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Differentiation between conventional methods and MALDI-TOF in Klebsiella species isolated from Pet animals and humans.

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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 increasing 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 cloacae, Enterobacter species, Acinetobacter baumanii, Acinetobacter spp. and E.coli). The MALDI-TOF was more effective and reliable in differentiation of Klebsiella species than conventional method.

1. INTRODUCTION

Klebsiella is Gram negative bacteria, belongs to family *Enterobacteriacea*, worldwide, zoonotic, opportunistic and one of dangerous "ESKAPE" microorganisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter* species, *Acinetobacter baumanii and Pseudomonas aeruginosa*). They called by this name due to skipped and hiding from antibiotics (Marques et al., 2019, Osman et al., 2020, Sherif et al., 2021).

Klebsiella causes many diseases in pet animals as respiratory infection, urinary infection and abscess formation (Wareth et al., 2022).

Also, it is life-threating infection in human causing abscess formation in different body organ, sepsis, injury or surgical wound sites infection, respiratory tract infections, lower biliary tract infection, ankylosing spondylitis, thrombophlebitis, urinary tract infection, cholecystitis, Bacteremia, empyema, osteomyelitis, meningitis, septicemia pleural adhesions which causes increase in death rate up to 50% even with antimicrobial treatment (Rashid and Ebringer, 2007, Ghaith et al., 2020,ElBaradei and Shawky, 2022).

Klebsiella is a facultative anaerobic bacterium, which grows between 35-37 °C and at pH 7.2. Also, the most strains of Klebsiella required carbon sources (citrate and glucose) and nitrogen source (ammonia). Its colony appear as large, mucoid, white color on nutrient agar, mucoid, lactose fermenting (pink) colony on Macconkey's agar, pink to purple without metallic green colony on Eosin Methylene Blue agar, mucoid pink with beta or gamma hemolysis on blood agar, metallic blue on CHROM agar Extended Spectrum Beta-Lactamase, Large, yellow, mucoid on Cystine Lactose Electrolyte Deficient Agar (MacConkey, 1905, Bruce et al., 1981, Hornsey et al., 2013, Rawy et al., 2020).

There are some limitations in availability of accurate conventional microbiological identification methods which depend on enzyme production due to the similarity of Klebsiella biochemical reaction to that of other coliforms. Recently matrix associated laser desorption ionization – time of flight (MALDI-TOF) which is consider as the first-line or primary diagnostic tool for microorganism identification in the last few years due to it can quickly determine the pathogen's species level from a single colony and at the subspecies level in clinical diagnostic laboratories in short time depending on protein determination (Khater et al., 2021).

Klebsiella pneumonia is widely spreading in Egypt in different animal species as pet animals, goat, sheep, and chicken. Also, it is one of most predominant pathogens in human which considered as the third main cause of Hospital Acquired Infections (HAI) (21.8%) (Hamza et al., 2016, Elmonir et al., 2021, Al-Baz et al., 2022).

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So, the current study aimed to identify *Klebsiella spp.* obtained from pet animals and humans in Qalyubia and Giza Governorates by using conventional microbiological technique and MALDI-TOF for accurate confirmation and differentiation between *Klebsiella spp*.

2. MATERIAL AND METHODS

2.1. Sampling:

A total of 100 clinical samples were collected from Qalyubia and Giza Governorates, Egypt at summer season. Seventy-five clinical samples were obtained from diseased pet animals (Dogs n=52, Cats n=23) from urinary tracts, respiratory tracts, and abscesses. Also, 25 clinical human samples were randomly collected from different private laboratories from patients suffered from urinary and respiratory infections (ethical number: BUVFTM 36-10-22) (Table,1).The samples were quickly transported on transport media (peptone water) (Himedia, india) to Microbiology lab, Faculty of Veterinary Medicine Benha University, Egypt under aseptic condition (Rawy et al., 2020).

Table 1 Number and type of samples collected from different species.

Host	Sample No.
Dog	52
Nasal swab	46
Urine	2
Pus	4
Cat	23
Nasal swab	13
Urine	7
Pus	3
Total No.	75
Human	25
Urine	23
Nasal swab	2

2.2. Bacteriological identification of Klebsiella spp. (Rawy et al., 2020):

The inoculated transported media (peptone water) incubated at 37ºC for 24 hr. then streaked on MacConkey's agar plates which incubated at 37°C for 24 hr. The suspected colonies of Klebsiella purified by sub-cultured onto MacConkey's agar plate, Eosin Methylene Blue (EMB) agar and sterile blood agar plate (Himedia, india).

