Sir, we read with interest the recent publication by Ensor et al. and concur with their concerns regarding the high frequency of extended-spectrum β-lactamase (ESBL) genotypes in India. However, in 2002 The Indian Antimicrobial Resistance Study Group published their findings on broad-spectrum β-lactam resistance and ESBL phenotypes in Enterobacteriaceae including Salmonella spp. The isolates were part of the MYSTIC Programme and were collected prior to 2000; at least 3 years before the aforementioned study. This study consisted of non-duplicate samples from 10 different Indian sites in six cities (New Delhi, Mumbai, Indore, Lucknow, Bangalore and Vellore). From our initial phenotypic screening, 65/71 (92%) Escherichia coli and 46/48 (96%) Klebsiella spp. were confirmed as being ESBL producers. These isolates were subjected to detailed ESBL genotyping the findings from which were presented in 2003.

We screened a subset of these isolates (23 E. coli and 24 Klebsiella spp.) for known ESBL genes including CTX-M by PCR analysis using custom-designed primers and the PCR products were sequenced and analysed as previously reported. Results from the molecular screen are shown in Table 1. In contrast to Ensor et al., we screened numerous ESBL genotypes although sequence analysis on the TEM- and SHV-positive amplicons indicated that these were not responsible for the ESBL phenotype (Table 1). Surprisingly, 83% of the E. coli and 75% of the Klebsiella spp. examined produced CTX-M-15 and no other type of CTX-M enzyme, or for that matter any other ESBL. These findings are similar to the recent report that found 73% and 72% for E. coli and Klebsiella spp., respectively. The blaCTX-M-15 genes were distributed evenly among the isolates from the 10 Indian medical centres. Karim et al. first reported the CTX-M-15 genotype from India in six Enterobacteriaceae strains isolated in 2000 and our data indicates that the blaCTX-M-15 genotype was widespread in India during this period and prior to the study of Ensor et al. Furthermore, these Enterobacteriaceae commonly carry multiple β-lactamases including CTX-M-15 with CMY-series enzymes.

These isolates were subsequently examined to investigate the genetic context of blaCTX-M-15. Custom primers were designed for IScpl1 and IS26 and the strains analysed by PCR. All strains possessing the CTX-M-15 genotype possessed IScpl 5-prime to blaCTX-M-15 with the established 48 bp inverted repeat (IR) region between blaCTX-M-15 and IScpl. None of the isolates was positive for IS26, concurring with the finding of Karim et al. but in contrast to the finding reported by Ensor et al. Therefore, it is possible that although blaCTX-M-15 was widely established in India before 2000, the IS26 element had not yet inserted within IScpl (tnpA), and that this is a later event. Ensor and colleagues identified three IS26 insertion sites and have postulated that such an insertion into IScpl traps the ESBL gene thus preventing its expression. Our data also indicated that blaCTX-M-15 is always associated with a large (>100 kb) plasmid, and similarly to those reported by Ensor et al., the isolates often were ciprofloxacin-resistant and also refractory to several aminoglycosides (data not shown).

The data from our studies indicate that the gene pool for blaCTX-M-15 was firmly established in India before 2000, and moreover, was widely distributed throughout that nation. The recent findings indicate that whilst the level of CTX-M-15-positive strains has not generally increased since the earlier sampled period, the genetic environment of blaCTX-M-15 has changed through the insertion of IS26 although the consequences of these events remain uncertain.

Acknowledgements

We wish to thank the following scientists for their contributions to these studies: D. M. C. Bennett, D. Mathai, D. J. Biedenbach, L. M. Deshpande and P. R. Rhomberg.

Transparency declarations

We have not received any personal financial payments from any external body that is relevant to the body of information contained within this article.

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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm017
Advance Access publication 9 March 2007

**Occurrence, prevalence and genetic environment of CTX-M β-lactamases in Enterobacteriaceae from Indian hospitals—authors’ response**

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Keywords: CTX-M-15, extended-spectrum β-lactamases, IS26

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Sir, It is interesting that Walsh et al.¹ report in their commentary on our paper (Ensor et al.)² that further work published as a conference abstract on Indian isolates obtained from the MYSTIC Program collected prior to 2000 found only the *bla*_CTX-M-15 genotype.³ This would add further support to our observations that *bla*_CTX-M-15 appears to be the only CTX-M genotype present in the Indian sub-continent. This is unusual when compared with the situation in other large geographical areas such as China and Europe, where multiple genotypes are seen.⁴,⁵ We read the paper cited by Walsh et al. from the Indian Antimicrobial Resistance Study Group⁶ with interest but, as this communication does not have any genotypic characterization of the isolates, we noted their findings and compared them with a number of Indian extended-spectrum β-lactamase (ESBL) surveys also in which only phenotype characterization has been undertaken. We were required by the Editor to reduce our reference list, and for this reason Mathai et al. 2002 was removed from our first draft manuscript in order to shorten our paper. We are very familiar with the paper by Karim et al.,⁷ which was the first report of *bla*_CTX-M-15 as a genotype, the gene being characterized in six isolates of Enterobacteriaceae and it was the complete lack in the literature of further genotyping surveys from different sites in the Indian sub-continent that led us to undertake our study. The apparent acquisition of IS26 by the more recent examples of plasmids in Indian strains is interesting. It perhaps suggests a ‘turnover’ of plasmids in the population over time, the IS26 insertion creating a selective advantage in those strains carrying the IS26 insertion. Although the numbers are small, the geographical consistency of the observation could make it broadly applicable and worthy of further investigation. It is very useful that Walsh et al. in their commentary provide further evidence for the remarkable finding that only one genotype of CTX-M appears to be present in the Indian sub-continent, particularly bearing in mind the very large population of that region and the high prevalence of ESBL genes in Enterobacteriaceae which represents a large relatively poorly studied reservoir compared with Europe and the Americas.

**References**


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