Comparison of Beta-lactamase Resistance Gene detection in MDR *Escherichia coli* Isolates in Nepal

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ABSTRACT

Objectives: To compare ESBL and carbapenemase gene detection using phenotypic (double disc diffusion and VITEK-2AES), and genotypic (PCR) methods in the *E. coli* isolates in Nepal.

Methods: 132 multidrug resistant *E. coli* isolates with phenotypic extended-spectrum-β-lactamase (ESBL) and carbapenemase archived during 2018 to 2023 at National Public Health Laboratory were analyzed to detect three extended-spectrum-β-lactamase genes (*CTX-M*, *SHV* and *OXA 10/11*) and eight carbapenemase genes (*NDM, KPC, IMP, VIM, OXA-51, OXA-23, OXA-48* and *OXA-58*). Vitek 2 generated Advanced Expert System™ reports were compared with PCR findings, to evaluate their predictive diagnostic values.

Results: Out of the 132 isolates tested, *CTX-M* gene was detected in 60/80 (75%), *OXA 10/11* and *SHV* gene in none (0/80); while no genes were detected in 20 ESBL-non producers. *NDM* gene was detected in 12/22(55%) and *OXA-48* in 2/22(9%) metallo-beta-lactamase (MBL) producers, while other carbapenemase genes were not detected in 10 MBL non-producers. Five (5%; 5/102) of the isolates had both *CTX-M* and *NDM* genes. The Vitek 2 detection of *CTX-M*-like ESBLs showed a sensitivity of 75% (60/80) and specificity of 100% (20/20) PPV of 100% and NPV of 50% for the *E. coli* isolates.

Conclusion: *blaCTX-M* and *blaNDM* were the most common genes responsible for third-generation cephalosporins and carbapenem resistance respectively with low co-existence. Vitek2 AES detects ESBL with adequate sensitivity, however more evidences are required for use in routine screening.

Keywords: Antimicrobial susceptibility testing; Extended-spectrum beta-lactamase (ESBL); Advanced Expert System; Vitek2 Genotyping, Nepal

INTRODUCTION

Antimicrobial resistance due to various mechanisms like enzymes production via extended-spectrum beta lactamases (ESBL), AmpC beta-lactamases (ACBL), metallo-beta lactamases (MBL) and carbapenamases, cell target modifications and antibiotic efflux pumps (Noster et al., 2021) are a common cause of multi-drug resistance in Enterobacterales and *E. coli*. Mobile genetic elements (Dunn et al., 2019) increase the resistance towards third-generation cephalosporins and carbapenem

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need to be used for treatment (Mancuso et al., 2023).

As many ESBL-producing organisms are also resistant to other antibiotic classes, high rates of ESBL infections result in increased use of carbapenems, which, in turn, selects for carbapenem-resistant organisms, for which there are few therapeutic options. Carbapenemases and MBLs are not inhibited by β-lactamase inhibitors and result in resistance to most available carbapenems (Nordmann and Poirel, 2019) leading to extremely drug-resistant (XDR) phenotypes, with resistant to most aminoglycosides, fluoroquinolones, and other β-lactams(Boyd et al., 2020). MBLs are common antibiotic resistance mechanism reported in Nepal in *E. coli* and the numbers are significantly high in the past years (Shrestha et al., 2022, 2015).

The most clinically relevant MBLs are the Verona integron-encoded MBL (*VIM*), imipenemase (*IMP*), and the New Delhi metallo-betalactamase (*NDM*)(Sawa et al., 2020). Genes that confer ESBL activity (*blaTEM, blaSHV, blaCTX-M*) and carbapenamase activity (*blaOXA, blaNDM, blaVIM, blaKPC and blaIMP*) have been reported among the Enterobacterales(Fleece et al., 2018). Though *blaTEM* and *blaSHV* were common in the past, *blaCTX-M* have become more prevalent in recent years (Koirala et al., 2021; Sah et al., 2021) (Nachimuthu et al., 2021).

Antimicrobial susceptibility testing (AST) reports are useful in guiding appropriate antimicrobial therapy, in addition to generating evidences for national antimicrobial resistance surveillance systems. As the broth and agar dilution methods are labor-intensive, automated susceptibility testing systems such as the Vitek 2 (BioMerieux, France) have become more popular in recent years (Carvalhaes et al., 2023). These provide the MIC values along with antibiotic resistance phenotypes (Livermore et al., 2002).

In routine diagnostic laboratories, phenotypic methods for the detection of ESBLs are based on the inhibition of ESBL enzymes by β-lactamase inhibitors and on the comparison of cephalosporin activity with or without a β-lactamase inhibitor (Das et al., 2023).

VITEK 2, a new automated bacterial identification and susceptibility testing system that uses fluorescence-based technology for the identification of routine clinical isolates with high discrimination between the species, low rate of multiple choice and mis-identified species and minimal number of off-line tests and considered as the test with accurate fingerprint. The system includes an ADVANCED EXPERT SYSTEM (AES) and VITEK® 2 AST cards, essentially a miniaturized, abbreviated and automated version of the doubling dilution technique, that analyzes MIC patterns based on the broth micro-dilution minimum inhibitory concentration technique and detects phenotypes for most organisms tested with optimum laboratory efficiency. The test results allow clinicians to take evidence-based decision to continue or discontinue empiric therapy and prescribe targeted therapy, resulting in improved patient outcomes and enhanced antibiotic stewardship (Biomeriux, 2024).

