Laboratory Diagnosis of NDM-1 and Other Carbapenem-Resistant Enterobacteriaceae

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Background

Carbapenems belong to the beta-lactam group of antimicrobials. Compared with earlier beta-lactams, carbapenems have a wide spectrum of anti-bacterial activities and are stable against many types of beta-lactamases produced by bacteria. With the emergence of antimicrobial resistance against multiple agents among bacteria, carbapenems have come to be considered as the last line of defence against resistant infections. As a result, the development of reduced susceptibility of bacteria in the Enterobacteriaceae family to carbapenems, not uncommonly linked to resistance to other antimicrobial classes including fluoroquinolones (such as ciprofloxacin) and aminoglycosides (such as gentamicin and amikacin), heralds the era of untreatable infections.1

Laboratory Methods for Detection of Antimicrobial Resistance

In the microbiology laboratory, detection of antimicrobial resistance is mainly by means of phenotypic and genotypic methods. Phenotypically, antimicrobial disk diffusion methods and determination of minimum inhibitory concentrations (MICs) are the major techniques employed. In the routine diagnostic laboratory, the disk diffusion method is the most practical choice. Standard guidelines have been promulgated for the performance and quality control of the disk diffusion test, and the most commonly adopted standard in laboratories in Hong Kong is that from the Clinical and Laboratory Standards Institute (CLSI), USA.2 Another less commonly used standard locally is that from the European Committee on Antimicrobial Susceptibility Testing (EUCAST).3

The disk diffusion test yields results in categories of “susceptible”, “intermediate” and “resistant”. Bacterial strains exhibiting the latter two result categories are collectively considered as being “non-susceptible” to an agent. As for MIC determination, results are in terms of mg/L of the antimicrobial agent required to inhibit growth of the strain, and are presented in a continual scale of 2-fold dilutions. MIC determination can be conveniently performed in a routine diagnostic laboratory using commercially available strips impregnated with a gradation of an antimicrobial (such as the Etest), or using automated systems (such as the Vitek system). More conventional standard methods for MIC determination include the broth dilution (micro or macro format) and agar dilution tests. Apart from the phenotypic methods above, genotypic methods can detect the presence of particular genes conferring resistance to specific antimicrobials. An example is the presence of the NDM gene, encoding for the New Delhi metallo-beta-lactamse, conferring resistance to carbapenems. Genetic testing can also determine the locus and architecture of the resistance mechanism, its transferability and the co-existence of resistance mechanisms of other antimicrobials.

Carbapenemases in Enterobacteriaceae

Although carbapenems are characteristically resistant to hydrolysis by many beta-lactamases, a few groups of enzymes that exhibit carbapenemase activity have been detected among various families of bacteria. An important feature of these enzymes is that they are mostly encoded by mobile genetic elements which may be transferable to other bacterial strains. These enzymes have differential effects on different carbapenems, including imipenem, meropenem, ertapenem and doripenem which are available for clinical use in Hong Kong. Three major classes of carbapenemases of clinical relevance can be detected in members of Enterobacteriaceae (such as Escherichia coli, Klebsiella spp. and Enterobacter spp.).2 Class A carbapenemases comprise enzymes such as KPC (K. pneumoniae carbapenemase) and IMI (imipenem-hydrolysing beta-lactamase). Their activity is inhibited by boronic acid in the laboratory. Class B carbapenemases are also called metallo-beta-lactamases (MBLs). Examples include NDM and IMP (active on imipenem). These enzymes can be inhibited by metal-chelating agents such as EDTA in the laboratory. Another group of carbapenemases found in Enterobacteriaceae is the OXA (active on oxacillin) enzymes, mainly OXA-48. They do not have a consistent inhibitor on laboratory testing.

