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PHENOTYPIC CHARACTERIZATION OF *K. PNEUMONIAE* FROM ANIMAL AND HUMAN CLINICAL SAMPLES; POTENTIAL HEALTH HAZARD

By

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ABSTRACT

A total of 1511 samples were aseptically collected from diseased human suffering urinary tract infection (UTI), respiratory tract infection (RTI) [blood (190), sputum (300), urine (637), wound (360) and pus (24) , also 640 samples were aseptically collected from sick infant in intensive care unit (ICU) [blood (216), pharyngeal swab (72) and mechanical ventilator (52) from Neonatal unit , while animals samples were 265 milk samples from mastitic animals [cows (120), buffaloes (120) and goats (25), also 41 nasal passage samples were collected from dead chick, 42 lung and liver tissue samples were collected from infected sheep and 230 bone samples were aseptically collected from clinically infected dog. The results revealed that *K. pneumoniae* was isolated in 87 (6%) out of (1511) human patients, blood samples were11 (6%), sputum samples 13 (4%), urine samples were 36 (6%) wound samples 24 (7%) and pus samples were 3 (12.5%) from isolates that confirmed *K. pneumoniae.* The number of *K. pneumoniae* isolates from milk sample was 5 (1.9%) out of (265) from isolates were *K. pneumonia , K. pneumoniae* were isolated in111 (33%) out of 640 infant samples, blood samples were 87(40%), pharyngeal swab21 (29%), mechanical ventilator 3 (6%) from isolates that confirmed *K. pneumoniae, K. pneumoniae* were isolated in 8 (19.5%) out of (41) chicken nasal passage samples ,1(2.4%) out of 42 sheep lung and liver tissue samples , 5(2%) out of 230 dog bone marrow samples and 3(33%) out of rabbit nasal swab samples.

INTRODUCTION

Klebsiella pneumoniae is considered as a saprophyte in humans and other mammals, colonizing the gastrointestinal tract, skin, and nasopharynx is a member of the *Klebsiella* genus of *Enterobacteriaceae. K. Pneumoniae* is found in the environment and as a harmless commensally, but is also a frequent nosocomial pathogen causing urinary, respiratory and blood infections **(Tzouvelekis *et al.*, 2012**). The emergence of *K.pneumoniae* as a nosocomial pathogen in the US and Europe may be due in part to the acquisition of antibiotic resistance markers providing a selective advantage in hospital settings, and it is responsible for 6-17% of urinary tract infection (UTI's), 7-14% of pneumonia, 4-15% of septicemia, 2 - 4% of wound infections, 4-17nosocomial infections in intensive care units, and 3-20 % of all neonatal septicemia cases **(Fodah *et al*., 2014).** Among Enterobacteriaceae, *Klebsiella pneumoniae* has been identified as the most important pathogens causing drug-resistant infections in hospital setting, especially in intensive care unit. Recent investigation **(Popy *et al.*, 2011**) in Bangladesh showed that, the prevalence of 6.03% *Klebsiella* sp. was present in both trachea (n=50) and nasal sinuses (n=50) of dead chickens. Thus, it is revealed that, the *klebsiella* cause loss to the farmers. So, the present investigation has been undertaken to estimate the prevalence of the *K. pneumoniae* in clinical infection of animals and human.

MATERIAL AND METHODS

Samples were transported to the laboratory in cool conditions and processed within two hours of collection.

Collection of human samples:

One thousand five hundred and eleven samples from patients suffered from urinary tract infection and respiratory tract were collected during the study period from (October/2015 - April/2016). In addition to three hundred and forty samples from infants in intensive care unit.

Collection of animal samples:

Five hundred eighty and seven samples were collected randomly from different species with apparently healthy and non-healthy, of different age and sex, and were collected during the study period from (October /2015 May / 2016).