Vitek 2 consists of a database that contains a number of algorithms in addition to the MIC distribution for various antibiotic combinations and possible mechanisms of resistance including ESBL, AmpC/cephalosporinases and carbapenemase in various species. The MIC phenotype detected by the Vitek 2 for a particular isolate is then compared with all the patterns in the database and the best match is identified. It also provides list of aminoglycoside resistance with probable genes and decreased susceptibility or quinolone resistance indication.

While most studies include the MIC values given by Vitek 2 for reporting, very limited studies have fully utilized the information given by the Vitek 2 Advanced Expert System™ (Carvalhaes et al., 2023).

National Public Health Laboratory (NPHL) is the national reference laboratory for AMR surveillance in Nepal. It has Vitek-MS and Vitek 2 (Biomeriux) automated systems, used for identification and measurement of minimum inhibitory concentrations of antibiotics against common bacteria and yeasts.

The current study was designed to compare ESBL and carbapenemase gene detection using phenotypic (double disc diffusion and VITEK-2AES), and genotypic (PCR) methods in the *E. coli* isolates in Nepal.

METHODS

Study design

This was a laboratory based cross-sectional study, where clinical specimens received at NPHL were processed following standard methods for the identification and antimicrobial susceptibility testing of the *E. coli* (Isenberg, 2004).

Place and duration of study

The study population was *E. coli* isolates from the suspected patients visiting NPHL, for diagnostic purpose, during 2018-2023. Laboratory testing was performed in

Microbiology Laboratory at NPHL.

Inclusion and exclusion criteria

E. coli isolates obtained from clinical samples processed at the Microbiology Laboratory of NPHL were included for further genotypic analysis. Duplicate, unlabeled, improperly transported, contaminated and samples lacking patients' clinical data were excluded.

Sample size and sampling

Convenience sampling method was followed, where 132 MDR isolates phenotypically considered ESBL or carbapenemase producers (80 ESBL & 22 MBL positive) and 20 ESBL non-producers and 10 MBL non-producers were further analyzed for detecting genes conferring related resistance. Same isolates were analyzed using Vitek2 AES, and compared for the two enzymes ESBL (*CTX-M* like) and carbapenemases. ACBL and other resistance enzymes were not analyzed.

Labotratory analysis

Bacterial isolates: All multi-drug resistant *E. coli* isolated at National Public Health Laboratory (NPHL), during 2018-2023 with the criteria of ESBL positivity by combination disc method, or mCIM positive in accordance with the CLSI guidelines(Pierce et al., 2017), were further tested by Vitek 2 compact (Biomeriux, France) for MICs of selected antibiotics, using Gram Negative cards N280.

Tests for extended-spectrum β-lactamase production: ESBL was detected by combination disc method and interpreted according to CLSI criteria (Lewis et al, 2023). Appropriate quality control organisms, namely *E. coli* ATCC® 25922 and *K. pneumoniae* ATCC® 700603, were included for each method in each run. Disc diffusion testing was performed using the modified Kirby–Bauer method (Wayne, 2012). Cefotaxime and ceftazidime discs with or without clavulanic acid were used, with an increase of ≥5 mm in zone inhibition diameter for either cephalosporin in the presence of the inhibitor, indicating the presence of an ESBL (Lewis et al, 2023).

Vitek 2 AES results were compared with standard AST methodologies like combination disc method and various β-lactamase gene detection. Clinical *E. coli* isolates (n=132) that were phenotypically positive for either ESBL (n=80) or MBL(n=22) were tested for the various β-lactamase genes along with 20 ESBL non-producers and 10 MBL non-producers respectively for comparison.

The STROBE chart reveals the recruitment and laboratory analysis of the study samples (Figure 1).

The third-generation cephalosporin resistant *E. coli* isolates from various types of specimens received at NPHL, were stored at -80°C in TSB glycerol stocks, till further genetic analysis. The isolates were sub-cultured and re-tested with the Vitek 2 for confirmation of the isolates and comparison of the susceptibility test results. The same inoculum was used for concurrent phenotypic and genotypic testing using PCR and MIC by VITEK 2 using AST-N280 cards. For this study, only routine antibiotic susceptibility testing panels were used i.e., AST-N280, which comprised β-lactam antibiotics including cefotaxime, ceftazidime, and cefepime; it does not include testing of a cephalosporin in the presence of CA. The interpretation of the results were obtained from advanced expert system (AES) designed to analyze the results generated by the VITEK 2 system. Control strains for ESBL detection were tested in parallel.

Quality control of the phenotypic tests and PCR: Standard ATCC® strains of *E. coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603) were used as negative and positive control respectively for the ESBL test while *Klebsiella pneumoniae* ATCC® BAA-1705 and ATCC® BAA-1706 for MBL test. *E. coli* isolates confirmed to be positive for *blaSHV*, and *blaCTX–M* by whole-genome sequencing were taken as positive control, wild strains (ATCC 25922) as negative control and nuclease-free water used as no template control.

VITEK 2 Advanced Expert System™ (AES) interpreted the results obtained with Vitek 2 into nine different phenotypes, viz. Acquired Penicillinase, ESBL, ESBL(*CTX-M*-like), *SHV* hyperproduction, Acquired Penicillinase + Cephalosporinase (AmpC), cephalosporinase, Impermeability Carba (+ESBL or +HL AmpC), and carbapenemase (±ESBL). Vitek 2 definition of ESBL producer included isolates that were interpreted by the Vitek2 AES as ESBL/ESBL (*CTX-M*-like) and not an ESBL if only wild type or β-lactamases other than ESBLs were suggested by AES (Garrec et al., 2011).