The Status so Far for NDM-1 and Other Acronyms in Hong Kong

NDM was first reported in the literature in late 2009.5 In Hong Kong, the Microbiology Division of the Public Health Laboratory Services Branch (PHLSB), Centre for Health Protection (CHP), Department of Health provides diagnostic microbiology and reference laboratory testing services locally to both the public and private sectors. In September 2009, a urinary specimen from a male patient 64 years of age grew an E. coli strain with intermediate susceptibility to imipenem. Among
first-line antimicrobials for urinary tract infections, although the strain was resistant by the routine disk diffusion test to ampicillin and amoxicillin-clavulanate, and intermediate to cefotaxime and ceftriaxone. Regarding second line agents, apart from being intermediate to imipenem, the strain was resistant to cefepime (gentamicin and amikacin). It was “susceptible” to meropenem and intermediate to ertapenem. The patient recovered after a course of ciprofloxacin. Retrospective testing of the isolate showed that it produced a carbapenemase and harboured the NDM-1 gene. Since 2009 and up to October 2010, carbapenemase-producing Enterobacteriaceae isolates have been confirmed by the Microbiology Division from 12 patients. There was one patient with an Enterobacter cloacae isolate harbouring the Class A carbapenemase IMI-3. For the remaining 11 patients with isolates containing Class B carbapenemases, apart from the NDM-1 strain mentioned above, the remaining 10 patients had strains with the IMP-4 enzyme (two strains of E. coli, seven strains of Klebsiella spp. and one strain of Citrobacter freundii).

**Laboratory Surveillance of Enterobacteriaceae Non-susceptible to Carbapenems**

In order to monitor the emergence of Enterobacteriaceae with reduced susceptibility to carbapenems, the Microbiology Division has been collating data on such bacterial strains in collaboration with other microbiology laboratories in Hong Kong. Nevertheless, close liaison of the microbiology laboratories with the hospital infection control teams is essential such that isolation precautions for individual patients are adopted immediately on detection of bacterial strains with reduced susceptibility to carbapenems, prior to availability of further characterisation results.

Through discussion among a CHP working group with the participation of clinical microbiologists, clinicians and laboratory scientists, a laboratory protocol has been prepared, recommending testing strategy to screen for Enterobacteriaceae isolates potentially harbouring genes encoding for carbapenemases. As Proteus spp., Providencia spp. and Morganella morgani might exhibit intermediate or frank resistance to imipenem due to non-carbapenemase-mediated mechanisms, these genera were excluded from the enhanced surveillance protocol. In brief, hospital laboratories were requested to be vigilant for any Enterobacteriaceae strains showing non-susceptibility to any carbapenems. Nevertheless, some bacterial strains producing carbapenemase might still yield “susceptible” results to some carbapenems, such as the NDM-1 strain, being susceptible to meropenem as described above. On detection of any Enterobacteriaceae isolate with non-susceptibility to any carbapenem agent using routine susceptibility testing methods, it is recommended to perform the modified Hodge test for carbapenemase production using imipenem, meropenem and ertapenem as substrates. This test detects the presence of any enzyme that hydrolysates the carbapenem used in the test (Figure 1a). However, results of this test can be difficult to be interpreted, with occasional indeterminate and false positive/negative findings. For any strain positive or indeterminate in the modified Hodge test, laboratories are requested to perform the combination disk test, essentially routine disk diffusion susceptibility testing with each carbapenem, together with the same carbapenem disk incorporating the inhibitor of Class A carbapenemases (boronic acid) and that of Class B carbapenemases (EDTA) (Figure 1b). Strains found to possess Class A or B carbapenemase activity should undergo molecular testing for the presence of respective genes by polymerase chain reaction, with confirmation and further characterisation by nucleotide sequencing as necessary. Clinicians may check with their service laboratories regarding adoption of the protocol as recommended by the working group.

Currently, as there is no readily available screening protocol for Class D carbapenemases, and Enterobacteriaceae strains harbouring this resistance mechanism are not yet globally widespread, systematic surveillance in Hong Kong has not been initiated. Nevertheless, all laboratories should watch out for resistant strains exhibiting carbapenemase activity and being negative for known carbapenemase genes, and subject the isolates for further characterisation as appropriate. Furthermore, as in the case of the NDM gene, new genes mediating resistance will be discovered with time. Any isolate exhibiting uncharacterised resistance mechanisms should be archived for further testing for subsequent newly discovered genes.

**Prospect**

It is certain that bacteria will continue to evolve, and multi-drug resistance will further emerge. The laboratory plays a crucial role in detecting and monitoring the problem. With changing epidemiological patterns and discovery of new resistance mechanisms, the laboratory needs to be proactive in adopting the most appropriate and up-to-date technology and protocols and surveillance strategies, so as to continue to embrace the challenge against infections by resistant micro-organisms with few therapeutic options.

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References