Genotypic resistance profile of ESBL-producing enterobacterales from wound infections in Salem, India

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Summary

The Extended Spectrum β-lactamases (ESBLs) have been abruptly increasing in hospital and community settings. The present study aimed to detect ESBL producers from wound infections and to determine the associated ESBL genotypes. Gram-negative bacilli obtained from wound infections for a period of one year from November 2020 to October 2021 were included. Isolates with inhibition zone size ≤ 27 mm for Cefotaxime and ≤ 22 mm to Ceftazidime were subjected to phenotypic confirmation by Vitek 2 ID / AST. Extended spectrum β-lactamase genes OXA-10/11, TEM, SHV & CTX-M were detected by Real Time PCR. The CTX-M enzyme was the most common ESBL genotype observed among Enterobacterales in our study. The co-expression of ESBL genes was observed in clinical isolates of *Klebsiella pneumonia* and *Escherichia coli*. CTX-

M & TEM genes were observed in 40% of *E. coli* isolates. All isolates of *E. coli* with CTX-M & TEM genes were susceptible to carbapenems and amikacin. 8.33% of *E. coli* isolates with CTX-M genotype alone were resistant to carbapenems and amikacin. 50% of *K. pneumoniae* isolates with SHV, CTX-M & TEM genes showed resistance to carbapenems, β-lactam/ β-lactamase inhibitor combinations, cefepime and aminoglycosides. In conclusion ESBL associated infections are becoming a public health issue with respect to wide dissemination of ESBL genes and limited therapeutic options.

Key words
wound infections, ESBL Enterobacterales, ESBLs genotypes

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Introduction

Enterobacterales have emerged as the most common etiological agent associated with nosocomial and community acquired infection. Extended spectrum β-lactamases (ESBLs) are commonly plasmid encoded β-lactamases that can inactivate narrow and extended spectrum cephalosporins, monobactams and penicillins but are susceptible to inhibition by clavulanic acid.[1] The frequent co-expression of resistance to various classes of antibiotics such as fluoroquinolones, tetracyclines, aminoglycosides and trimethoprim/sulfamethazole limits the wide range of active antimicrobials against ESBL isolates.[2]

The first plasmid encoded β-lactamase, TEM 1, was described in 1960s after the introduction of first and second generation cephalosporins in clinical practice. The TEM enzyme is found in Enterobacterales, *Pseudomonas aeruginosa, Haemophilus influenzae* and *Neisseria gonorrhoea*.[3] With the use of third generation cephalosporins in clinical settings, SHV-2 ESBL type evolved. This type could hydrolyze third generation cephalosporins or oxy-imino cephalosporins. The SHV ESBL type β-lactamases are most often found in *Klebsiella pneumoniae* clinical isolates as well as in other Enterobacterales and *P. aeruginosa*.[2,3] The inhibitory activity of β-lactam/ β-lactamase inhibitors may vary depending on the type of inhibitor and ESBL type. Clavulanic acid and tazobactam have been found to be more potent than sulbactam in inhibiting SHV and TEM ESBL types.

The CTX-M β-lactamases hydrolyze cefotaxime and are susceptible to inhibition by clavulanate, sulbactam and tazobactam, even though CTX-M-15 and CTX-M-19 can hydrolyze ceftazidime, which might complicate their phenotypic recognition.[3,4] Compared to the CTX-M type, TEM and SHV β-lactamases show higher hydrolytic activity for ceftazidime than cefotaxime. The CTX-M variants have been reported among members of Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* species. The community acquired urinary tract infection has been highly associated with CTX-M enzymes.[4] The OXA β-lactamases are frequently found in *P. aeruginosa* than in members of Enterobacterales. Most OXA ESBL type exhibit resistance to β-lactam inhibitors and are characterised by their high hydrolytic activity against oxacillin and cloxacillin.[5]

Rapid detection and identification of ESBLs are essential to the epidemiology of antibiotic resistant isolates. Molecular detection of ESBL producers helps in rapid diagnosis and obtaining epidemiological information during outbreaks. The detection of ESBL mediated resistance in Gram-negative bacilli is of high importance due to its clinical significance and the lim-

ited therapeutic options.[6] The emergence of drug resistance in bacteria has made the treatment of the wound infections more difficult and also expensive. The phenotypic confirmatory tests are highly sensitive and specific but there are instances where the phenotypic confirmatory tests fail to detect ESBL phenotypes.[7] The determination of the predominant ESBL phenotype is necessary for improved therapeutic management of wound infections as well as for strengthening the hospital infection control measures and antibiotic stewardship practices.

