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Carbapenem resistance in a rural part of southern India: *Escherichia coli* versus *Klebsiella* spp.

Sir,

The emergence of carbapenem resistance in *Enterobacteriaceae* is a growing public health problem worldwide. Among the *Enterobacteriaceae*, *Escherichia coli* and *Klebsiella* pose greatest risk to public health, because of their high prevalence, wide range of clinical infections, multidrug resistance and rapid dissemination of resistance to other organisms[1,2].

Carbapenems are regularly used as the last choice for the management of multidrug-resistant *E. coli* and *Klebsiella* infections[3]. However, the recent emergence and dissemination of carbapenem resistance raise a question on the effectiveness of empirical therapy with carbapenems[4,5]. In India, there are no valid data on the prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE)[6]; although a few reports have been published from metropolitan cities which are biased in sampling towards the selection of resistant strains[7,8]. Therefore, this hospital-based study was aimed to assess the occurrence of CRE in a rural part of Tamil Nadu, south India.

A cross-sectional prospective study was conducted in the microbiology department to measure the carbapenem resistance in *E. coli* and *Klebsiella* spp. among the patients attending a 900-bedded tertiary care teaching hospital (Government Theni Medical College, Theni, India). The study was conducted from January 2012 to December 2014. All clinical isolates of *E. coli* and *Klebsiella* spp. recovered from the clinical specimens such as urine, pus, sputum, blood, body fluids, stool and others, collected from the patients of this hospital were studied. All isolates were tested for ertapenem (ETP) (Merck, India) susceptibility by disc diffusion antimicrobial susceptibility test (AST) method using in-house prepared 10 µg ETP disc by following the Clinical and Laboratory Standards Institute (CLSI) recommendations[9] with ATCC 25922 *E. coli* as susceptible quality control. Isolates showing the zone of inhibition of <22 mm to ETP disc were identified as non-susceptible (NS)[10,11], and randomly selected ETP NS isolates were preserved for minimum inhibitory concentration (MIC) testing. Four carbapenems including ETP (Merck), imipenem (IPM), meropenem (MEM) and doripenem (DOR) (Sigma, USA) were tested for MIC by agar dilution AST method[12] for the concentration extending from 0.016 to 64 µg/ml. The susceptibility breakpoints (intermediate range) were >0.5 to <2 µg/ml for ETP, and >1 to <4 µg/ml for IPM, MEM and DOR, and results were interpreted as per the CLSI guidelines[11]. The susceptibility related information was saved and analyzed with WHONET software ver. 5.6 (*www.whonet.org*); further, the statistical analysis was performed by Chi-square test. The study protocol was approved by the ethics committee of Government Theni Medical College, Theni.

During the study period, a total of 2292 non-repetitive clinical isolates of *E. coli* (n = 1338) and *Klebsiella* spp. (n = 954) were isolated. Of these, 444 isolates were identified as ETP NS by disc diffusion method, and these included 207 (15.5%) isolates *E. coli* and 237 (24.8%) *Klebsiella* spp. Among the 444 ETP NS isolates, randomly selected 198 (*E. coli* -103 and *Klebsiella* spp. - 95) isolates were tested for MIC of carbapenems and 150 were confirmed (*E. coli* - 73 and *Klebsiella* spp. - 77) as ETP NS isolates. These isolates were recovered from 71 (47.3%) male and 79 (52.7%) female patients with the median age of 42 years (range - one day to 78 yr).

Forty-eight (24.2%) of 198 ETP NS isolates detected by disc diffusion method were found susceptible by agar dilution method. Further, 33

Table. Carbapenem resistance by minimum inhibitory concentration in *Escherichia coli* and *Klebsiella* spp.

Organism	Antibiotic	R (%)	I (%)	S (%)	Estimated	
					R (%)	(95% CI)
Escherichia coli (n=103)	ETP	50 (48.5)	23 (22.3)	30 (29.1)	100 (7.47)	80.1-120.2
	IPM	10 (9.7)	9 (8.7)	84 (81.6)	20 (1.49)	11.1-35.1
	MEM	13 (12.6)	5 (4.9)	85 (82.5)	26 (1.94)	15.6-42.2
	DOR	11 (10.7)	3 (2.9)	89 (86.4)	22 (1.64)	12.6-37.5
Klebsiella spp. (n=95)	ETP	67 (70.5)	10 (10.5)	18 (18.9)	167 (17.51)	143.8-186.6
	IPM	21 (22.1)	8 (8.4)	66 (69.5)	52 (5.45)	35.4-74.5
	MEM	20 (21.1)	17 (17.9)	58 (61.1)	50 (5.24)	33.3-71.8
	DOR	22 (23.2)	11 (11.6)	62 (65.3)	55 (5.77)	37.5-77.2
Total (n=198)	ETP	117 (59.1)	33 (16.7)	48 (24.2)	267 (11.65)	238.5-298.6
	IPM	31 (15.7)	17 (8.6)	150 (75.8)	72 (3.14)	57.3-90.2
	MEM	33 (16.7)	22 (11.1)	143 (72.2)	76 (3.32)	60.9-94.7
	DOR	33 (16.7)	14 (7.1)	151 (76.3)	77 (3.36)	61.8-95.8

ETP, ertapenem; IPM, imipenem; MEM, meropenem; DOR, doripenem; R, resistant; I, intermediate; S, susceptible; CI, confidence interval

(16.7%) were identified as intermediate resistant. Hence, only 117 of 198 isolates were confirmed as ETP resistant (Table).

