

REVIEWER'S REPORT

Manuscript No.: IJAR-59191

Title: Characterization of Microbiome Incidence and Antibacterial Resistance Profiles in Urinary Tract Infected Patients

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

Reviewer Report

Manuscript Title

“Characterization of Microbiome Incidence and Antibacterial Resistance Profiles in Urinary Tract Infected Patients”

Overall Assessment

The manuscript addresses an important clinical microbiology problem: **uropathogen diversity and antimicrobial resistance among UTI patients**, using culture, biochemical characterization, antimicrobial susceptibility testing, and 16S rRNA sequencing. The study includes a reasonably large initial sample size of **385 urine specimens** and reports **121 bacterial isolates**, with 20 representative isolates subjected to molecular identification.

The combination of conventional microbiological characterization with molecular identification is potentially useful. However, the manuscript in its present form has **substantial methodological**,

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statistical, data-reporting, terminology, and interpretation problems that should be addressed before the work can be considered for publication.

1. Strengths of the Manuscript

1.1 Relevant and clinically important topic

Antimicrobial resistance among UTI-associated bacteria is an important public-health and clinical concern. The manuscript addresses this issue in a population containing children, adults, diabetic patients, and pregnant women.

1.2 Reasonable initial sample size

The study involves **385 urine samples**, which provides a useful initial dataset for investigating the bacterial profile of clinically diagnosed UTI cases.

1.3 Multiple identification approaches

The study does not rely exclusively on one identification technique. It combines:

- culture characteristics,
- Gram staining,
- biochemical tests,
- antimicrobial susceptibility testing, and
- 16S rRNA sequencing.

This integrated approach is a positive feature of the study.

1.4 Molecular confirmation

Twenty representative isolates were subjected to 16S rRNA sequencing, with reported sequence similarities above 99%.

This provides an additional level of confirmation beyond conventional phenotypic identification.

1.5 MAR-index analysis

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The manuscript attempts to quantify multidrug resistance using the Multiple Antibiotic Resistance (MAR) index. Fourteen of the 20 tested isolates are reported to have MAR values ≥ 0.30 .

This can be a useful descriptive component if the interpretation is appropriately qualified.

1.6 Practical antimicrobial-stewardship relevance

The manuscript connects antimicrobial-resistance findings with the need for culture-guided therapy and antimicrobial stewardship. This gives the study potential practical relevance.

2. Major Weaknesses

2.1 *The title is scientifically problematic*

The term “**Microbiome**” is not appropriate for the study as currently designed.

The study is based on:

- conventional bacterial culture,
- biochemical identification,
- antibiotic susceptibility testing, and
- 16S rRNA sequencing of 20 selected isolates.

This is **not a microbiome study in the usual sense** because there is no culture-independent community profiling, metagenomic analysis, microbiome sequencing, alpha/beta diversity analysis, or comprehensive microbial-community characterization.

Also, “**incidence**” is potentially inappropriate because the study does not appear to establish incidence of UTI in a defined population over a defined period.

Reviewer suggestion

A more appropriate title would be:

“Characterization and Antimicrobial Resistance Profiles of Uropathogens Isolated from Urinary Tract Infection Patients”

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or

“Phenotypic and Molecular Characterization of Uropathogens and Their Antimicrobial Resistance Profiles in UTI Patients”

3. Major Methodological Concerns

3.1 *The study population is insufficiently described*

The manuscript states that 385 samples came from:

- children,
- adults,
- diabetic patients, and
- pregnant women.

However, the manuscript does not provide adequate information about the number of participants in each group.

A reviewer would expect a table containing:

Patient group	Number tested	Male	Female	Culture positive	% positive
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Children	—	—	—	—	—
Adults	—	—	—	—	—
Diabetic	—	—	—	—	—
Pregnant	—	—	—	—	—
Total	385	—	—	—	—

Without these data, the comparison among the claimed high-risk groups cannot be adequately evaluated.

3.2 *Culture-positive rate is not clearly reported*

The manuscript states that **121 morphologically distinct isolates** were recovered.