Materials & Methods

This prospective cross sectional study was carried out for a period of one year from November 2020 to October 2021. Under strict aseptic conditions, wound swabs were collected from patients with clinically suspected wound infections. Wound swabs were inoculated on blood agar and MacConkey agar and incubated overnight at 37°C.

Isolates were further identified by standard biochemical methods.[8] All Gram-negative bacilli isolates from wound infections were subjected to antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method using the following discs (HiMedia Laboratories Pvt. Ltd., Mumbai, India): gentamicin (10µg), amikacin (30µg), cefotaxime (30µg), cefepime (30µg), ceftazidime (30µg), ciprofloxacin (5µg), cefoperazone/sulbactam (75/30µg), imipenem (10µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), amoxicillin/clavulanate (20/10 µg) and co-trimoxazole (25 µg).

All Gram-negative bacilli with inhibition zone size ≤ 27 mm for cefotaxime and ≤ 22mm to ceftazidime were subjected to phenotypic confirmation by Vitek 2 ID / AST.[9] Turbidometrically controlled bacterial suspension was used to inoculate Vitek 2 ID/AST cards. The minimum inhibitory concentration of amikacin, gentamicin, ciprofloxacin, imipenem, meropenem, piperacillin/tazobactam, amoxicillin/clavulanate, co-trimoxazole, cefotaxime, ceftazidime, ceftriazone and cefepime were determined by Vitek 2 ID/AST cards.

For extraction and purification of DNA, HiPur A Bacterial Genomic DNA Purification Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was used. Extended spectrum β-lactamase genes OXA-10/11, TEM, SHV & CTX-M were detected by Real Time PCR using the following cyclic condition: Initial denaturation at 95°C for 10 minutes followed by denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 30 seconds. Fluorescence reading for all the channels was taken at the end of the extension stage. The cycle threshold (Ct) is determined as the point at which the fluorescence exceeds the threshold limit. The cycle threshold (Ct) value of ≤ 40 was interpreted as positive for OXA-10/11, TEM, SHV & CTX-M genes. Lack of amplification curve in the target genes channel was interpreted as negative.

Results

A total of 100 Gram-negative bacilli obtained from wound samples during the study period was subjected to ESBL screening (Fig 1). Out of 100 Gram-neg-

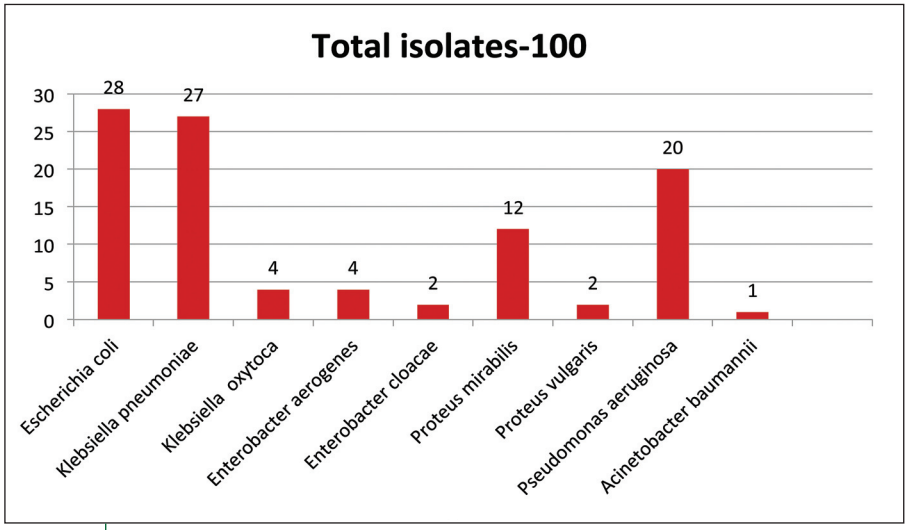

Figure 1 Distribution of Gram-negative bacilli from wound infections.