Other interpretations such as aminoglycoside resistance, acquired penicillinase and AmpC cephalosporinase were not analyzed in the study. From the isolates showing ESBL by CTX-M-like mechanism, 80 were selected for PCR while 22 were selected from the isolates showing Carbapenemase or impermeability carbapenemase. Thirty isolates with Vitek2 AES reports not showing CTX-M like or Carbapenemase (either lacking detectable β-lactamase activity or hyper-producers of *SHV* or AmpC) were also

selected for PCR to detect any repressed genes.

Genotypic detection of extended-spectrum beta-lactamases

Bacterial DNA was extracted from colonies grown on 2% blood agar using the QIAamp DNA Mini kit following the protocol for bacteria, according to the manufacturer's instructions (Qiagen, Hilden, Germany). ESBL genes *blaCTX-M*, *blaSHV* and *blaOXA10/11*, were detected using real-time PCR assays from HiMedia (Hi-PCR® Extended Spectrum ß-Lactamases (ESBLs) Gene (Multiplex) Probe PCR Kit, Ref: MBPCR131-10R)("ESBL HiMedialabs," n.d.) were used. The kit includes positive and negative controls while external controls using the ATCC strains were included in each run. In the master mix *SHV, CTX-M, OXA-10/11* were detected in FAM, HEX, and Cy5 channels respectively, and Internal Control is detected in Cy5.5 channel. The cycling method consisted of initial denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds with 40 cycles of annealing and extension at 60°C for thirty seconds and the final holding stage was carried out in Biorad CFX-96 Real-time Thermocycler. A cycle threshold value of ≤40 and sigmoid curve was considered positive.

Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit (Ref: MBPCR132-10R, HiGenoMB from HiMedia, India) was used for carbapenemase genes detection following manufacturer's instructions("CPMHiMedialabs," n.d.). In master mix-1, *NDM, KPC, IMP* and *VIM* were detected in FAM, HEX, Texas Red and Cy5 channels respectively, while in master mix-2 *OXA-51, OXA-23, OXA-48* and *OXA-58* in FAM, HEX, Texas Red and Cy5 channels respectively. Internal Control was detected in Cy5.5 channel in both the master mixes. The cycling method consisted of initial denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for 5 seconds, and 45 cycles of annealing and extension at 60°C for one minute carried out in Bio-rad CFX-96 Thermocycler. A sigmoid curve with cycle threshold (Ct) value of ≤33 for *SHV* & *CTX-M* and ≤31 for *OXA10/11* was considered positive.

Statistical analysis

All samples related and machine generated data were entered and analyzed using the Statistical Package for Social Science (SPSS) version 25. The Sensitivity, Specificity, Positive and Negative predictive values (PPV and NPV) of the Vitek 2 AST-N280 Gram-negative susceptibility card for ESBL detection was compared with the phenotypic combination disc method and RT-PCR based genotypic method.

Ethics statement

The study proposal received ethical approval from Ethical Review Board of Nepal Health Research Council (NHRC)-Regd. 428-2018, and adhered to the protocol

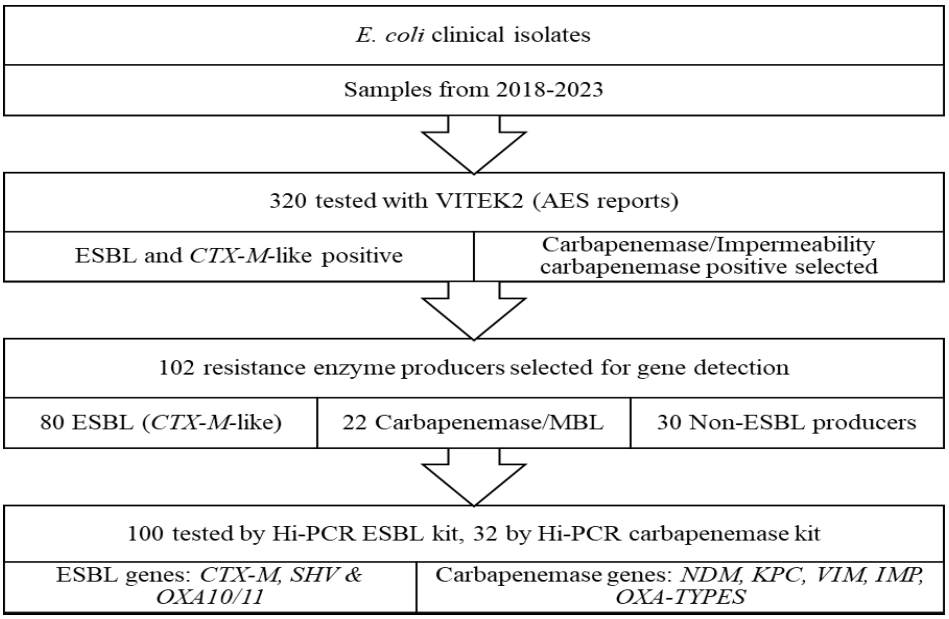

Figure 1: The STROBE flowchart of the study.

