To the Editors:

Phenotypic detection of metallo-β-lactamase producing *Klebsiella pneumoniae* by an inhibitor based method

We report a case of metallo-β-actamase producing *Klebsiella pneumoniae* isolated from the urine of a 9 month old child. As far as we are aware, this is the first reported case of a metallo-β-lactamase producer (MBL) isolated in Sri Lanka. The increased prevalence of bacterial pathogens producing different β-lactamases necessitates clinical laboratories to detect strains producing these enzymes accurately. Our method is technically simple and inexpensive for MBL detection.

A 9-month old girl was admitted to Anuradhapura Teaching Hospital with high fever, rigors and urinary symptoms. She had three culture positive urinary tract infections with coliforms in the preceding month and had been treated with many different combinations of antibiotics. On examination she was febrile, but had no other signs. Her C-reactive protein was 5.3 mg/dl and white blood cell count was 24.7×10^6/ml. A coliform $>10^5$ cfu/ml was isolated from her urine and the organism was resistant to all antibiotics tested including meropenam and imipenam (Stokes disk diffusion method). Ultrasound of the abdomen was normal.

As metallo-β-lactamase (MBL) production was a possibility, we used a double disk synergy test with three substrates, ceftazidime in addition to imipenam and meropenam, to increase the detection sensitivity and EDTA (ethylene-diamine-tetraacetic acid) as an MBL inhibitor. For confirmation we used a combined disk with EDTA using the same substrates. We also used a blank disk with EDTA on the plate to exclude direct toxicity of EDTA to the organism. We identified the coliform isolate as *Klebsiella pneumonia spp* using API 20E identification method.

Her fever continued and she was given netilmicin and ciprofloxacin empirically. After ten days of netilmicin and ciprofloxacin the child became clinically well. A supra-pubic aspirated specimen of urine showed no growth.

Carbapenems are often used as antibiotics of last resort for treating infections due to multidrug-resistant gram-negative bacilli, because they are stable to ESBLs and AmpC β-lactamases. However, gram-negative bacilli producing the MBLs are resistant to almost all beta-lactam antibiotics including carbapenems. Hence, therapeutic options are limited.

MBL-producing organisms are found in *Pseudomonas* and *Acinetobacter* species. MBL hydrolyses all penicillins, cephalosporins and carbapenems except monobactams [1]. MBL genes are usually transmitted via plasmids which may also carry genes encoding resistance to non-betalactam agents such as cotrimoxazole and aminoglycosides.

MBLs require metallic iron zinc for their action and hence the use of EDTA and thiol-based compounds which absorb the zinc ion rendering the enzyme incapable of antibiotic hydrolysis [1, 2]. Carbapenem or cephalosporin resistance reversed by the addition of EDTA is the essential phenotypic requirement for MBL confirmation. MBL resistance vary among enterobactereae, therefore addition of ceftazidime to carbapenem increases detection sensitivity [3, 4].

References

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