ative bacilli screened for ESBL production by the disk diffusion method, 43 isolates were subjected to phenotypic confirmation by Vitek 2 ID/AST. 37 (86.05%) out of 43 isolates were phenotypically confirmed by Vitek 2 ID/AST (Fig 2). Among the ESBL producers, 23 (62.16%) were *E. coli*, 9 (24.32%) were *K. pneumoniae*, 2 (5.41%) were *Proteus mirabilis* and one isolate each was *Enterobacter aerogenes* (2.70%), *Enterobacter cloacae* (2.70%) and *P. aeruginosa* (2.70%). Out of 43 isolates, 27 ESBL genes were detected by PCR (Fig 3). Among the ESBL isolates identified by PCR, 20 (74.00%) were *E. coli*, 6 (22.22%) were *K. pneumoniae* and a single isolate (3.70%) was *E. aerogenes*. ESBL genotype, CTX-M was detected in 48.15% (13/27) isolates. TEM and CTX-M were detected in 29.63% isolates (8/27). The presence of the SHV, CTX-M & TEM genotype was detected in 22.22% (6/27) isolates. The CTX-M ESBL type was the most predominant among ESBL producing Enterobacterales in our study. The co-expression of ESBL genes was observed in isolates of *E. coli* and *K. pneumoniae*. All *K. pneumoniae* isolates harboured SHV, CTX-M & TEM genes. CTX-M and TEM genes were observed in 40% of the *E. Coli* isolates. (Table 1)

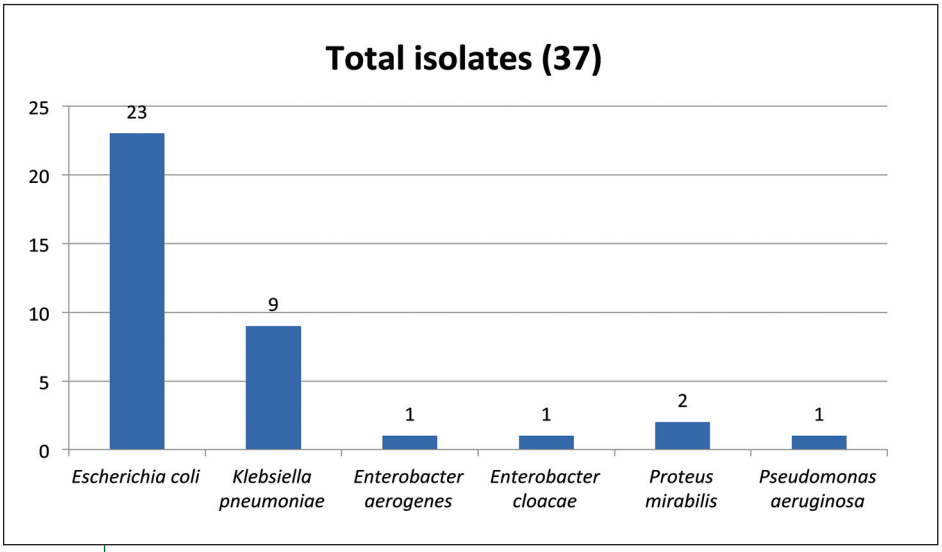

Figure 2 Number of ESBL-producing isolates detected by the Vitek 2 automated method.