RESULTS

The isolates where Vitek 2 N280 cards were used for MIC analysis totaled 320, however AES reports were not generated in two due to inconsistent reports. The enzyme and gene indication through AES reports showed *CTX-M*-like ESBL presence in 50% (159/318) and SHV hyperproduction in 4.4% (14/318) of the isolates. A total of 65 (26%) isolates were wild type where no acquired β-lactamase resistant genes was detected by the AES. Aminoglycoside resistant genes were detected in either Gentamicin alone (12.3%) or among other aminoglycosides, namely, Tobramicin, Netilmicin and Amikacin (Range 11.3-12.3%) as shown in Table 1. Only 100/159 isolates with *CTX-M* like ESBL detected by the Vitek 2 AES, selected conveniently due to time and resource constraints, were further analyzed. Though carbapenemase and impermeability carbapenemase were detected in 28 and 26 isolates respectively, only 22 MBL positives (by CLSI-recommended eCIM method) (Acharya et al., 2024)were further tested for the carbapenemase genes by PCR.

Comparison of VITEK 2 AES and Combination disc tests: The sensitivity of Vitek 2 AES for detection of ESBLs as compared to the gold standard-CLSI-recommended combination disc method was 99% (192/195), while specificity was only 56% (68/122). Positive Predictive Value (PPV) was 78%, Negative Predictive Value (NPV)was 96% and the association was significant (P-value of <0.0001). (Table 2)

Table 1: Antibiotic resistance mechanisms detected by VITEK 2 AES

Type of resistance	Positive	Negative	%Positive	Total
Acquired Penicillinase	45	273	14.2	318
ESBL	247	71	77.7	318
ESBL (*CTX-M*-like)	159	159	50.0	318
SHV hyperproduction	14	304	4.4	318
AmpC	56	262	17.6	318
Impermeability Carbapenemase	26	292	8.2	318
Carbapenemase+/-ESBL	28	290	8.8	318
GEN (aac(3)-I)	39	279	12.3	318
GEN TOB (ant (2"))	38	280	11.9	318
GEN TOB NET (aac(3)-II)	36	282	11.3	318
GEN TOB NET (aac(3)-IV)	38	280	11.9	318
GEN TOB NET AMI (Gene not specified)	33	259	11.3	292
GEN TOB NET AMI (aac(6')) only	26	0	NA	26
Wild	65	253	25.6	318

AAC, aminoglycoside acetyltransferase; AES, Advanced Expert System; AmpC, ampicilinase C; AMI, Amikacin; CTX-M, cefotaximase-Munich; ESBL, extended-spectrum beta-lactamase; GEN, Gentamicin; NET, Netilmicin; SHV, sulfydryl variable; TOB, Tobramicin.

Table 2: Comparison of VITEK 2 AES ESBL and combination disc methods

	Combination disc method			
	ESBL Positive	**ESBL Negative**	**Total**	**P-value**
VITEK 2 AES ESBL Positive	192 (98.5%)	54 (44.3%)	246 (82.0)	<0.001
VITEK 2 AES ESBL Negative	3 (1.5%)	68 (55.7%)	71 (18.0)	
Total	195 (100%)	122 (100%)	317 (100%)	

Table 3: Comparison of Multiplex PCR and VITEK-AES

		Hi-Multiplex PCR		
		CTX-M Positive	**CTX-M Negative**	**Total**
VITEK2	**CTX-M Positive**	60	0	60
	CTX-M Negative	20	20	40
	Total	80	20	100

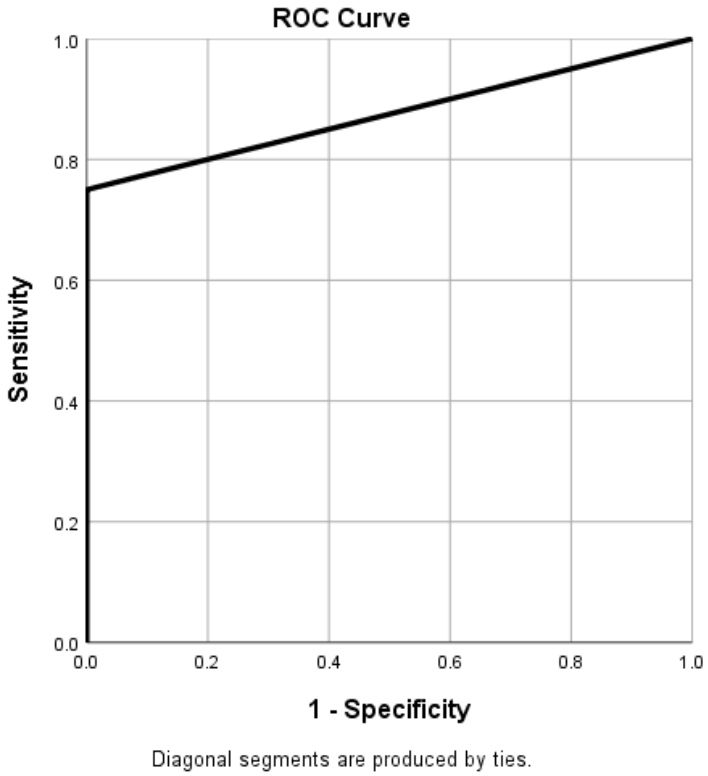

Figure 2: ROC Curve showing CTX-M detection by Vitek AES and Multiplex Hi-PCR in *E. coli* isolates

Comparison of Multiplex PCR with VITEK-AES

Vitek 2 AES for detection of ESBLs (*CTX-M*-like) in comparison to Multiplex PCR showed the specificity as high as 100%, and the sensitivity of 75%, PPV - 100, NPV - 50, and p-value<0.0001 (Table 3). The Vitek 2 AES did not detect 20 CTX-M genes detected by PCR, indicating the high degree of difference between the PCR and VITEK-AES results.