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However, this is not equivalent to saying that 121 patients had culture-positive UTI.

The manuscript needs to clearly distinguish:

patients → culture-positive samples → isolates → representative isolates

For example:

385 urine samples → X culture-positive samples → 121 bacterial isolates → 20
molecularly characterized isolates.

At present, this relationship is unclear.

4. Important Numerical Inconsistency

There is a clear inconsistency between the **Methods/Table 1** and the **Results**.

Methods

The manuscript states:

- MacConkey: 135 countable plates = **35.06%**
- Blood agar: 159 countable plates = **41.30%**

Results

The manuscript subsequently states:

- MacConkey: **37.5% countable**
- Blood agar: **44.2% countable**

These cannot both be correct.

Reviewer comment

Major revision required: The authors should recalculate all percentages and ensure complete consistency between Methods, Tables, Results, Discussion and Abstract.

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5. The 121 isolates need better characterization

The manuscript reports 121 isolates but does not provide a comprehensive distribution such as:

Organism	Number	Percentage
E. coli	—	—
Klebsiella spp.	—	—
Proteus spp.	—	—
Pseudomonas spp.	—	—
Staphylococcus spp.	—	—
Enterococcus spp.	—	—
Others	—	—
Total	121	100

This is one of the most important datasets in the study and should be presented.

6. Antibiotic susceptibility testing is a major concern

The study states that antimicrobial susceptibility was conducted on **20 representative isolates**, rather than all 121 isolates.

This substantially limits the conclusions.

The manuscript sometimes appears to generalize the resistance findings to the entire study population, whereas the susceptibility results actually come from only 20 selected isolates.

The authors should explicitly state:

“Antimicrobial susceptibility testing was performed on 20 representative isolates selected for phenotypic and molecular characterization.”

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The Abstract, Discussion and Conclusion should not imply that the reported resistance percentages represent all 121 isolates.

7. Antibiotic panel requires scientific justification

The antibiotic panel includes:

- ciprofloxacin,
- ofloxacin,
- ampicillin,
- penicillin G,
- oxacillin,
- piperacillin,
- cefuroxime,
- gentamicin,
- erythromycin,
- vancomycin,
- imipenem,
- chloramphenicol,
- trimethoprim-sulfamethoxazole.

The appropriateness of individual agents depends heavily on the bacterial species.

For example, **vancomycin, oxacillin, erythromycin and penicillin G cannot simply be interpreted in the same manner across Gram-negative and Gram-positive uropathogens.**

The authors should provide:

1. species-specific antibiotic panels,
2. applicable breakpoint standards,
3. antibiotic disk concentrations,
4. CLSI/EUCAST edition used,
5. quality-control strains, and
6. organism-specific interpretation criteria.

REVIEWER'S REPORT**8. The susceptibility guideline is outdated/inadequately cited**

The manuscript cites **NCCLS 2002** and later refers to “NCCLS/CLSI breakpoint criteria.”

For a contemporary antimicrobial-resistance study, the authors should clearly identify the **current CLSI or EUCAST standard and version used during the experiments.**

This is particularly important because susceptibility breakpoints can change over time.

9. MAR index interpretation is overstated

The manuscript states that 14/20 isolates with $MAR \geq 0.30$ indicate:

“widespread multi-drug resistance”

and subsequently suggests antibiotic exposure and acquisition of mobile resistance determinants.

The MAR index can describe resistance patterns, but **$MAR \geq 0.30$ alone does not demonstrate the presence of mobile resistance determinants.**

No resistance genes, plasmid analysis, ESBL genes, carbapenemase genes, or other molecular resistance mechanisms are reported.

Therefore, statements about:

“acquisition of mobile resistance determinants”

should be removed or appropriately qualified unless molecular evidence is provided.

10. The molecular identification section requires clarification

The manuscript reports 20 isolates with 16S rRNA sequence identities ranging from approximately 99.05–99.84%.

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However, the authors should provide:

- GenBank accession numbers in a verifiable format,
- accession numbers for the **newly submitted sequences**, if applicable,
- sequence lengths,
- percentage query coverage,
- E-values,
- reference accession numbers,
- BLAST results,
- phylogenetic tree,
- bootstrap values,
- sequence alignment information.