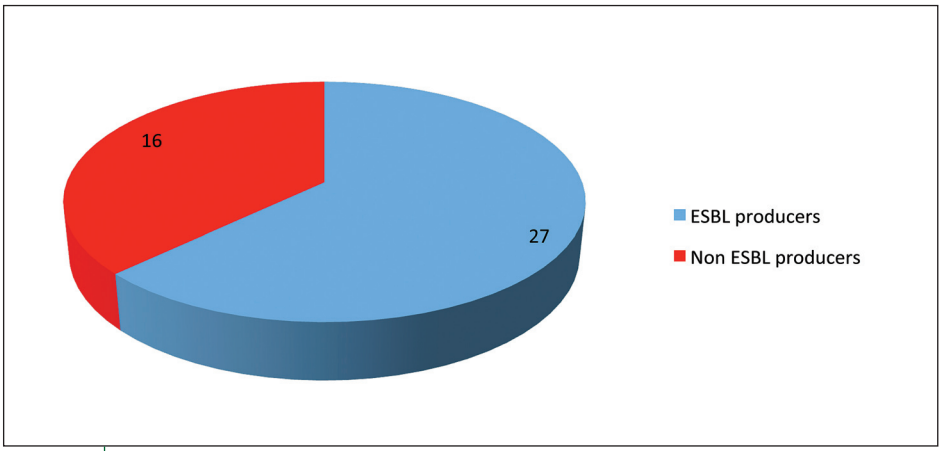

Figure 3 Distribution of ESBL producers from wound infections.

Out of 27 ESBL producers, 14.81% isolates showed resistance to carbapenems, 81.48% were resistant to fluoroquinolones and 74.07% to trimethoprim-sulfamethoxazole. Regarding the aminoglycoside susceptibility testing, 14.81% of the isolates showed resistance to amikacin and 40.74% were resistant to gentamicin. Of the β-lactam- β-lactamase inhibitor drugs tested, 40.74% isolates showed resistance to piperacillin–tazobactam and 25.93% were resistant to amoxicillin-clavulanate (Table 2). 50% of *K. pneumoniae* isolates with SHV, CTX-M & TEM genes showed resistance to carbapenems, β-lactam/ β-lactamase inhibitor combinations and aminoglycosides. All *E. coli* with CTX-M & TEM enzymes showed susceptibility to carbapenems and amikacin. 8.33% of *E. coli* isolates with CTX-M genotype alone were resistant to carbapenems and amikacin. Among the β-lactam/ β-lactamase inhibitor drugs tested, more than 30% of *E. coli* isolates with either CTX-M ESBL type or with CTX-M & TEM ESBL enzymes were resistant to piperacillin-tazobactam combination whereas more than 10% *E. coli* isolates with either CTX-M ESBL type or with CTX-M & TEM ESBL enzymes showed non susceptibility to amoxicillin-clavulanate combination.

Discussion

In this study, the prevalence of ESBL producers and associated genes were studied by phenotypic and genotypic methods. The prevalence of different ESBL genes in clinical isolates varies worldwide depending on geographical location, sample size and period of study.[10] In our study, 27 out of 100 Gram-negative bacilli from

Table 1 1-Distribution of ESBL genes in the study's isolates.

ESBL genotype	No of isolates		
	***Escherichia coli* (20)**	***Klebsiella pneumoniae* (6)**	***Enterobacter aerogenes* (1)**
CTX-M	12 (60.00%)	0 (0.00%)	1 (100.00%)
CTX-M TEM	8 (40.00%)	0 (0.00%)	0 (0.00%)
SHV, CTX-M &TEM	0 (0.00%)	6 (66.67%)	0 (0.00%)

Table 2 Antimicrobial resistance pattern of ESBL producers.

ESBL genotype	AMC	PIT	AK	GEN	CIP	COT	IMI	MER
CTX-M (13)	3 (23.08%)	5 (38.46%)	1 (7.69%)	6 (46.15%)	12 (92.31%)	10 (76.92%)	1 (7.69%)	1 (7.69%)
CTX-M & TEM (8)	1 (12.50%)	3 (37.50%)	0 (0%)	2 (25%)	5 (62.50%)	4 (50%)	0 (0%)	0 (0%)
SHV,CTX-M & TEM (6)	3 (50%)	3 (50%)	3 (50 %)	3 (50%)	5 (83%)	6 (100%)	3 (50%)	3 (50%)
Total (27)	7 (25.93%)	11 (40.74%)	4 (14.81%)	11 (40.74%)	22 (81.48%)	20 (74.07%)	4 (14.81%)	4 (14.81%)