The ROC curve analysis (Hajian-Tilaki, 2013) was used to quantify how accurately Vitek 2 AES systems could detect ESBL by *CTX-M*-like genes. Area under the ROC curve was 0.875, and it shows that Vitek CTX-M like has overall good performance for the detection of ESBL producers (Figure 2). The Vitek 2 AES detected SHV hyperproduction in 14 isolates, in which CTX-M-like was not detected. However, SHV gene was not detected with Multiplex PCR in these isolates.

Comparison of extended-spectrum beta-lactamase detection methods

Comparison of the phenotypic and genotypic tests for ESBL testing shows a good association between the methods. The correlation between the Vitek 2 AES reports of ESBL (*CTX-M* like) and *CTX-M* gene detection by PCR had significant association ($P < 0.001$).

Vitek 2 AES have sensitivity of 99% for detection of ESBL, with sensitivity of 75% in detecting ESBL (CTX-M like) genes. So, VITEK-2AES could be used as a screening test for ESBL screening based on its availability for MIC determinations. The PCR test could not detect ESBL genes in 20 Vitek 2 AES ESBL (CTX-M like) isolates, and non-ESBL producers included in the study.

DISCUSSION

Antimicrobial resistance attributable to ESBL and carbapenemase resistance is high with detection of different genes responsible for resistance, in clinical *E. coli* isolates which significantly adds to difficulties in patient treatment. This study detected high proportions (50%) of *CTX-M* genes in the ESBL producers, which has been reported in comparable numbers in neighboring countries in Asia (Abrar et al., 2019; Ghenea et al., 2022; Nachimuthu et al., 2021; Narendrakumar et al., 2022). No *SHV* detection in this study may be attributable to the inclusion of larger proportion of isolates that were ESBL positive but were CTX-M-like in Vitek 2 AES reports. Studies from various countries have reported low percentages (4-9%) (Hassuna et al., 2020; Sami Michael and Saadi, 2018) while higher percentages (21-44%) have been reported from other some countries (Balaky, 2018; Ehsan et al., 2023). Studies in other countries showed higher *SHV* isolation from animals or food sources with some geographic restriction than clinical isolates (Liakopoulos et al., 2016). *E. coli* isolates that lack ESBLs but hyperproduce SHV-1 may also give false-positive confirmatory test results (Rawat and Nair, 2010).

Out of 36 carbapenem-resistant isolates, 22 (61%) were MBL producers. Among the 22 MBL producers, *blaNDM* gene was the most common (12/22, 54.5%), followed by *blaOXA-48* (2/22; 9%) whereas, PCR-amplification products related to *blaIMP, blaVIM* or *blaKPC* genes were not detected. There was co-production of *blaNDM* and *blaCTX-M* in five (5%) isolates. The *blaNDM* is a concern in the Indian subcontinent (Junaid, 2021; Thapa et al., 2021; Uddin et al., 2022) having reported similar results (7.2%-25.3%) while the countries in other regions have not reported NDM genes (Al-Mayahie et al., 2022; Rashedi et al., 2023). Acquired *OXA-48*-type carbapenemases are important causes of carbapenem resistance among Enterobacterales and their numbers have been increasingly reported in *E. coli* globally (Findlay et al., 2022; Pitout et al., 2020), while only 9% of the MBL isolates were *OXA-48* positive in the current study. The Carbapenemase producing isolates in the current study were also found to be resistant to cephalosporins and fluoroquinolones. Low carbapenem MICs have been widely reported in *OXA-48*-producing *E. coli* (Findlay et al., 2022), though in the current study only two isolates had high carbapenem MICs. CLSI-recommended combination disc test and the MICs using more than one third-generation cephalosporin in combination with clavulanic acid provides overall high sensitivity of ESBL detection (Das et al., 2023; Wiegand et al., 2007). The Vitek 2 AES detected 99% of ESBL but only 75% of the CTX-M-like isolates; in addition, the specificity was lower to 56% for ESBL and higher 100% for CTX-M-like enzymes.

A study comparing automated susceptibility testing systems with conventional testing methods reported that the combination disc test had the highest sensitivity and positive predictive values 97% and 98%, respectively, which compares well with the results of this study(Young et al., 2019), which has been asserted by another study comparing conventional and automated methods

(Wiegand et al., 2007). Our study results are limited only to the Vitek 2 Automated antibiotic susceptibility platform and specifically Gram-negative AST-N280 cards.

