%) has shown greater than 8 fold reduction in MIC for ciprofloxacin. In the presence of reserpine change in MIC values was not observed in any of the clinical isolates of *E. coli*. These results indicate the need to identify overexpressing efflux pumps at diagnostic level.

Keywords: *E. coli*, Efflux pumps, Reserpine, MC 207-110 (PAβN).

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Corresponding Author: DR. C. R. SURENDRANATH

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INTRODUCTION

Antibiotic resistance is the biggest challenge to the medical profession in the treatment of infectious diseases. Resistance has been documented not only against antibiotics of natural and semi-synthetic origin, but also against purely synthetic compounds (such as the fluoroquinolones) or those which do not even enter the cells (such as vancomycin). The wide range of occurrence of antibiotic resistance suggests that, in principle, any organism could develop resistance to any antibiotic (1).

Increasing gram-negative resistance has also negatively impacted the physician's alternatives when choosing adequate initial therapy: increased reliance on the carbapenem class as empirical agents has led to the emergence of multidrug resistance in non-enteric gram-negative bacilli. The problem of resistance among gram-negative pathogens is clearly exemplified by the production of β-lactamases by *Enterobacteriaceae*. Fluoroquinolone antibiotics have been available since the 1980s, when ciprofloxacin and norfloxacin were licensed (2).

In most hospitals, fewer than 5% of isolates of the *Enterobacteriaceae* are resistant to carbapenem. However, in recent years, there have been pockets of increased resistance to this antibiotic class. (3, 4) .Quinolones are used widely for the treatment of serious *E. coli* urinary tract infections (UTIs) and may also be used to treat other infections caused by other members of the *Enterobacteriaceae* family (5).

Hence, quinolone resistance in *Enterobacteriaceae* may lead to treatment failures and is a significant concern, as is the recent emergence of plasmid-mediated resistance (6).Several mechanisms are known by which this microorganism escapes the toxic effects of antimicrobial agents. These include production of inactivating enzymes, mutations of target enzymes, and multidrug efflux pumps (7).

The efflux must be considered as a common and basic mechanism of resistance, and perhaps more ubiquitous than target modification or production of antibiotic-inactivating enzymes (8). The pumps, especially the Resistance Nodulation-Division (RND) family, have received particular recent attention because they can extrude multiple structurally unrelated compounds, and thus are involved in multidrug resistance (17). By the Inhibition of activity of efflux pumps will thus have clear benefits for therapy since this will increase the susceptibility of gram negative bacilli, thus increasing the therapeutic efficacy of antibiotics used for treating such infections by these pathogens (9). In the present study multidrug resistance (MDR) clinical isolates of *E. coli were* tested with ciprofloxacin and with efflux pump inhibitors (Reserpine, and PAβN). Clinical isolates of multidrug resistant *Escherichia*

coli, were collected from the cultures of blood, urine, pus, stool, catheter tip and body fluids etc, Isolates were tested for species conformation, sensitivity, MIC with ciprofloxacin, in presence and in the absence of efflux pump inhibitor.

METHODS:

An antimicrobial susceptibility test was done by Kirby Bauer disk diffusion method (10). Isolates resistant to at least three classes of antimicrobial agents were considered as multidrug resistant (11). *Escherichia coli ATCC-25922 (Hi-Media)* was taken as reference strain.

All isolates were tested for reduction in MIC of ciprofloxacin, in presences and in absence of efflux pump inhibitors Reserpine (25µg/ml) and PAβN (25µg/ml) separately in triplicates (12, 13, 14, 15, 16, 17, 18).

RESULTS :

The antibiogram results of *E. coli* showed that, all the 54 *E. coli* isolates were resistant to gentamicin, ciprofloxacin, nalidixic acid, cefotoxime. 53 isolates were resistant to amikacin, 52 isolates were resistant to co-trimoxazole, 49 isolates were resistant to tetracycline, 41 isolates were resistant to piperacillin/ tazobactum , 35 isolates were resistant to cefaperazone/ sulbactum, 26 isolates were resistant to nitrofurantoin, and 15 isolates were resistant to meropenem (Table: 1).