AMC-amoxicillin/clavulanate, PIT-piperacillin/tazobactam, CIP-ciprofloxacin, COT-cotrimoxazole, AK-amikacin, GEN-gentamicin, IMI-imipenem, MER-meropenem

wound infections were found to be ESBL producers. In a similar report from south India, 10.76% of isolates from pus samples were ESBL producers. In a study on ESBL associated community acquired infection, 27% were wound isolates.[11] Betsy Andrews et al. have reported 32.2% ESBL isolates from wound infections.[12]

By genotypic method, the most common ESBL producer among Enterobacterales were *E. coli* 20/23 (86.95%), *K. pneumoniae* 6/9 (66.67%) and *E. aerogenes* 1/1 (100.00%). None of the *Proteus* isolates in our study harboured ESBL genes. In a similar study from North India, Sahoo et al have reported the predominant ESBL producers among Enterobacterales as *E. coli* (44.00%), *K. pneumoniae* (42.60%) and *Enterobacter* species (48.00%).[13]

In this study, the CTX-M ESBL type was more predominant among Enterobacterales. The co-expression of ESBL genes was observed in isolates of *E. coli* and *K. pneumoniae*. All *K. pneumoniae* isolates harboured SHV, CTX-M & TEM genes. CTX-M and TEM genes were observed in 40% of *E. coli* isolates. In a multicentre study from India, the predominant ESBL genotype among *E. coli* and *K. pneumoniae* isolates were reported as TEM and OXA.[7] CTX-M enzymes have become predominant worldwide with CTX M-15 reported as the most common variant detected in nosocomial and community acquired infection.[14] The high frequency of CTX-M enzymes among *E. coli* and *K. pneumoniae* isolates and their drug resistance to multiple antibiotics have been reported from previous Indian studies.[15,16] On the other hand, absence of CTX-M enzymes in ESBL-producing *K. pneumonia* clinical isolates was reported in an Indian study in 2019.[17]

In this study, 50% of *K. pneumoniae* isolates with SHV, CTX-M & TEM genes showed resistance to carbapenems (imipenem and meropenem), aminoglycosides (amikacin and gentamicin), β-lactam/ β-lactamase inhibitor combinations (amoxicillin/clavulanate and piperacillin/tazobactam) and fourth generation cephalosporin (cefepime). In *E. coli* isolates with CTX-M genotype, 16.67% of isolates showed resistance to amoxicillin/clavulanate and 33.33% were resistant to piperacillin/tazobactam. Resistance to carbapenems (imipenem and meropenem) and amikacin was observed among 8.33% of isolates. 50% of isolates showed resistance to gentamicin and 40% of isolates were resistant to cefepime.

All isolates of *E. coli* with CTX-M & TEM enzymes showed susceptibility to carbapenems and amikacin. 12.50% of the isolates showed resistance to amoxicillin/clavulanate, whereas 37.50% showed resistance to piperacillin/tazobactam combinations. A single isolate of *E. aerogenes* with CTX-M ESBL type showed susceptibility to carbapenems, cefepime and aminoglycosides, but was resistant to β-lactam/ β-lactamase inhibitor combinations. Nosocomial isolates of ESBL-producing *E. coli* with high level resistance to ampicillin, cephalosporins, amoxicillin/clavulanate, quinolonones and piperacillin /tazobactam have been already reported.[18]

The *bla* $_{CTX-M}$ type is identified to be the highest disseminated ESBL resistant gene.[19] Gram-negative bacteria that harbour the CTX-M gene have the ability to transfer plasmid borne resistance to other bacterial cells. The *bla* $_{CTX-M}$ type confers resistance to third/fourth generation cephalosporin groups.[20] The choice of drug for ESBL producers varies between β-lactam/ β-lactamase inhibitors and carbapenems. β-lactam/ β-lactamase inhibitors have been the preferred agents for less severe infection whereas carbapenems are preferred for more severe infections. However the production of multiple ESBL's, the co-expression of Amp C β-lactamases and the inoculum effect may limit the efficacy of β-lactam/ β-lactamase inhibitor combinations.[21]

The prevalence of ESBL varies from <1% to >70% across the globe.[22] The extended use of cephalosporins in health care settings favourites the spread of ESBL-encoding genes. Thus, the determination of predominant ESBL genotypes could aid in the rapid selection of the appropriate treatment. The knowledge of ESBL genotypes that are prevalent in healthcare settings probably helps in clinical decision making.

