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REVIEWER'S REPORT

Manuscript No.: IJAR-57940

Title: Phenotypic and Molecular Characterization of ESBL-Producing Escherichia coli and Klebsiella spp. in a Tertiary Care Teaching Hospital in South India.

Recommendation:
Accept as it is .....
Accept after minor revision.....
Accept after major revision .....YES
Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality			√	
Clarity		√		
Significance			√	

Reviewer's ID: JPR-094

Detailed Reviewer's Report

Strengths

1. Addresses an important public health issue related to antimicrobial resistance and ESBL-producing Enterobacteriaceae.

2. Combines both phenotypic and molecular methods for ESBL detection.

3. Includes detection of major ESBL genes (blaCTX-M, blaTEM, blaSHV).

4. Ethical approval is clearly documented.

5. Results are presented in a simple and understandable format.

6. Findings provide local epidemiological data from a tertiary care hospital in South India.

Weaknesses

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1. **Outdated Data: The study was conducted during 2015–2016, making the findings nearly 10 years old and limiting current relevance.**
2. **Small Sample Size: Only 100 isolates were included, reducing statistical power.**
3. **Lack of Statistical Analysis: No p-values, confidence intervals, or comparative analyses are presented.**
4. **Incomplete Molecular Methodology: PCR primer sequences, cycling conditions, amplicon sizes, and quality control procedures are missing.**
5. **No Species-Level Identification of Klebsiella: The manuscript groups all Klebsiella spp. together without species differentiation.**
6. **Limited ESBL Gene Coverage: Important genes such as OXA, PER, VEB, and carbapenemase genes were not investigated.**
7. **Questionable References: Several recent references (2024–2025) require verification and may not be traceable.**
8. **Inadequate Figure Presentation: Figure 1 is mentioned but not provided.**
9. **No Multidrug Resistance Analysis: MDR, XDR, or PDR classifications are not reported.**
10. **Keywords Section Missing: Keywords heading is present but no keywords are provided.**
11. **Discussion is Superficial: Comparison with regional and international studies is limited.**
12. **No Study Limitations Section: Important limitations are not acknowledged.**

Key Points

1. ESBL prevalence was 70% among *E. coli* and 46% among *Klebsiella* spp.

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2. Complete resistance was observed against cefotaxime, ceftazidime, and ciprofloxacin among ESBL-positive isolates.

3. Amikacin and imipenem retained comparatively good activity.

4. blaCTX-M was the predominant ESBL gene detected.

5. Multiple ESBL gene coexistence suggests plasmid-mediated dissemination of resistance.

6. The study emphasizes the need for routine ESBL surveillance and antimicrobial stewardship.

Significance

*** The study contributes regional data on ESBL-producing pathogens and their genetic determinants.**

*** Molecular characterization enhances understanding of resistance mechanisms.**

*** Findings may support local antibiotic policy development and infection-control practices.**

*** However, the scientific impact is reduced by the age of the data and limited methodological details.**

Major Issues Requiring Revision

1. Provide detailed PCR methodology including primers, annealing temperatures, and amplicon sizes.

2. Add statistical analysis and significance testing.

3. Verify and update all references, especially citations from 2024–2025.

4. Include the missing figure and improve table formatting.

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5. Add a dedicated limitations section.

6. Expand discussion with comparison to recent national and international studies.

7. Justify publication of 2015–2016 data in 2026 and explain its current relevance.

8. Include keywords and improve manuscript formatting.

9. Clarify whether isolates were consecutively collected and describe inclusion/exclusion criteria.

10. Consider additional resistance gene analysis or justify the limited gene panel.

Recommendation

****Decision: MAJOR REVISION****

Justification

Although the topic is important and the study combines phenotypic and molecular characterization of ESBL-producing isolates, significant methodological, analytical, and reporting deficiencies limit its scientific rigor. The age of the dataset, absence of statistical analysis, incomplete molecular methods, and insufficient discussion must be addressed before the manuscript can be considered for publication.