Table: 1. Antibiotic resistance pattern of 54 *E. coli* isolates.

Antibiotics	Resistant (%)	
	No	%
Ct	52	96.2
Gm	54	100
Ak	53	98.1
Na	54	100
Ci	53	98.1
Mr	15	27.7
Cs	35	64.8
Pt	41	75.9
T	49	90.7
Cx	54	100
Nf *	26	76.4

* % based on total number of urinary isolates.

(Ct: Co-trimoxazole, Gm: Gentamicin, Ak: Amikacin, Na: Nalidixic acid, Ci: Ciprofloxacin, Mr: Meropenem, Cs: Cefaperazone/ Sulbactum, Pt: Piperacilin/ Tazobactum, T: Tetracycline, Cx: Cefotoxime, Nf: Nitrofurantoin. R: Resistance, S: Sensitive.* for urinary isolates only).

The minimum inhibitory concentration results of 54 multidrug resistance clinical

isolates of *E. coli* for ciprofloxacin in presence and in absence of efflux pump inhibitors PAβN and reserpine were performed.

In the presence of PAβN, only one strain of *E. coli* (1.8%) has shown greater than 8 fold reductions in MIC for ciprofloxacin, while remaining 53 isolates have shown no change in MIC. In the presence of reserpine change in MIC values was not observed in any of the 54 clinical isolates of *E. coli*.

DISCUSSION:

The multidrug resistance phenomenon is at times associated with the over expression of the drug transporters that recognize and efficiently expel a broad range of structurally unrelated compounds from the cells. The available genome sequences of various bacteria revealed that known and putative drug efflux transporters constitute 6% to 18% of all transporters (19).

In this study, the reduction in MIC in the presence of reserpine was not observed in any of the clinical isolates of *E. coli,* which indicates that there can be absence of expression of monocomponent efflux pumps.

Reserpine is an effective inhibitor of monocomponent efflux pump system like SMR, MFS, MATE, ABC family commonly found in gram positive bacteria and in few gram negative bacteria in which the mechanism is plasmid mediated (15, 20, 21, 22, 23).

The MIC analysis in the current study has shown that in the presence of PAβN has shown reduction in MIC in 1 clinical isolate of *E. coli.* These results clearly indicate the presence of over expressing Resistance Nodulation cell-Division (RND) efflux pumps (24, 17).

In *Escherichia coli,* the predominant FQ efflux system is encoded by the *acrAB-tolC* genes. This system is broadly specific and accommodates a number of clinically relevant antimicrobials in addition to FQs, where it provides for intrinsic resistance, and its hyper expression in mutants results in elevated resistance to FQs and other agents (25). The gene *acrR* is transcribed divergently from the acrAB genes and encodes a repressor of the TetR repressor family. AcrR is known to repress both its own and acrAB transcription (26, 27, 28, 29). In this study one *E.coli* isolate has shown the reduction in MIC which may be due to the presence of mutations in *acrAB* regulatory gene *acrR*, however these results needs to be accentuated. The role of AcrR in the fluoroquinolone resistant of *E.coli* clinical isolates showed by Hui Wang *et al.,* (30) a link between mutations in *acrR* and high level of fluoroquinolone resistance as a result of *acrAB* overexpression (30).

It has been suggested that apart from local regulators (AcrR) of *E. coli*. In other isolates which has not shown reduction in MIC to ciprofloxacin in presence of efflux pump inhibitors may be due to mechanisms other

than efflux mechanism like target modification (26).

CONCLUSIONS:

Numerous articles have been published for a variety of bacterial species showing MIC data of antibiotics with and without an EPI such as reserpine or PaβN reveal enhancement of antibiotic activity, this has been interpreted to indicate the presence of an efflux pump. The use of efflux pump inhibitors is necessary in diagnosing the multidrug resistant isolate particularly in tertiary care hospital.

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