**Abstract:**

*Klebsiella species* are one of the major challenges that have been continuously increasing in recent years in human and animals, that causing economic loss and decrease in general health condition. Also, the antibiotic resistance, rapid spreading and high virulence of bacteria is increases the risk level. The aim of this study is to compare the incidence rate of *Klebsiella spp.* by conventional microbiological technique and modern technique (MALDI-TOF). The samples were collected from diseased pet animals (cats and dogs) as pus, respiratory and urinary tracts samples (n=75) while human samples collected from patients that had infection in respiratory and Urinary tracts (n=25) at Qalyubia and Giza Governorates

By conventional techniques, 35/75 isolates of *Klebsiella spp.* were identified from pet animals, (26 from Dogs and 9 from cats) and 14/25 isolates from human samples. On the other hand, application of matrix\_associated laser desorption ionization – time of flight (MALDI-TOF) as a modern technique for identification of *Klebsiella spp.* The results were 29/75 isolates from pet animals sample (16 from Dogs and 4 from cats) and 9/25 isolates from human samples.

The total 49 isolates of *Klebsiella spp.* confirmed by MALDI-TOF gave *Klebsiella pneumoniae* 10/49 (20.4%), *Klebsiella oxytoca* 1/49 (2.04%), *Klebsiella species* 18/49 (36.7%) and 20/49 (40.81%) differentiated other than *Klebsiella species* (*Raoultella ornithinolytica*, *[Enterobacter](https://doi.org/10.1601/nm.3148" \t "xrefwindow" \o "Enterobacter - Click to open Names for Life widget)*cloacae, [*Enterobacter*](https://doi.org/10.1601/nm.3148)species, *Acinetobacter baumanii, Acinetobacter spp. and* [*E.coli*](https://doi.org/10.1601/nm.2553)).

The MALDI-TOF was more effective and reliable in differentiation of *Klebsiella species* than conventional method.

**Keywords:** Klebsiella, Pets, Human, conventional methods, MALDI-TOF.