Limitation of the study

This study has limitations such as selection of samples and shorter duration of research. Existence of Amp C betalactamases and carbapenemase enzymes among the study isolates were not evaluated in our study. Future research on exploration of β-lactamases from multiple samples for a prolonged period will overcome the drawbacks of present study.

Conclusion

CTX-M type was the predominant ESBL genotype detected among the clinical isolates in our study. Existence of multiple ESBL genotypes, SHV, CTX-M & TEM was observed among Klebsiella pneumoniae isolates. The knowledge on ESBL genotypes that are prevalent in a geographical region is of great importance for epidemiological surveillance and for determining effective measures of infection control. The high burden of antimicrobial resistance in Indian settings is challenging for clinical decision making.

Περίληψη

Genotypic resistance profile of ESBL-producing enterobacterales from wound infections in Salem, India

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Οι β-λακταμάσες ευρέως φάσματος (Extended Spectrum β-lactamases, ESBL) έχουν αυξηθεί εκρηκτικά, τόσο στο νοσοκομείο όσο και στην κοινότητα. Η παρούσα μελέτη είχε στόχο να ανιχνεύσει τους μικροοργανισμούς που παράγουν ESBL σε λοιμώξεις μαλακών μορίων και να καθορίσει τα είδη γονιδίων ESBLς. Μελετήθηκαν αρνητικοί κατά Gram βακτηρίδια που απομονώθηκαν από λοιμώξεις μαλακών μορίων για διάστημα ενός έτους, από τον Νοέμβριο του 2020 έως τον Οκτώβριο του 2021. Μικροοργανισμοί με ζώνη αναστολής ≤ 27 mm για την Κεφοταξίμη και ≤ 22 mm για την Κεφταζιδίμη υποβλήθηκαν σε φαινοτυπική επιβεβαίωση με Vitek 2 ID / AST. Τα γονίδια β-λακταμάσης εκτεταμένου φάσματος OXA-10/11, TEM, SHV & CTX-M ανιχνεύθηκαν με Real Time PCR. Το ένζυμο CTX-M ήταν ο πιο κοινός γονότυπος ESBL που παρατηρήθηκε μεταξύ των Εντεροβακτηριακών. Συν-έκφραση των γονιδίων ESBL παρατηρήθηκε σε κλινικά στελέχη των *Klebsiella pneumoniae* και *Escherichia coli*. Τα γονίδια CTX-M & TEM παρατηρήθηκαν στο 40% των απομονώσεων *E. coli*. Όλα τα στελέχη *E. coli* με γονίδια CTX-M & TEM ήταν ευαίσθητα σε καρβαπενέμες και αμικασίνη. Το 8,33% των απομονωθέντων *E. coli* με μόνο γονότυπο CTX-M ήταν ανθεκτικά στις καρβαπενέμες και την αμικασίνη. Το 50% των απομονωθέντων στελεχών *K. pneumoniae* με γονίδια SHV, CTX-M & TEM εμφάνισαν ανθεκτικότητα στις καρβαπενέμες, σε συνδυασμούς αναστολέων β-λακτάμης/β-λακταμάσης, κεφεπίμη και αμινογλυκοσίδες. Συμπερασματικά, οι λοιμώξεις που σχετίζονται με την παρουσία ESBL αποτελούν σοβαρό θέμα δημόσιας υγείας όσον αφορά την ευρεία διάδοση των γονιδίων ESBL και τις περιορισμένες θεραπευτικές επιλογές.

Λέξεις κλειδιά
λοιμώξεις μαλακών μορίων, Εντεροβακτηριακά που παράγουν ESBL, γονίδια ESBLs

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