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Prevalence of bla$_{CTX-M-3}$-like genotypes producing Enterobacteriaceae in a tertiary care centre in South India

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KEYWORDS

bla$_{CTX-M-3}$-like gene, CTX-M-3 β-lactamases, antibiotic resistance

A B S T R A C T

Cefotaxime belonging to third generation cephalosporins are commonly used in India for the treatment of *Enterobacteriaceae* which has been attributed to emergence of these strains carrying these enzymes. To determine the distribution of *bla*$_{CTX-M-3}$-like genotypes produced by *Enterobacteriaceae*, this study was undertaken in a tertiary care hospital, Chennai, South India. A total of 9 *Escherichia coli* and 10 *Klebsiella pneumoniae* out of 46 *Enterobacteriaceae* (recovered from 81 culture positive isolates), collected from various intensive care units of this centre were screened for extended-spectrum β-lactamases (ESBL) production using ceftazidime incorporated MacConkey agar. The 18 (39.13%) isolates were confirmed for *bla*$_{CTX-M-3}$-like genotypes using primer specific PCR. Remaining twenty eight (60.87%) non- ESBL strains served as negative control for PCR. The results revealed the steady increase of CTX-M genes harboured by *Enterobacteriaceae*. Also, it supported the urgent need for regular screening and national surveillance characterizing the CTX-M types and to implement strict antibiotics policy to limit the irrational use of cephalosporins, so as to minimize antibiotic selective pressure.

Introduction

Plasmid encoded *bla*$_{CTX-M}$ enzymes represent an important sub-group of class A β-lactamases causing the extended-spectrum β-lactamases (ESBLs) phenotype which is increasingly found in *Enterobacteriaceae*.

These are the pathogens responsible for various types of infections, such as respiratory tract infections, urinary tract infections, bacteremia and meningitis,

particularly in intensive care, neonatal and surgical units[1].

A new variant of *bla*$_{CTX-M,}$ the *bla*$_{CTX-M-3}$ gene on 60kb plasmid (downstream of an ISEcp1B element), possesses a T instead of A at nucleotide position 663 and is responsible for cefotaxime resistivity in bacterial pathogens [2].

In recent years, bacterial pathogens producing CTX-M extended-spectrum β-lactamases have very rapidly disseminated in several parts of the world & are now frequently reported from countries all over Europe & much of Asia, both in hospital environments and in the community playing a major role in outbreaks [3].

In India, the first gene of the *bla*$_{CTX-M}$ type, *bla*$_{CTX-M-15}$, was identified in clinical isolates of *Enterobacteriaceae* in mid-1990. Since then, several studies have revealed that the level of CTX-M-positive strains has not generally increased since the earlier sampled period [4,5,6,7,8,9,10,11].

The aim of our study was to investigate the distribution of *bla*$_{CTX-M-3}$-like genes in the isolates of *Enterobacteriaceae* collected from a hospital at Chennai, South India during 2008. It was an effort to control the incidences of this particular resistant gene in hospital isolates through a reliable diagnostic process.

Materials and Methods

Study Design

Eighty one culture positive isolates were collected from patients in various intensive care units of a speciality hospital between September 2008 and October 2008. These comprised 46 *Enterobaceriaceae*, identified using rapid ID 32E galleries (bioMérieux, Marcy l'Etoile, France) as 23 *Escherichia coli*, 21 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca* and 1 *Citrobacter diversis*. They were screened for phenotypes consistent with possible ESBL production based on their growth on MacConkey agar is supplemented with ceftazidime (2 mg/L)[12]. *Escherichia coli* ATCC 25922 was included as positive control strain.

Nineteen ESBL confirmed strains were subjected to polymerase chain reaction (PCR) as positive controls, while the phenotypically confirmed non-ESBL producers were included as negative controls in PCR.

This study was approved by the management of the tertiary care centre.

PCR

A loopful of bacterial colonies harvested from a blood agar plate was suspended in 0.5 ml of sterile water and boiled at 95°C to 100 °C for 10 min. After centrifugation at 5,000 rpm for 5 min at 4°C, the DNA-containing supernatant was used as the source of template for further amplification.

