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DETECTION OF INHIBITOR RESISTANT BETA-LACTAMASES IN A TERTIARY CARE CENTRE OF MANIPUR, INDIA: A REPORT OF THREE CASES

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ABSTRACT: The emergence of inhibitor resistant beta-lactamase producing isolates might be related to the frequent use of beta-lactamase inhibitors in hospitals and general practice. During a routine identification and antibiogram of gram negative isolates by the Vitek 2 system, two urinary and one sputum isolates of inhibitor resistant beta-lactamases showing concordant result with the Kirby Bauer disk diffusion method were detected. Paucity of data regarding the occurrence of these enzymes in north-east India is the rationale for reporting these cases.

KEYWORDS: Inhibitor resistant β -lactamases, IRT, OXA.

INTRODUCTION: The synergistic effect of β -lactamase inactivators (Clavulanic acid, sulbactam and tazobactam) and a penicillin or cephalosporin has been employed to combat β -lactamases.¹ Reports have established regarding the development of resistance to these inhibitors which might be caused by the hyperproduction of unmodified TEM (TEM named after the patient, Temoniera, from whom it was isolated)-type β -lactamase, or modification of the outer membrane proteins, or production of OXA (Oxacillinase)-type enzymes, or hyperproduction of cephalosporinases.²⁻⁶ Since 1990, the emergence of inhibitor-resistant TEM β -lactamases (IRT) has further compromised the effect of β -lactamase inhibitors.⁷

Although the organisms possessing these enzymes are resistant to combinations of beta-lactams and beta-lactamase inhibitors, they remain susceptible to narrow spectrum (Cephalothin) and extended spectrum cephalosporins (Ceftazidime, Ceftriaxone), carbapenems and monobactams⁸. Most of the cases showed resistance to amoxicillin/clavulanate and ampicillin/sulbactam as when compared to piperacillin/tazobactam.⁸

We report three isolates of Enterobacteriaceae that resist the activity of β -lactamase inhibitors.

CASE REPORTS: Case 1: A 34 years old lady from an urban area of Manipur, India presented to the outpatient department of medicine, JNIMS, Imphal on 7th December, 2012 with complaints of intermittent lower pain abdomen, frequency of micturition, dysuria and nausea for about 1week. On examination, body temperature was 38⁰C, hypogastrium was tender and other systems were normal. She was advised urine microscopy with culture and sensitivity, and ultrasonography of whole abdomen. USG showed normal study.

Case 2: A 67 years old man, farmer, smoker from a rural area of Manipur was admitted to male medicine ward, JNIMS, Imphal with fever, productive cough of pink-tinged sputum, exertional dyspnea and malaise for 3 weeks. There was no significant past or family history. On examination, he

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was underweight, body temperature 39°C, pulse 120 beats/min, BP-134/90 mmHg and respiratory rate 38 breaths/min. Auscultation of the chest revealed diffusely reduced breath sounds, rhonchi and faint inspiratory crackles in the lower right lung. There was no clubbing. Other systems were normal. Routine blood, blood sugar (Random), sputum microscopy and culture sensitivity, chest X-ray were sent. Empirical treatment of ceftriaxone and azithromycin was started. Blood test revealed leucocytosis (17,000/mm³), blood sugar of 90mg% and consolidation in right lower lobe of lungs on chest X-ray.

Case 3: A 28 year old lady from a rural area attended the outpatient department of medicine, JNIMS, Imphal on 12th August, 2013 with fever, chills, abdominal discomfort, urinary frequency and urgency, and dysuria for 5 days. She had similar episode one month back for which she took medicines from a pharmacy without consulting doctor. Examination showed body temperature of 39°C, tachycardia, mild pallor, mild tenderness of hypogastrium. Routine blood, urine microscopy and culture sensitivity, and USG of lower abdomen were advised. Her Hb was 9gm%, WBC-8000/mm³ and normal USG.

Microbiological Studies of the Cases: Grossly, both the urine specimens were turbid with pH of 5.5 and 7 in case 1 and 3 respectively. Microscopically, plenty of pus cells were seen.

The sputum was purulent with pinkish tinge and direct gram stain revealed gram negative bacilli with plenty of pus cells and few epithelial cells.

Identification and antibiotic sensitivity pattern of the causative bacteria from the clinical samples were performed by the Vitek 2 system (Bio Me'rieux, Marcy l'Etoile, France) following presence of pure culture of isolated colonies of the microorganisms on blood agar and Mac Conkey's agar.⁹ Following the manufacturer's instructions, they were inoculated onto the identification and the AST-N280 cards, on which different concentrations of eighteen antibiotics were assayed. This system also features an Advanced Expert System (AES) that interprets the antibiotic resistance patterns, validates the results and reports the resistance phenotype, and has proved useful in calculating MIC values.