Isolation of *Klebsiella pneumoniae:*

All sample were immediately streaked on to MacConkey, and incubated at 37˚C for 24hours., and streaked again on Brilliant Green Agar and Xylose lysine Deoxycholate Agar (XLD) and

incubated at37˚C for 24hr., and streaked again on Hi chrom uti agar this medium is selective which used for the identification of *K. pneumoniae* **(Morelloc *et al.,* 2006**). Then confirm the isolates by using biochemical tests and API 20 E system.

RESULTS

The results from (Table 1) to 7 revealed that *K. pneumoniae* were isolated in 87 (6%) out of (1511) human patients, blood samples were11 (6%), sputum samples 13 (4%), urine samples were 36 (6%) wound samples 24 (7%) and pus samples were 3 (12.5%) from isolates that confirmed *K. pneumoniae.* The number of *K. pneumoniae* isolates from milk sample were 5 (1.9%) out of (265) from isolates were *K. pneumoniae, K. pneumoniae* were isolated in111 (33%) out of 640 infant samples, blood samples were 87 (40%), pharyngeal swab21 (29%), mechanical ventilator 3 (6%) from isolates that confirmed *K.pneumoniae, K. pneumoniae* were isolated in 8 (19.5%) out of (41) chicken nasal passage samples,1(2.4%) out of 42 sheep lung and liver tissue samples ,5 (2%) out of 230 dog bone marrow samples and 3 (33%) out of rabbit nasal swab samples .

DISCUSSION

This results show that, the rate of infection in a neonatal intensive Care unit were 32.6% (Pharyngeal swab 29%, vent 6% and blood 40 %), human 6.2 %, this low rate about **Moore *et al* 2005** may because the duration of isolation Of sample occurs in cold weather from (November / 2015 - April / 2016) this is agreement with, **Khan *et al* ., (2016)** who recorded that, the prevalence rate of *K. pneumoniae* was 1.6times higher during the 4 warmest months of the year as compared to the rest of the year. Data suggest that rates of *K. pneumoniae* infection were associated with changes in temperature and humidity. **Khan *et al*., (2016)** also observed that there is Seasonal Variation in *K. pneumoniae* Blood Stream Infection: for the observed higher rates of *K. pneumoniae* during warm months remains elusive. Finally, *K. pneumoniae* survives better at higher humidity, as experimental models have shown that dehydration is an important factor in inactivating the organism, also **Anderson *et al* ., (2007)** that showed the rate of *K. Pneumoniae* BSI and other were 1.5 times higher during the 4 warmest months of the year. The present result approached to that mentioned by **(Abadullah and Zghair 2016**), their results showed that, the rate of infection in urine Samples of humane were 2%, this study show that, the rate of infection in urine samples of human were 6%. The raising systems, the hygiene and the implementation of

control programs are responsible for the great variability in the obtained results, *K. pneumoniae* isolated from milk leading to losses in milk production and quality, as well as to public health problems due to the presence of these agents in milk for consumption. Another significant aspect for public health is the possible occurrence of strains that are multi-resistant to antimicrobials administered for both animals and humans. The proportional occurrence of bacteria in trachea (n = 50) and nasal sinuses (n = 50) of dead chickens was *Klebsiella* sp. (6.0%) **(Popy *et al.* 2011)**. Thirty nasal passage swabs from 30 dead birds (20 from SK Veterinary Diagnostic Centre (SKVDC) and 10 from the Department of Pathology (n=30) of dead chickens. The prevalence of *Klebsiella* was 8.69% (2 *Klebsiella* spp. in 23 isolates from 30 nasal passage swabs) in the study of **(Hossain *et al*., 2012**) which was higher that, the values reported by **(Popy *et al*., 2011)** in Bangladesh, In this study the prevalence of *Klebsiella* spp. was less the present work, the proportional occurrence of *klebsiella* spp. in tracheal swab (n=36) of dead chickens was *klebsiella pneumoniae* 8 (22%) and *klebsiella oxytoca* 2 (5.6%) which is higher than the values reported by **(Popy *et al.* 2011)** in Bangladesh and **(Hossain *et al*., 2012)**. Also **Khalda *et al*. (2000)** found that, the isolation rate was (10.2%), while **(Dashe *et al*., 2013)** mentioned that, the isolation rate was (8.8%) and **Dashe *et al*. (2008)** recorded lower isolation rate of *Klebsiella pneumoniae* than our study. Also lower prevalence rate was recorded by **Hajieh (2008)** results who isolated *klebsiella* form (1%) only of samples tested, **Dashe *et al*., (2008)** detected *klebsiella* in (1.5%) of samples **(Aher *et al*., 2012**) isolated *klebsiella* in (6.5%) of collected samples. But the result received by **(Fielding *et al*., 2010), and Rajaa, *et al*., (2011)** who recorded higher isolation rate of *Klebsiella pneumoniae* (40.4), **Botchris *et al*., (2012).** *Klebsiella* isolated associated with bile and intestinal content of slaughtered chickens (63%) of samples. The overall proportion of *Klebsiella* spp. (6%) was lower than from other authors **(Ibrahim *et al*., 2004; Trkyilmaz. 2005).** This might be due to age and breeds of the chickens, geographic variation and management, vaccination and nutrition.