The oligonucleotide sequences and protocol as described elsewhere was used for the PCR amplification of *bla*$_{CTX-M-3}$-like gene[13]. One novel primer sets, CTX-F (5'-TCCCAGAATAAGGAATCCCAT-3') and CTX-R1 (5'-CCCATTCCGTTTCCGCTA-3'), were used to detect *bla*$_{CTX-M-3}$-like genes.

PCR amplification was carried out in a 25 µl total volume with the PCR reaction mixture containing 2µl of template DNA from the samples, 1µM of each forward and reverse primer, 200 µM of deoxynucleotide trisphosphate and 1.25U of Taq DNA polymerase in PCR buffer (Bangalore Genei pvt Ltd).

Amplification was done with an initial denaturation at 94°C for 2 min, 35 cycles with denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min. A final extension step for 10 min at 72°C was performed and the products were stored at 4°C until used.

The PCR products were analyzed by standard gel electrophoresis with 100 bp DNA marker using 1% agarose gels stained with ethidium bromide, visualized and documented in a gel documentation unit (Vilber Lourmet, France).

Result and Discussion

Out of 81 culture positive isolates, 46 were identified as *Enterobacteriaceae* (56.79%) (Figure 1). Taking into account the resistance phenotypes of 19 enterobacterial isolates (41.3%) on antibiotic containing media (not illustrated), these were tested for the presence of genes coding for the CTX-M family of ESBLs with primers that are specific for $bla_{\text{CTX-M-3}}$ like genes (479 bp) (Figure 2). The results of PCR amplification of ESBL-encoding genes are shown in Table 1. No $bla_{\text{CTX-M-3}}$-like gene was detected in any of the ESBL negatve isolates.

CTX-M enzymes emerged in the late 1980s, shortly after the introduction of cefotaxime (Ctx) in clinical practice. Since then, wide dissemination of strains carrying these enzymes has occurred, and led to several outbreaks in hospitals. CTX-M enzymes comprise a rapidly growing family of enzymes disseminated in several parts of the world and the diverse CTX-M types often exist within a single country[14,15].

CTX-M-15 ESBL enzyme has been found almost exclusively in *E. coli* and *Klebsiella spp.*, and has been a cause of concern for many clinical microbiologists and infectious diseases experts in many countries of the world as it is associated with multiple drug resistance and is spreading rapidly[16].

In the year 2001, Karim A et al[8] were first to report the presence of CTX-M producing *Enterobacteriaceae* from New Delhi. All of the six isolates from 2000 were found to be unrelated and all produced CTX-M-3-like genes (CTX-M-15).

Ensor VM et al[5] in their first systematic molecular survey report from India confirmed that 72 of 98 (73%) *E. coli* and 23 of 32 (72%) *K. pneumoniae* carried $bla_{\text{CTX-M}}$.

Datta P et al[4] conferred that the rates of ESBL carrying isolates vary widely (12.6–71%) with most prevalence rate of around 50% and upwards.

Walsh TR et al[10] reported that 83% of the *E. coli* and 75% of the *Klebsiella spp.* could produce CTX-M-15.

The PCR assay in the present study detected $bla_{\text{CTX-M-3}}$-like genes in 18 of 19 ESBL-producing isolates (94.7%) while no PCR products were observed in one isolate (5.3%).

As for ESBL producing *Escherichia coli*, all of the 9 strains (100%) harbored $bla_{\text{CTX-M-3}}$-like genes. For *Klebsiella pneumoniae* (ESBL-KP), 9 of 10 strains (90%) possessed $bla_{\text{CTX-M-3}}$-like genes. This study supports the reported observation that CTX-M genotypes are now the most common ESBL type among the members of the *Enterobacteriaceae*, with group 1 CTX-M producers predominating[17].

As depicted by Table 1, the one CTX-M PCR-negative ESBL-producing *Klebsiella pneumoniae* (isolate: BP321) (Figure 2) assumed to be a non-CTX-M ESBL

producers but these aspects were not analyzed as these were out of the study objective.