While ESBL screening using more than one third-generation cephalosporin is still a sensitive method, the choice of method to detect will depend on the cost and availability of automated equipment. The Vitek 2 has the added advantage of screening for ESBL without using extra clavulanic acid MICs and corelating well with other gene detection and phenotypic methods (Chan and Leroi, 2021). The ESBL detection sensitivity of Vitek2 AES system using N280 cards was 99%, while the specificity was 56% in the limited number of isolates tested. Studies using different Vitek AST cards with confirmatory wells for ESBL have reported better sensitivities (Carvalhaes et al., 2023; Chan and Leroi, 2021). The performance of VITEK AES in current study findings was not equivalent to the CLSI-recommended combination disc method, although other studies showed better results and utilization for routine diagnostic purposes (Carvalhaes et al., 2023; Chan and Leroi, 2021; Young et al., 2019). In this study, the performance of the Vitek 2 AST-N280 card was comparable to previous studies with sensitivity of 92% (78% – 97%) and specificity of 100% (72% – 100%) for *E. coli* against the composite reference standard (Young et al., 2019). The small number of non-ESBL isolates included in the current study meant that confidence intervals for all specificity estimates were relatively wide, ranging between 56% and 100%, for ESBL and ESBL-CTX-M like respectively.

The conventional CLSI-recommended combination disc method, is more specific, and sensitive when more than one cephalosporins are used for detection (Rawat and Nair, 2010). The conventional method is less expensive than the automated system, however it might not be able to detect ESBL when masked by other antibiotic resistance enzymes such as ACBL or MBL (Das et al., 2023).

Considering the variable sensitivity and specificity of VITEK-AES observed in this study, the continuation of double disc diffusion test based on CLSI-guideline for the confirmation of ESBL is still efficient, till further scientific evaluation evidence for VITEK-2-AES, with higher representative number of samples in country context is available, with supporting evidence for inclusion in routine testing in the national system.

Limitations of this study includes not testing the isolates in duplicate, and using only Vitek-N280 cards for analysis. Genotypic testing was limited to detection of only three ESBL genes, *blaCTX-M*, *blaSHV* and *blaOXA10/11*. Genotypic detection of resistance genes was taken as reference to assess Vitek 2 AES but the genes detected might not be expressed which could have led to non-concordance among the phenotypic and genotypic methods. The samples in current study were limited to a single outpatient facility site based in Kathmandu, and the tested isolates may only provide a snap-shot of the problem in Nepal.

Conclusion

The *blaCTX-M* and *blaNDM* are the most common genes responsible for ESBL and Carbapenem resistance, though *blaOXA-48* was also observed in *E. coli* with low co-production of *NDM* and *CTX-M* genes in Nepal. VITEK-2-AES sensitivity for ESBL (*CTX-M* like) and carbapenemase detection is comparable with CLSI recommended method for ESBL detection, while specificity is limiting for recommending in routine use, till further limited evaluation results are available.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abrar, S., Ain, N.U., Liaqat, H., Hussain, S., Rasheed, F., Riaz, S., 2019. Distribution of bla CTX-M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrob Resist Infect Control 8. https://doi.org/10.1186/s13756-019-0536-0

Acharya, J., Ghimire, P., Banjara, M.R., Sharma, S., Rijal, K.R., Rijal, N., Shretha, A., Jha, R., 2024. Multidrug resistant Escherichia coli isolated at National Public Health Laboratory, Nepal.

Al-Mayahie, S.M.G., Al-Guranie, D.R.T., Hussein, A.A., Bachai, Z.A., 2022. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. PLoS One 17. https://doi.org/10.1371/journal.pone.0262984

Balaky, S.T., 2018. Molecular detection of SHV-Type ESBL in E. coli and K. pneumoniae and their antimicrobial resistance profile.

Biomeriux, 2024. VITEK® 2 Advanced Expert System™ [WWW Document]. URL https://www.biomerieux.ca/en/product/vitek-2-advanced-expert-system (accessed 9.19.24).

Boyd, S.E., Livermore, D.M., Hooper, D.C., Hope, W.W., 2020. Metallo-β-lactamases: Structure, function, epidemiology, treatment options, and the development pipeline. Antimicrob Agents Chemother 64. https://doi.org/10.1128/AAC.00397-20

Carvalhaes, C.G., Shortridge, D., Woosley, L.N., Gurung, N., Castanheira, M., 2023. Performance of the Vitek 2 Advanced Expert System (AES) as a Rapid Tool for Reporting Antimicrobial Susceptibility Testing (AST) in Enterobacterales from North and Latin America. Microbiol Spectr 11. https://doi.org/10.1128/spectrum.04673-22

Chan, E., Leroi, M., 2021. Evaluation of the VITEK 2 Advanced Expert System performance for predicting resistance mechanisms in Enterobacterales acquired from a hospital-based screening program. Pathology 53, 763–767. https://doi.org/10.1016/j.pathol.2021.01.009

CPMHiMedialabs [WWW Document], n.d. URL https://www.himedialabs.com/us/mbpcr132-hi-pcr-carbapenemase-gene-multiplex-probe-pcr-kit.html (accessed 9.16.24).

Das, P., Mahapatra, D., Mazumder, S. Sen, 2023. A Guide Towards the Phenotypic Detection of Extended-spectrum b-lactamases Production in Enterobacteriaceae: Alone or in Presence of Other Interfering Enzymes. J Pure Appl Microbiol 17, 1410–1421. https://doi.org/10.22207/JPAM.17.3.31

Dunn, S.J., Connor, C., McNally, A., 2019. The evolution and transmission of multi-drug resistant Escherichia coli and Klebsiella pneumoniae: the complexity of clones and plasmids. Curr Opin Microbiol 51, 51–56. https://doi.org/10.1016/j.mib.2019.06.004

Ehsan, B., Haque, A., Qasim, M., Ali, A., Sarwar, Y., 2023. High prevalence of extensively drug resistant and extended spectrum beta lactamases (ESBLs) producing uropathogenic Escherichia coli isolated from Faisalabad, Pakistan. World J Microbiol Biotechnol 39. https://doi.org/10.1007/s11274-023-03565-9

ESBL HiMedialabs [WWW Document], n.d. URL https://www.himedialabs.com/us/mbpcr131-hi-pcr-extended-spectrum-ss-lactamases-esbls-gene-multiplex-probe-pcr-kit.html (accessed 9.16.24).

