Comparing conventional, biochemical and genotypic methods for accurate identification of Klebsiella pneumoniae in Sudan


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Figure s1: Sample processing at the microbiology laboratories for *K. pneumoniae* identification.

*Please note this is performed routinely in the hospitals and not part of the current research project*
250 isolates received from 4 hospitals in Khartoum identified as *K. pneumoniae* as per methods presented in figure s1

All isolates cultured in MacConkey Agar at 37°C for 18-20 hours to confirm by colony morphology, lactose fermenting, and to confirm no contamination of organism

79 isolates selected randomly for API-20

26 confirmed *K. pneumoniae*

Amplification of 16S-23S rDNA internal transcribed spacer (ITS) for all 250 isolates (including those selected for API-20)

No amplicon produced with either primer: other Gram-negative lactose fermenters

Amplicon at 260bp with the Pf/Pr2 primer pair, in addition to a 130bp amplicon with the Pf/Pr1 primer pair

Only produced amplicon with one but not the other primer pair: non-pneumoniae *Klebsiella*

Amplification and sequencing of *rpoB, gapA* and *pgi* housekeeping genes

N = 139 confirmed *K. pneumoniae*
**Method of API 20E/NE:**

The isolates were cultured on MacConky agar at 37°C over-night, followed by suspension of the colonies in sterile normal saline. The solution was used to fill up the strip until the brim. Sterile oil was added to ODC, LDC, ADH, H₂S and urease test, and then the strips incubated over-night in 37°C. The following day one drop of Kovacs reagent was added for the Indole test, one drop of 40 % KOH (VP reagent 1) and one drop of VP Reagent 2 (α-Naphthol) were added for Voges-Proskauer test, and one drop of Ferric Chloride was added for the Tryptophan deaminase test. The results were read by using API Reading Scale (color chart) to determine a positive and negative result for each test, in the chart there are score for each positive test and each three reactions are added together at time to give a seven digital number. Then by using the apiwib (through the 7 digital numbers in to [https://apiweb.biomerieux.com](https://apiweb.biomerieux.com)) the organisms were identified.