E. coli was isolated in both the urine samples and *Klebsiella pneumoniae* in the sputum. However, AES findings showed all of them to be inhibitor resistant beta-lactamases. The antibiogram is shown in Table-1.

Susceptibility of the three isolates to amoxicillin (30µg), amoxicillin/clavulanic acid (20/10µg), piperacillin (100µg), piperacillin/tazobactam (100/10µg), cefoxitin (30µg), ceftazidime (30µg) was further evaluated by the Kirby Bauer disk diffusion method.⁹ The phenotype amoxycillin-resistant, amoxycillin plus clavulanate-resistant, piperacillin-intermediate, piperacillin/tazobactam-susceptible, cefoxitin-susceptible and ceftazidime-susceptible was observed in these isolates.

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Antimicrobial	Case 1		Case 2		Case 3	
	MIC	Inter-pretation	MIC	Inter-pretation	MIC	Inter-pretation
Ampicillin	≥32	R	≥32	R	≥32	R
Amoxicillin/ Clavulanic acid	≥32	R	≥32	R	≥32	R
Piperacillin/Tazobactam	≤4	S	64	I	8	S
Cefuroxime	≤4	S	≤4	S	4	S
Ceftriaxone	≤1	S	≤1	S	2	S
Cefepime	≤1	S	≤1	S	≤1	S
Cefoperazone/ Sulbactam	≥64	R	≥64	R	≥64	R
Ertapenem	≤0.5	S	≤0.5	S	0.5	S
Imipenem	≤1	S	≤1	S	1	S
Meropenem	≤0.5	S	≤0.25	S	≤0.5	S
Amikacin	≤2	S	≤2	S	≤2	S
Gentamicin	≤1	S	≤1	S	≤1	S
Ciprofloxacin	≥4	R	2	I	2	I
Nalidixic acid	≥32	R	2	S	≥32	R
Tigecycline	≤0.5	S	≤0.5	S	≤1	S
Nitrofurantoin	≤16	S	64	I	32	S
Trimethoprim/ Sulfamethoxazole	≥320	R	≤20	S	80	R
Colistin	≤0.5	S	≤0.5	S	≤0.5	S

Table 1: Susceptibility reports

S-Sensitive, I-Intermediate, R-Resistant

DISCUSSION: Despite the extensive use of β -lactam/ β -lactamase inhibitor combinations in most countries, inhibitor resistant β -lactamases have been more frequently isolated in Europe than in the USA, where they have been rarely reported.¹⁰ Their occurrence have also been detected in Malaysia from *E. coli* isolates.¹¹ However, there is a paucity of information about IRT geographical distribution, probably due to inadequate phenotypic techniques using standard laboratory susceptibility tests. It was demonstrated that the automated system was able to detect 86.7% of IRT-producing *E. coli*.¹² Livermore DM et al. observed that for Enterobacteriaceae with IRT, the vitek 2 AES achieved 60% full and 40% partial agreement with the genotypic analysis, and penicillinases were considered as the alternative cause for the partial agreement strains.¹³ However, the characterization must be completed by iso-electric points of β -lactamases, determination of kinetic parameters and the use of molecular biology techniques.¹⁴

These variants of β -lactamases occur among both community and hospital-acquired isolates. They have been recovered from urine (Predominantly), blood, sputum and stool.⁸ So far, only one isolate has been reported from an agricultural source.¹⁵

In our cases, the Vitek 2 showed concordant result with the disk diffusion method but the characterisation could not be carried out due to lack of infrastructure of molecular techniques in our institute. These isolates could be IRT or OXA enzymes or cephalosporinase hyperproduction. Despite our inability to confirm the findings, we report these cases to show the possibility of strains

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possessing these enzymes in Manipur and they were susceptible to ceftriaxone, cefepime, cefuroxime, imipenem, meropenem, ertapenem, colistin, amikacin, gentamicin and piperacillin-tazobactam.

CONCLUSION: The occurrence of inhibitor resistant β -lactamases has been under-reported from this region. The reasons being, unreliable detection by the routine susceptibility tests, lack of automation like Vitek2 which could at least help in preliminary detection or molecular biology methods for confirmation in most institutes. In view of inadequate data regarding the incidence of these beta-lactamases in our region, we report these cases.

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