CONCLUSION

Klebsiella pneumoniae considered as a member of *Enterobacteriaceae* with important clinical problems and potential public health hazard.

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Table (1): Results of the examined human samples.

Hospitals	Samples					
	Blood	Sputum	Urine	Wound	Pus	
Al Kaser Al Aini Hospital	80	100	440	200	24	844
Naser Institute Hospital	40	93	127	100	-	360
New Al Kaser Al Aini teaching Hospital	70	107	70	60	-	307
Total	190	300	637	360	24	1511
No. of *K. pneumoniae* Isolates	11 (6%)	13 (4%)	36 (6%)	24 (7%)	3 (12.5%)	87(6%)

Table (2): Results of the examined milk samples.

Health condition of the Animals	Animals			
	Cow	Buffaloes	Goat	
Sub clinically Mastitic Animals	8	25	25	58
Clinically Mastitic Animals	112	95	-	207
Total	120	120	25	265
No. of *K. pneumoniae* isolated (%)	3	1	1	5 (1.9%)

Table (3): Results of the examined infant samples.

Hospitals	Samples			
	Blood	Pharyngeal swab	Vent	
Neonatal Unit at Al - Kaser Al -Aini Hospital	90	-	-	90
Neonatal Unit at Naser Institute Hospital	-	30	12	42
Abo Al - Rish Pediatric Hospital	126	42	40	208
Total Samples	216	72	52	340
No. of *K. pneumoniae* Isolates	87 (40%)	21 (29%)	3 (6%)	111 (33%)

Table (4): Results of the examined chicken samples.

Health condition of the Animals			
Apparently Healthy poultry	**Giza**	**Behira**	
Clinically Infected poultry	**-**	**-**	**-**
Total no. of isolated bacteria	**28**	**13**	**41**
No. of isolated *K. pneumoniae* (%)	**9**	**1**	**10 (24.3%)**
No. of isolated *K. oxytoca* (%)	**8**	**-**	**8 (19.5%)**
Apparently Healthy poultry	**1**	**1**	**2 (4.8%)**

Table (5): Results of the examined sheep samples.

Origin of samples	**No. of. Samples**	**No. of. *K. pneumoniae* isolates**	**(%)**
Lung and liver sheep samples	**42**	**1**	**2.4**

Table (6): Results of the examined dog samples.

Origin of. samples	**No. of. Samples**	**No. of. *K. pneumoniae* isolates**	**(%)**
Dog bone marrow samples	**230**	**5**	**2%**

Table (7): Results of the examined rabbit samples.

Origin of samples	**No. of samples**	**No. of *K. pneumoniae* isolates**	**(%)**
Rabbit nasal swab samples	**9**	**3**	**33%**