



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	Ms_BMRJ_23733
Title of the Manuscript:	Phenotypic Identification, Plasmid Profiling and Detection of Resistance Genes in Klebsiella sp. from Urine Samples of In- and Out-Patients in Ile-Ife.
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	This is an interesting work on the presence of <i>Klebsiella</i> strains in urine samples, with clear implications human health. The authors use appropriate methodology but some results are not clear or correct, Species assignation for example requires more than the phenotypic tests used. Plasmid profiles are not good and think should be improved. The quality of the plasmid profiles is important because it must be clear, for example, that the largest plasmid band is indeed plasmid DNA. Even if this is so, this would be a too small plasmid to be conjugative and its cell to cell transfer would depend on the presence of a mobilizing plasmid in the same cell. Authors should comment on this.	Identification of isolates was strictly done using Bergey's Manual of Determinative Bacteriology (Breed <i>et al.</i>, 1957) which is widely accepted all over the world. The plasmids detected in the isolates were indeed plasmid DNA. The plasmid DNA were detected using TENS buffer.
Minor REVISION comments	Line 8 mentions 11 Antibiotics while Materials and methods include 12 Lines 9 and 99 The word sterile is not necessary Line 119 Ethidium bromide should be expressed as a final concentration Line 126 An approximate concentration of template DNA should be used instead of a 3 microliter volume. Inside the parenthesis total dna	The number of antibiotics have been corrected to twelve in materials and methods. The word sterile has been deleted from lines 9 and 99. Ethidium bromide has been expressed in final concentration of 10mg/ml . The DNA concentration has been included as 11µg/ml (total DNA of bacteria). The brand of master mix (Solis Biodyne, Estonia) has been included. Identification of the <i>Klebsiella</i> isolates was done



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	should be read instead chromosomal DNA. Indicate what brand or type of master mix was used Line 152 Biochemical tests do not differentiate between Klebsiella species. This s Line 240 Plasmid size is probably underestimated. Lambda/H3 fragments are linear and therefore this is not a good marker to estimate plasmid size, supercoiled DNA should be used as a marker. There is a contradiction between this line and lines 18 and 282. Line 244 The 9416 bp band is present in several strains, this could be chromosomal DNA. Is there an evidence that this a plasmid? It should be easy to differentiate these two types of DNA. Line 245 Plasmid extraction and/or gel quality should be improved. Line 248 What is the negative control? Line 265 Check the position of the lane numbers Line 303 Plate 2b is not necessary Line 368 The putative 9416 base-pair plasmid corresponds probably to chromosomal DNA. In line with this is the lack of correlation between the presence of this band and the antibiotic resistance observed in the strains. Line 379 would improve adding the underlined word “β-lactamases produced by bacteria are	according to Bergey's Manual of Determinative Bacteriology (Breed <i>et al.</i>, 1957). The plasmid size has been correctly estimated. Plasmid DNA was extracted by alkaline lysis using TENS (25 mM Tris, 10 mM EDTA, 0.1 N NaOH and 0.5% SDS) buffer. This contains steps to precipitate out the chromosomal DNA, leaving the plasmids in solution and allowing them to be separated. All corrections on lines 240 to 412 have been effected. The plasmid extraction has been improved. The strains with plasmid size 9416 base pairs had some common antibiotics to which they showed resistance, as observed from the results.
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	known to protect <u>them</u> against...” Line 383 There are several clases of <i>tet</i> genes. Any comments about why was this particular class of <i>tet</i> gene was searched for? Line 386 <u>etc.</u> References should be unifirmed. Line 412 <u>Singapore</u>	Ribosomal protection proteins confer tetracycline resistance by binding to the ribosome, thus preventing drug interaction with its binding site. TetM gene was detected because it mediates resistance by ribosomal protection. Line 412 has been corrected.
<u>Optional/General</u> comments		