Furthermore, in this study, the adopted MacConkey agar-ceftazidime appeared as an excellent medium for the screening and presumptive identification of ESBL-producing *Enterobacteriaceae* in overnight cultures. Finally, PCR method enables a simple, rapid, reliable and faster detection of CTX-M-producing members of the *Enterobacteriaceae*. However, it is only possible to say that genotypes matched reference sequences over the 497bp amplified region of the bla_{CTX-M} gene. However, definitive assignment of any CTX-M-positive isolates to the allelic level would require full sequencing of the entire bla_{CTX-M} gene. Thus, PCR did not discriminate among different variants of $bla_{CTX-M-3.}$

Conclusion

In conclusion, the result of this study shows the phenomenal increase in the prevalence of *E. coli* and *Klebsiella* clinical isolates producing CTX-M enzyme in South India since early sampled period. But, considering the detection of small number of $bla_{CTX-M-3}$-like genes, the result of this study cannot be generalized without further studies for the same. At the same time, all clinical laboratories should be aware of and able to detect Ctx resistance in hospitals on routine basis till molecular level for prevention of the dissemination of ESBL-producers among hospital environment.

Table.1 Distribution of blaCTX-M-3 types by species

Strain (n)	Number of ESBLs (growth on MacConkey Agar + Ceftazidime)	$bla_{CTX-M-3}$
Escherichia coli (23)	09	09
Klebsiella pneumoniae (21)	10	09

Figure.1 Distribution of Enterobacteriaceae in different specimen

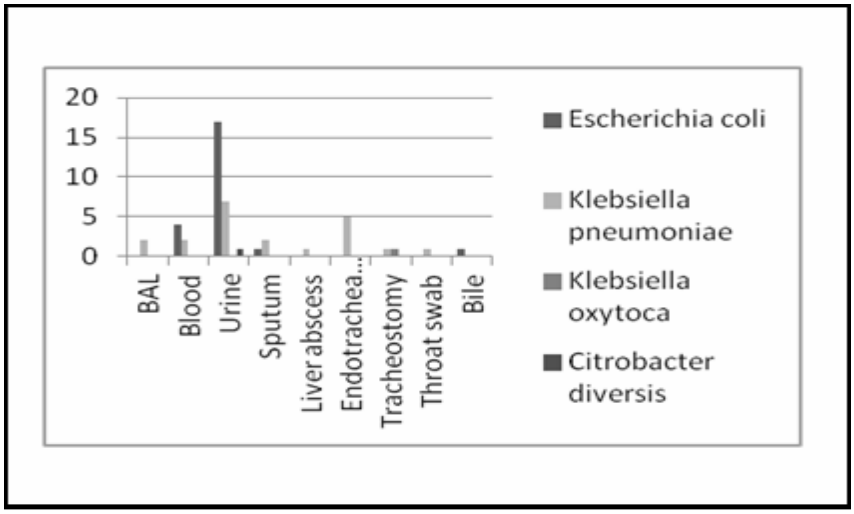
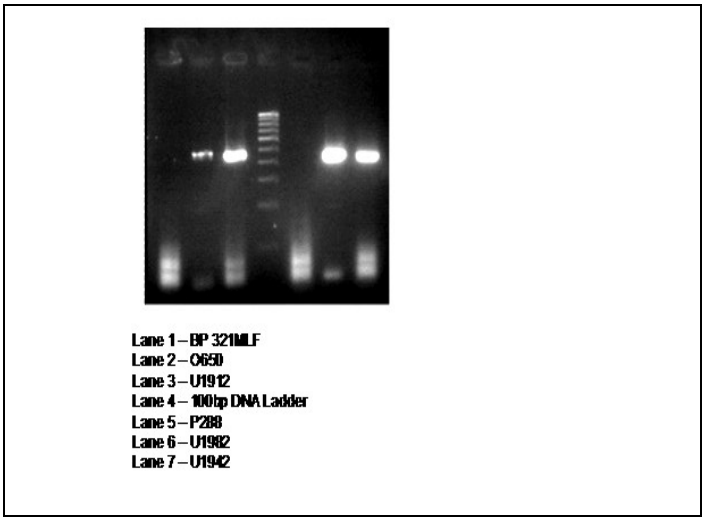


Figure.2 Detection of blaCTX-M-3-like genes using PCR



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