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Letter to Editor

Microbiology Section

Simplified Protocol for the Phenotypic Identification of Carbapenem Resistance Mechanism in *Enterobacteriaceae*

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Dear Editor,

Carbapenems are often one of the last resorts available for the treatment of multi-drug resistant *Enterobacteriaceae* infections. However, in the recent past, reports were accumulating on the emergence and dissemination of carbapenem resistance [1]. Therefore, carbapenem resistance in *Enterobacteriaceae* is considered as one of the major public health problems worldwide. According to recent report [2], there are three major mechanisms involved in carbapenemase production, which confers carbapenem resistance among *Enterobacteriaceae*. They are, production of *Klebsiella pneumoniae* carbapenemase (bla_{KPC}), production of metallo β-lactamase (MBL) encoded by bla_{NDM}, bla_{IMP} and bla_{VIM} genes, and production of Oxacillinase-48(bla_{OXA-48}) or its derivatives. However, the other mechanisms of carbapenem resistance often observed among *Enterobacteriaceae* are loss of porin channel along with the co-production of Extended Spectrum β-lactamase (ESBL) or Ambler Cass C (AmpC) β-lactamase [1]. Identification and documentation of carbapenem resistance in *Enterobacteriaceae* is imperative to contain their spread.

Based on the literature review and minor modifications from the previously published protocols [1,3] herewith we present a simplified protocol for the easy detection and differentiation of the mechanism of resistance in Carbapenem Resistant *Enterobacteriaceae* (CRE) by combined Disk Test (CDT), a phenotypic method. In CDT, the synergism is identified as the significant enlargement (≥5 mm) of the Zone of Inhibition (ZOI) around the antibiotic disk with specific inhibitor, compared to the ZOI around the antibiotic disk without inhibitor.

All clinical isolates of *Enterobacteriaceae* could be initially screened with ertapenem (ETP) disk (10μg), which is reportedly having high sensitivity in detecting carbapenemases among all carbapenems [4]. Then, the clinical isolates showing ZOI <25mm around ETP disk [3] could be further tested with following disks in single 150mm petri plate: ETP (10 μg), ETP+clavulanate (10 + 10μg), ETP+cloxacillin (10 + 750μg), ETP+boronate (10 + 400μg), ETP+EDTA (10 + 585μg), and temocillin (30 μg); optionally aztreonam (30 μg) disk may be included for susceptibility testing. Then the mechanism of carbapenem resistance can be identified based on the synergy observed with specific inhibitor and/or resistance to temocillin as described in [Table/Fig-1].

Although the above protocol is highly reliable in detecting most isolates expressing any one of the major carbapenem resistance mechanisms, it may fail in detecting the isolates expressing more than one type of resistance mechanism. That is, production of more than one type of carbapenemases and/or ESBLs or AmpC enzymes with porin loss results in the loss of synergism. Further, this may lead to the false identification of KPC or MBL as OXA-48. Therefore, again we are dependent to molecular tools for the confirmation of OXA-48. However, this protocol would be helpful to reduce the need of molecular diagnosis to selective isolates especially in resource-limited settings. In conclusion, this protocol facilitates timely detection of CRE and identification of the mechanism of carbapenem resistance in *Enterobacteriaceae*; thus, it could help timely implementation of infection control measures.

Carbapenem Resistance Mechanism	≥5 mm Enlargement of ZOI (Synergy)				Resistance to	
	Ertapenem + Clavulanate	Ertapenem + Cloxacillin	Ertapenem + Boronate	Ertapenem + EDTA	Temocillin*	Aztreonam (Optional)
ESBL + Porin loss	+	-	-	-	-	+
AmpC + Porin loss	-	+	+	-	-	+
KPC	+/-	-	+	-	+/-	+
MBL (NDM, VIM, IMP)	-	-	-	+	+	-
OXA-48	-	-	-	-	+	-

[Table/Fig-1]: Combined disk tests for the detection of common mechanisms of carbapenem resistance in *Enterobacteriaceae* (important test for each mechanism is shaded with blue colour) (*ZOI<10mm around the disk)

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