Although the disc diffusion method revealed 19.4 per cent (444/2292) ETP NS isolates, these included a considerable proportion of susceptible isolates, which were later identified by agar dilution method. Hence, the presence of CRE was estimated based on the MIC results of sample data (n = 103 for *E. coli* and 95 for *Klebsiella* spp.). The estimated carbapenem resistance was about three per cent with the notable exception of ETP, which had 4-fold higher resistance rate (~12%) when compared with other carbapenems[13]. Further, the resistance was higher among *Klebsiella* spp. and the difference was significant (P<0.05). The resistance of ETP [17.51 vs. 7.47%; odds ratio (OR) 2.63; 95% confidence interval (95% CI) 2.01-3.42] and MEM (5.24 vs. 1.94%; OR 2.79; 95% CI 1.72-4.52) was 2-fold higher in *Klebsiella* spp.; besides, the resistance of IPM (5.45 vs. 1.49%; OR 3.80; 95% CI 2.25-6.41) and DOR (5.77 vs. 1.64%; OR 3.66; 95% CI 2.22-6.04) was 3-fold higher in *Klebsiella* spp. when compared with *E. coli*[14]. The carbapenem resistance seen in the present study was not considerably different from that reported by Gupta *et al*[15] in New Delhi. However, according to a recent report based on the systematic literature obtained from the Asian countries[14], the resistance rate of IPM and MEM was, respectively, 0.2 and 0.5 per cent in *E. coli*, and 1.9 and 2.4 per cent in *Klebsiella* spp. The current study showed higher carbapenem resistance in both *E. coli* and *Klebsiella* spp. when compared with the average of Asian countries necessitating timely detection and appropriate infection control measures to contain the spread of CRE in this region.

In conclusion, the present study documented carbapenem resistance in about three per cent clinically important members of *Enterobacteriaceae* from south India. The newer carbapenem ETP had 4-fold higher resistance rate. Further, molecular investigations need to be done to understand the mechanism of resistance.

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***Conflicts of Interest*:** None.

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References

1. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other *Enterobacteriaceae*: an evolving crisis of global dimensions. *Clin Microbiol Rev* 2012; *25* : 682-707.
2. Temkin E, Adler A, Lerner A, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: biology, epidemiology, and management. *Ann N Y Acad Sci* 2014; *1323* : 22-42.
3. McKenna M. Antibiotic resistance: the last resort. *Nature* 2013; *499* : 394-6.
4. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *Biomed Res Int* 2014; *2014* : 249856.
5. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, *et al*. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013; *13* : 785-96.
6. WHO. Antimicrobial Resistance: Global Report on Surveillance. World Health Organization; 2014. Available from: *apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf,* accessed on January 24, 2017.
7. Rahman M, Shukla SK, Prasad KN, Ovejero CM, Pati BK, Tripathi A, *et al*. Prevalence and molecular characterisation of New Delhi metallo-ß-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant *Enterobacteriaceae* from India. *Int J Antimicrob Agents* 2014; *44* : 30-7.
8. Kazi M, Drego L, Nikam C, Ajbani K, Soman R, Shetty A, *et al*. Molecular characterization of carbapenem-resistant *Enterobacteriaceae* at a tertiary care laboratory in Mumbai. *Eur J Clin Microbiol Infect Dis* 2015; *34* : 467-72.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests: Approved standard. 11th ed. CLSI document M02-A11. Wayne, PA: CLSI; 2012.
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty second informational supplement. CLSI document M100-S22. Wayne, PA: CLSI; 2012.
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty fifth informational supplement. CLSI document M100-S25. Wayne, PA: CLSI; 2015.
12. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard. 9th ed. CLSI document M07-A9. Wayne, PA: CLSI; 2012.
13. Wang JT, Wu UI, Lauderdale TL, Chen MC, Li SY, Hsu LY, *et al*. Carbapenem-nonsusceptible *Enterobacteriaceae* in Taiwan. *PLoS One* 2015; *10* : e0121668.
14. Xu Y, Gu B, Huang M, Liu H, Xu T, Xia W, *et al*. Epidemiology of carbapenem resistant *Enterobacteriaceae* (CRE) during 2000-2012 in Asia. *J Thorac Dis* 2015; *7* : 376-85.
15. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in North India. *Indian J Med Res* 2006; *124* : 95-8.