Findlay, J., Perreten, V., Poirel, L., Nordmann, P., 2022. Molecular analysis of OXA-48-producing Escherichia coli in Switzerland from 2019 to 2020. European Journal of Clinical Microbiology and Infectious Diseases 41, 1355–1360. https://doi.org/10.1007/s10096-022-04493-6

Fleece, M.E., Pholwat, S., Mathers, A.J., Houpt, E.R., 2018. Molecular diagnosis of antimicrobial resistance in Escherichia coli. Expert Rev Mol Diagn 0. https://doi.org/10.1080/14737159.2018.1439381

Garrec, H., Drieux-Rouzet, L., Golmard, J.L., Jarlier, V., Robert, J., 2011. Comparison of nine phenotypic methods for detection of extended-spectrum β-lactamase production by enterobacteriaceae. J Clin Microbiol 49, 1048–1057. https://doi.org/10.1128/JCM.02130-10

Ghenea, A.E., Zlatian, O.M., Cristea, O.M., Ungureanu, A., Mititelu, R.R., Balasoiu, A.T., Vasile, C.M., Salan, A.I., Iliuta, D., Popescu, M., Udriştoiu, A.L., Balasoiu, M., 2022. TEM, CTX-M, SHV Genes in ESBL-Producing Escherichia coli and Klebsiella pneumoniae Isolated from Clinical Samples in a County Clinical Emergency Hospital Romania-Predominance of CTX-M-15. Antibiotics 11. https://doi.org/10.3390/antibiotics11040503

Hajian-Tilaki, K., 2013. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation, Caspian J Intern Med.

Hassuna, N.A., Khairalla, A.S., Farahat, E.M., Hammad, A.M., Abdel-Fattah, M., 2020. Molecular characterization of Extended-spectrum β lactamase- producing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. Sci Rep 10. https://doi.org/10.1038/s41598-020-59772-z

Isenberg, H.D., 2004. Clinical Microbiology Procedures Handbook, 2nd ed. ASM Press, Washington, D.C.

Junaid, K., 2021. Molecular diversity of NDM-1, NDM-5, NDM-6, and NDM-7 variants of new delhi metallo-β-lactamases and their impact on drug resistance. Clin Lab 67, 1897–1904. https://doi.org/10.7754/CLIN.LAB.2021.201214

Koirala, S., Khadka, S., Sapkota, S., Sharma, S., Khanal, S., Thapa, A., Khadka, D.K., Poudel, P., 2021. Prevalence of CTX-M β -Lactamases Producing Multidrug Resistant Escherichia coli and Klebsiella pneumoniae

among Patients Attending Bir Hospital, Nepal. Biomed Res Int 2021. https://doi.org/10.1155/2021/9958294

Lewis, J.S., 2023. M100 performance standards for antimicrobial susceptibility testing, 33rd Edition, M100ED33. CLSI.

Liakopoulos, A., Mevius, D., Ceccarelli, D., 2016. A review of SHV extended-spectrum β-lactamases: Neglected yet ubiquitous. Front Microbiol. https://doi.org/10.3389/fmicb.2016.01374

Livermore, D.M., Struelens, M., Amorim, J., Baquero, F., Bille, J., Canton, R., Henning, S., Gatermann, S., Marchese, A., Mittermayer, H., Nonhoff, C., Oakton, K.J., Praplan, F., Ramos, H., Schito, G.C., Van Eldere, J., Verhaegen, J., Verhoef, J., Visser, M.R., 2002. Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests, Journal of Antimicrobial Chemotherapy.

Mancuso, G., De Gaetano, S., Midiri, A., Zummo, S., Biondo, C., 2023. The Challenge of Overcoming Antibiotic Resistance in Carbapenem-Resistant Gram-Negative Bacteria: "Attack on Titan." Microorganisms 11. https://doi.org/10.3390/microorganisms11081912

Mirkalantari, S., Masjedian, F., Irajian, G., Siddig, E.E., Fattahi, A., 2020. Determination of the frequency of β-lactamase genes (bla SHV, bla TEM, bla CTX-M) and phylogenetic groups among ESBL-producing uropathogenic Escherichia coli isolated from outpatients. Journal of Laboratory Medicine 44, 27–33. https://doi.org/10.1515/labmed-2018-0136

Nachimuthu, R., Kannan, V.R., Bozdogan, B., Krishnakumar, V., S, K.P., Manohar, P., 2021. CTX-M-type ESBL-mediated resistance to third-generation cephalosporins and conjugative transfer of resistance in Gram-negative bacteria isolated from hospitals in Tamil Nadu, India. Access Microbiol 3. https://doi.org/10.1099/acmi.0.000142

Narendrakumar, L., Chakraborty, M., Kumari, S., Paul, D., Das, B., 2022. β-Lactam potentiators to re-sensitize resistant pathogens: Discovery, development, clinical use and the way forward. Front Microbiol. https://doi.org/10.3389/fmicb.2022.1092556