Gram's stain was performed for all suspected isolates which examined by light microscope to determine the morphological characters.

Oxidase test, H_2S production test, Citrate utilization test and urea hydrolysis test (Himedia, india) were applied as a conventional biochemical identification for the suspected klebsiella isolated.

2.3. Application of MALDI-TOF (Khater et al., 2021):

The pure colonies of bacteria were collected by Amies agar gel (Oxoid Limited, Termo Fisher Scientific, Germany) with charcoal transport swabs and sent directly for identification of the microorganism then by using the MALDI-TOF in Friedrich loeffler Institute- Jena- Germany which performed as following: Bacterial swabs were cultured on blood agar media containing 7.5% blood and incubated for 24-48 hours at 37Cº at 5% of CO_2 incubator Keep Single fresh cultured colony in 1.5 ml Eppendorf tube. Each plate's contents were suspended in 300 µL of HPLC-grade water then homogenized by using a vortex. Each tube was filled with 900 µL of pure ethanol, which was then use vortex once more to inactivate the bacteria. Each sample's protein was extracted by using the methods previously discussed. Then by centrifuging at 11,000 xg for 2 minutes; the inactive bacterial pellets were obtained. The pellets were air-dried to eliminate any remaining ethanol before being reconstituted in a mixture of 50 µL of 70% formic acid and 50 µL of acetonitrile. For one minute, the samples were sonicated at 100% amplitude and 1.0 duty cycle. The clear supernatant was recovered after centrifuging the samples at 11,290g for 5 minutes at room temperature and 1 minute on ice. Each supernatant was found in one Liter. One L of saturated -cyano-4-hydroxycinnamic acid matrix solution (in 50% acetonitrile and 0. 25% trifluoroacetic acid) was then applied on top of the air-dried MALDI target (MSP 96 target polished steel (MicroScout Target) plate; Bruker Daltonik, Bremen, Germany). A Microfex LT (Bruker Daltonics, Bremen, Germany) device and MBT Compass Explorer 4.1 software were used to conduct the MALDI measurements. The log score value of 0–3 recommended by the MALDI Biotyper manufacturer for species identification was followed. Only bacterial species with score values of 2.300 or higher were deemed to be correctly identified by MS MALDI-TOF. The analysis excluded isolates identified with score values less than 2.300.

3. RESULTS

3.1. Incidence rate of Klebsiella isolated from pets and human:

Out of 100 clinical samples, 49 *Klebsiella isolates* (35/75 pets' samples and 14/25 human samples) (Table 2).

3.2. Bacteriological identification results:

The suspected colonies showed the characteristic properties of Klebsiella includes Gram-Negative bacilli, Capsulated, non-motile, appears with turbidity of sedimentation on peptone water, Mucoid pink colony on Macconkey's agar, pink to purple without metallic green colony on EMB agar, mucoid pink with beta or gamma hemolysis on blood agar. The 49 isolates showed positive result with Urea hydrolysis test and Citrate Utilization test and negative result with Oxidase test and H_2S production test (Table 2).

3.3. MALDI-TOF technique:

The *klebsiella isolates* obtained by conventional methods were confirmed by MALDI-TOF for identification and classification according to species level. The results showed that 29/49 isolates of *Klebsiella spp.* were classified to 10 isolates (20.4%) with score range from 2.300 to 2.460 were *Klebsiella pneumonia*, 1 isolate (1.3%) with score 2.330 was *Klebsiella oxytoca*, 18 isolates (36.7%) with score range 2.070 to 2.280 were other different *Klebsiella spp.* and the remain 20 isolates (40.81%) were other spp. Than Klebsiella as: *Raoultella ornithinolytica*, *Enterobacter cloacae*, *Enterobacter spp.*, *Acinetobacter baumannii*, *Acinetobacter spp.* and *E.coli*. Also, one isolate (2.04%) could not identify (Table 3).

Table 2 Incidence rate of K. spp. from different clinical samples.