The current table reports an "Accession No." column but does not clearly distinguish **new sequence accession numbers** from reference/database identifiers.

11. "Definitive confirmation" is too strong

The manuscript states that 16S rRNA sequencing provides:

"definitive confirmation of phenotypic identification"

and claims species-level confirmation.

This wording should be moderated.

16S rRNA sequencing can be highly useful for bacterial identification, but species-level discrimination is not equally reliable for every bacterial group.

A more appropriate expression would be:

"16S rRNA sequencing provided molecular support for the phenotypic identification of the selected isolates."

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12. Statistical analysis requires major revision

This is probably the **most serious methodological issue** in the manuscript.

The authors report:

$$F(12,234) = 2.246, P = 0.0106$$

for antibiotic activity.

However, the manuscript does not clearly describe:

- number of replicates,
- experimental unit,
- number of observations per antibiotic,
- whether observations were independent,
- whether normality was tested,
- whether variance homogeneity was tested,
- whether repeated measurements were used.

Because the same bacterial isolates may be tested against multiple antibiotics, ordinary one-way ANOVA may not be the most appropriate analysis if the observations are paired/repeated measurements.

The statistical method should be reconsidered by a statistician.

13. The molecular ANOVA is unclear and potentially inappropriate

The manuscript reports:

$$F(7,17) = 8968, P < 0.0001; R^2 = 0.9997$$

for molecular characterization.

This requires substantial explanation.

The manuscript does not explain:

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- what the dependent variable was,
 - what the eight groups A–H represent,
 - how molecular sequence data were converted into numerical observations,
-
- why ANOVA is appropriate for sequence data,
 - how R^2 was calculated,
 - what biological question this analysis answers.

This analysis should either be **fully justified and reconstructed** or removed.

14. “ $R^2 = 0.9997$ ” should not be interpreted as proof of discriminatory power

The manuscript states that the high R^2 demonstrates the discriminatory capability of molecular characterization.

That conclusion does not follow automatically from an ANOVA R^2 value.

The authors need to explain exactly what statistical model produced this R^2 and what variable it represents.

15. The discussion contains overinterpretation

For example, the manuscript states that the high proportion of TMTC plates:

“strongly indicate active infections rather than contamination, reflecting the clinical severity of UTIs.”

TMTC growth alone does not establish clinical severity.

Similarly, the conclusion refers to the dataset as:

“robust and representative.”

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Representativeness cannot be established without adequate sampling methodology and demographic information.

These claims should be moderated.

16. Important clinical information is missing

The study would be substantially stronger if the authors provided:

- age distribution,

- sex distribution,
- diabetic status,
- pregnancy status,
- previous antibiotic exposure,
- hospitalization status,
- recurrent versus first UTI,
- community-acquired versus hospital-acquired infection,
- clinical symptoms,
- culture positivity,
- organism distribution by risk group.

Without these data, the “high-risk group” comparison is incomplete.

17. Reference section requires substantial correction

Several references require verification and formatting correction.

For example:

“Kibret, M., & Abera, B. (2014). Antimicrobial susceptibility patterns of uropathogens in Ethiopia. (Journal name not provided).”

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This is incomplete.

The Leslie et al. reference also appears poorly matched to the methodology because it is cited in relation to urine specimen collection and susceptibility interpretation, while the listed reference concerns urine testing for nephrolithiasis.

All references should be checked for:

- correct authors,
- article title,
- journal,
- year,
- volume,
- issue,
- pages/article number,
- DOI where available.

18. Key Points / Novel Contributions

The potentially important contributions of the manuscript are:

1. **A dataset of 385 clinically diagnosed UTI patients.**
2. Recovery and characterization of **121 bacterial isolates.**
3. Evaluation of multiple uropathogenic groups.
4. Antimicrobial susceptibility characterization of selected isolates.
5. **MAR-index analysis** of 20 representative isolates.
6. **16S rRNA-based molecular identification** of representative isolates.
7. Integration of phenotypic and molecular approaches.
8. Identification of substantial resistance among the selected isolates.