Nordmann, P., Poirel, L., 2019. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. Clinical Infectious Diseases 69, S521–S528. https://doi.org/10.1093/cid/ciz824

Noster, J., Thelen, P., Hamprecht, A., 2021. Detection of multidrug-resistant enterobacterales—from esbls to carbapenemases. Antibiotics. https://doi.org/10.3390/antibiotics10091140

Pierce, V.M., Simner, P.J., Lonsway, D.R., Roe-Carpenter, D.E., Johnson, J.K., Brasso, W.B., Bobenchik, A.M., Lockett, Z.C., Charnot-Katsikas, A., Ferraro, M.J., Thomson, R.B., Jenkins, S.G., Limbago, B.M., Das, S., 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among enterobacteriaceae. J Clin Microbiol 55, 2321–2333. https://doi.org/10.1128/JCM.00193-17

Pitout, J.D.D., Peirano, G., Kock, M.M., Strydom, K.A., Matsumura, Y., 2020. The Global Ascendency of OXA-48-Type Carbapenemases. Clin Microbiol Rev 33. https://doi.org/10.1128/CMR.00102-19

Rashedi, F., Yazdanpour, Z., Khademi, F., Vaez, H., 2023. Prevalence of carbapenem-resistant Metallo-Beta-Lactamase-producing Escherichia coli strains isolated from urinary tract infections 17. https://doi.org/10.29252/mlj.17.6.19

Rawat, D., Nair, D., 2010. Extended-spectrum ß-lactamases in gram negative bacteria. J Glob Infect Dis 2, 263. https://doi.org/10.4103/0974-777x.68531

Rehman, N., Azam, S., Ali, A., khan, I., Asghar, M., Ali, M., Waqas, M., Ullah, F., Sehra, G. e., 2021. Molecular epidemiology of antibiotic-resistant genes and potent inhibitors against TEM, CTX-M-14, CTX-M-15, and SHV-1 proteins of Escherichia coli in district Peshawar, Pakistan. Saudi J Biol Sci 28, 6568–6581. https://doi.org/10.1016/j.sjbs.2021.07.028

Sah, R.S.P., Dhungel, B., Yadav, B.K., Adhikari, N., Thapa Shrestha, U., Lekhak, B., Banjara, M.R., Adhikari, B., Ghimire, P., Rijal, K.R., 2021. Detection of TEM and CTX-M Genes in Escherichia coli Isolated from Clinical Specimens at Tertiary Care Heart Hospital, Kathmandu, Nepal. Diseases 9. https://doi.org/10.3390/diseases9010015

Sami Michael, N., Saadi, A.T., 2018. Detection of bla CTX-M, bla TEM-01 and bla SHV Genes in Multidrug Resistant Uropathogenic E. coli Isolated from Patients with Recurrent Urinary Tract Infections. International Journal of Medical Research & Health Sciences 7, 81–89.

Sawa, T., Kooguchi, K., Moriyama, K., 2020. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care. https://doi.org/10.1186/s40560-020-0429-6

Shrestha, A., Acharya, J., Amatya, J., Paudyal, R., Rijal, N., 2022. Detection of Beta-Lactamases (ESBL and MBL) Producing Gram-Negative Pathogens in National Public Health Laboratory of Nepal. Int J Microbiol 2022. https://doi.org/10.1155/2022/5474388

Shrestha, B., Shrestha, S., Mishra, S.K., Kattel, H.P., Tada, T., Ohara, H., Kirikae, T., Rijal, B.P., Sherchand, J.B., Pokhrel, B.M., 2015. Phenotypic Characterization of Multidrug-resistant Escherichia Coli with Special Reference to Extended-spectrum-beta-lactamases and Metallo-beta-lactamases in a Tertiary Care Center 53, 89–95.

Thapa, S., Adhikari, N., Shah, A.K., Lamichhane, I., Dhungel, B., Shrestha, U.T., Adhikari, B., Banjara, M.R., Ghimire, P., Rijal, K.R., 2021. Detection of NDM-1 and VIM Genes in Carbapenem-Resistant Klebsiella pneumoniae Isolates from a Tertiary Health-Care Center in Kathmandu, Nepal. Chemotherapy 66, 199–209. https://doi.org/10.1159/000518256

Uddin, F., Imam, S.H., Khan, S., Khan, T.A., Ahmed, Z., Sohail, M., Elnaggar, A.Y., Fallatah, A.M., El-Bahy, Z.M., 2022. NDM Production as a Dominant Feature in Carbapenem-Resistant Enterobacteriaceae Isolates from a Tertiary Care Hospital. Antibiotics 11. https://doi.org/10.3390/antibiotics11010048

Wayne, P., 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition CLSI document M02-A11.

Wiegand, I., Geiss, H.K., Mack, D., Stürenburg, E., Seifert, H., 2007. Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. J Clin Microbiol 45, 1167–1174. https://doi.org/10.1128/JCM.01988-06

Young, A.L., Nicol, M.P., Moodley, C., Bamford, C.M., 2019. The accuracy of extended-spectrum beta-lactamase detection in Escherichia coli and Klebsiella pneumoniae in South African laboratories using the Vitek 2 Gram-negative susceptibility card AST-N255. S Afr J Infect Dis 34. https://doi.org/10.4102/sajid.v34i1.114