Host	Sample No.	Conventional method		MALDI-TOF	
		No. of *K. isolate*	%	No. of K. isolate	%
Dog	52	26	50	16	30.8
Nasal swab	46	23	44.2	14	26.9
Urine	2	2	3.8	1	1.9
Pus	4	1	1.9	1	1.9
Cat	23	9	39.1	4	17.4
Nasal swab	13	8	34.8	4	17.4
Urine	7	1	4.3	0	0
Pus	3	0	0	0	0
Total No.	75	35	-	20	-
Human	25	14	56	9	36
Urine	23	12	48	7	28
Nasal swab	2	2	8	2	8

% According to sample number of each species

Table 3 MALDI-TOF results of bacterial isolates.

Tested Microorganism	Number	%
Klebsiella pneumonia	10	20.4
Klebsiella oxytoca	1	2.04
Klebsiella spp	18	36.7
Raoultella ornithinolytica	1	2.04
Enterobacter cloacae	5	10.2
Enterobacter spp.	2	4.08
Acinetobacter baumannii	1	2.04
Acinetobacter spp.	2	4.08
E. coli	8	16.3
Not identified	1	2.04
Total *Klebsiella spp.*	29	59.2
Total others spp.	20	40.8

% According to the examined isolates (n=49)

4. DISCUSSION

Klebsiella (Gram-negative bacteria, zoonotic, opportunistic) causes several illnesses in pet animals as abscess formation, urinary infection, and lung infection and causing fatal infection in humans that raises the fatality rate (ElBaradei and Shawky, 2022, Wareth et al., 2022)

Our current results revealed that the isolation rate of Klebsiella was 46.66% (35/75) from pet animals samples (urine, pus, nasal swabs) at summer season. These results were in accordance with Marques et al., (2019) in Portugal which was (38.9%)

On the other hand, Klaper et al., (2021) who recorded higher incidence rate (61.7%) in Germany where the cold weather comparing to Egypt while other authors as Khalil et al., (2020) (20.8%) in Egypt, Banerjee et al., (2020) (22.9%) in India and Zhang et al.,(2022) (2.3%) in china where disagreed our results due to the difference of environmental condition and the types of samples collection.

In human, the incidence rate of *Klebsiella isolates* in current study was (14/25) 56%. Our results were in accordance with Osman et al., (2020) (55.6%) in Sudan and Abdelkader et al., (2022) (51.5%) in Egypt, while the current incidence rate was lower than obtained by Marques et al., (2019) (37.5%) in Portugal which have cold weather comparing to Egypt, the Yang et al., (2021) (22.9%) in china which collected the samples from (blood), but in Egypt Mohamed et al., (2017) (37%), Al-Baz et al., (2022) (21.8%) Elmonir et al., (2021) (14.4%) and Ahmed et al., (2022) (12.2%) which collected in different seasons and from different sample sources.

For the best of our knowledge, there are no related MALDI-TOF result in Egypt due to MALDI-TOF is highly expensive technique and the technique apparatus unavailable but, present in Egypt only in 57357 hospital which used for diagnosis of children cancer.

However, in the other countries, it is now widely considered one of first choice in routine diagnosis for pathogen identification due to it is more accurate in identifying microorganisms from pure colonies, reliable, requires low reagent costs and rapid technique comparing with conventional methods which based on culture and biochemical tests. So it will reduce time spent and the cost of consumables on other conventional diagnostics techniques (Khater et al., 2021).

By application of MALDI-TOF gave 29 Klebsiella isolates which were 16 from Dogs, 4 from cats and 9 from humans which classified to *Klebsiella pneumonia, Klebsiella oxytoa* and other *Klebsiella spp., but the remain examined isolates give Raoultella ornithinolytica, Enterobacter cloacae, Enterobacter spp., Acinetobacter baumannii , Acinetobacter spp.* and *E.coli.* On the opposite side by conventional methods obtained 26 *Klebsiella isolates* from Dogs, 9 from cats and 14 from humans. That indicate the MALDITOF is more reliable due to it is accurate in diagnosis and identification with time and effort saving than conventional methods**.**

5. CONCLUSION

The current study concluded that, the incidence of klebsiella depend on hygienic condition of environment around animals and humans. Also, traditional diagnostic methods affected by accuracy of microbiologist, type of used media and number of biochemical tests to more confirmation. Finally, the traditional diagnostic methods were replaced by MALDI-TOF due to it is a comparatively simple, effective technique and all known microorganisms databases containing spectra are existing. Also, can overcome many challenges in microorganism identification which allow the physicians and veterinarian to apply rapid and effective antimicrobials to the patient in an optimized time.

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