These are potentially publishable components if the data and methodology are presented rigorously.

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19. Scientific Significance

Potential significance: Moderate

The study has potential significance because antimicrobial resistance in UTI-associated organisms is clinically relevant and local resistance surveillance can contribute to antimicrobial stewardship.

However, the present manuscript does not yet demonstrate a sufficiently strong **epidemiological or molecular resistance contribution** because:

- the patient groups are not adequately quantified,
- susceptibility testing is restricted to 20 isolates,
- resistance genes are not investigated,
- prevalence of individual pathogens is not clearly presented,
- the statistical analysis requires clarification,
- and several interpretations exceed the data.

Therefore, the significance would be considerably strengthened by presenting the complete isolate distribution and resistance data and revising the statistical methodology.

20. Major Revision Required Before Publication

I would ask the authors to address the following **essential points**:

Essential revisions

- 1. Revise the title** and remove "Microbiome" unless genuine microbiome analysis was performed.
- 2. Provide complete demographic information** for all 385 participants.
- 3. Clearly report culture-positive samples versus total samples.**
- 4. Provide organism-wise distribution of all 121 isolates.**

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5. Resolve the numerical discrepancy between Methods and Results regarding countable plates.
6. Clearly state that antimicrobial susceptibility testing was performed on only 20 selected isolates.
7. Provide complete antibiotic susceptibility results, preferably organism-wise.
8. Justify the antibiotic panel for each organism.
9. Update the susceptibility-testing standard and clearly identify the version used.
10. Reconsider the statistical analysis, especially the two ANOVA analyses.
11. Explain the molecular ANOVA groups A–H and the origin of the R^2 value.
12. Provide complete 16S sequencing information and clarify accession numbers.
13. Include a phylogenetic tree with bootstrap values if phylogenetic analysis is claimed.
14. Reduce claims concerning mobile resistance determinants unless resistance genes were actually investigated.
15. Correct the references and verify all citations.
16. Moderate claims such as “definitive confirmation,” “representative,” “clinical severity,” and “robust dataset.”

Reviewer Evaluation Summary

Criterion	Reviewer assessment
Originality	Potentially useful, but novelty is not adequately established
Scientific relevance	Good
Clinical significance	Moderate to good
Sample size	Good initial sample size
Microbiological methodology	Moderate; requires clarification
Molecular component	Potentially useful but incomplete

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Criterion	Reviewer assessment
Antimicrobial-resistance analysis	Potentially valuable but limited to 20 isolates
Statistical analysis	Major concern
Data consistency	Major concern
Presentation	Requires substantial language/editing revision
References	Require correction and verification
Overall scientific quality	Major revision required

Recommendation to the Editor

MAJOR REVISION

Reviewer recommendation: Major Revision

The manuscript addresses an important subject and contains potentially useful microbiological and molecular data. The initial sample size of 385 patients and integration of culture, biochemical, antimicrobial susceptibility and 16S rRNA analyses are strengths. However, significant concerns regarding data consistency, incomplete demographic and organism-distribution data, limited antimicrobial susceptibility testing, antibiotic-panel interpretation, statistical methodology, molecular-data presentation, and overinterpretation currently prevent the manuscript from being considered acceptable in its present form.

If the authors satisfactorily resolve these major methodological and statistical issues, provide complete supporting data, correct the numerical inconsistencies, and substantially revise the interpretation, the manuscript could be reconsidered for publication.

Suggested final reviewer comment

The manuscript has potential merit, particularly because it combines conventional microbiological characterization with molecular identification and antimicrobial-resistance profiling. However, substantial revision is necessary before the scientific validity and reproducibility of the findings can be adequately assessed. The authors should particularly address the inconsistency in reported culture-plate percentages, provide complete demographic and organism-wise data, clarify that antimicrobial

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susceptibility testing was restricted to 20 representative isolates, reconsider the statistical analyses, clarify the 16S rRNA accession information, and avoid conclusions that are not directly supported by the data. Therefore, I recommend Major Revision.