****Overall Recommendation: Major Revision**.**

Major Revision Justification (Issue and Reason Line-by-Line)

Line No.	Issue Identified	Reason for Major Revision
9-14	Methods in abstract are incomplete	PCR methodology lacks primer details, amplification conditions, and quality controls, reducing reproducibility.
15-20	Results lack statistical support	No confidence intervals, p-values, or statistical comparisons are provided to support findings.
21-23	Conclusion is overly	Conclusions extend beyond the presented data

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Line No.	Issue Identified	Reason for Major Revision
	generalized	without demonstrating clinical impact or surveillance outcomes.
24	Keywords missing	Essential indexing keywords are absent, violating standard manuscript requirements.
32-35	Literature review outdated	Introduction relies heavily on older references and lacks current epidemiological evidence.
36-38	Novelty not established	Authors fail to explain how this study differs from previously published ESBL surveillance studies.
40-41	Study period (2015–2016)	Data are nearly a decade old; authors must justify current relevance and delayed publication.
44	Small sample size	Only 100 isolates were included, limiting generalizability and statistical robustness.
44-45	Sampling strategy unclear	No information on sample size calculation, consecutive sampling, or inclusion/exclusion criteria.
48-51	Outdated CLSI guideline	CLSI 2010 standards were used; authors should discuss implications compared with current guidelines.
52-55	Phenotypic ESBL testing inadequately described	Quality control strains and validation procedures are not reported.
56-58	Molecular methods incomplete	Primer sequences, PCR conditions, amplicon sizes, positive/negative controls, and sequencing confirmation are absent.
56-58	Limited gene panel	Only blaCTX-M, blaTEM, and blaSHV were tested; other relevant resistance genes were not evaluated or justified.
60-62	Ethical statement incomplete	No mention of patient consent waiver or confidentiality procedures.
68-70	Results presentation incomplete	Distribution of isolates by specimen type is not provided numerically.
71-74	Resistance data lack statistical analysis	No comparison between E. coli and Klebsiella resistance patterns.
71-74	Important susceptibility data missing	Zone diameters, MIC values, and interpretive categories are not reported.
75	Table 1 formatting issue	Table lacks sample numbers and confidence measures, reducing interpretability.
77	Table 2 incomplete	Gene prevalence percentages are presented without actual isolate counts.

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Line No.	Issue Identified	Reason for Major Revision
77	Coexistence data absent	Authors mention multiple gene coexistence but provide no detailed combinations or frequencies.
81	Figure missing	Figure 1 is cited but not included in the manuscript.
84-87	Discussion lacks depth	Findings are not critically compared with recent national and international studies.
85-86	Unsupported claim	Authors claim global dominance of blaCTX-M without sufficient contemporary evidence.
86-87	Mechanistic inference unsupported	Horizontal gene transfer is suggested without plasmid characterization studies.
88-90	Clinical implications underdeveloped	No discussion of treatment outcomes, infection control measures, or hospital policy implications.
92-96	Conclusion repetitive	Conclusion largely restates results without presenting broader scientific contributions.
113-120	Reference authenticity concern	Several 2024–2025 citations require verification and may not be adequately traceable.
Entire manuscript	No limitations section	Important limitations such as small sample size, single-center design, and outdated data are not acknowledged.
Entire manuscript	Lack of advanced statistical analysis	No multivariate analysis, risk-factor assessment, or epidemiological interpretation is provided.
Entire manuscript	Limited scientific novelty	Similar ESBL prevalence and gene distribution studies have already been extensively reported from India.

Overall Justification for Major Revision

The manuscript addresses an important topic in antimicrobial resistance; however, substantial deficiencies exist in methodology, statistical analysis, reporting quality, molecular characterization, data interpretation, and scientific novelty. The use of a decade-old dataset (2015–2016), incomplete PCR methodology, absence of statistical validation, missing figure, questionable recent references, and lack of a limitations section significantly weaken the manuscript. These issues must be comprehensively addressed before the work can be considered for publication.

Final Recommendation

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Major Revision

Editorial Decision: Major Revision Required.