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STUDY OF SOME AEROBIC BACTERIAL CAUSES OF RESPIRATORY AFFECTION IN SLAUGHTERED CAMELS IN DAKAHLIA GOVERNORATE

(With 5 Tables)

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**دراسة عن بعض المسببات البكتيرية لإصابة الجهاز التنفسي
في الجمال المذبوحة بمحافظة الدقهلية**

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أجريت هذه الدراسة علي عدد ٨٥ جمل مذبوح في سلخانات محافظة الدقهلية وإشتملت الدراسة علي ٢٥٥ عينة (٨٥ عينة من كل من الرئة والغدد الليمفاوية ومسحات من القصبة الهوائية) لفحصها بكتريولوجياً. أظهر الفحص الظاهري عن وجود إصابات تنفسية في عدد ٢٥ جمل والباقي سليم ظاهرياً. وقد أظهرت نتائج الفحص البكتريولوجي عن إيجابية ١١٨ (٤٦,٢٧%) للعزل البكتيري متمثلة في ٥٢ (٢٨,٨٨%) و ٦٦ (٨٨%) من العينات السليمة ظاهرياً والمصابة علي التوالي وأن ٩٣ (٧٨,٨١%) كانت إيجابية للعزل الفردي و ١٤ (١١,٨٧%) من هذه العينات كانت ثنائية العزل و ١١ (٩,٣٢%) ثلاثية العزل وكانت العدوي المختلطة في العينات المريضة فقط. وتم عزل ١٥٤ عترة بكتيرية صنفت إلي ٩٠ (٥٨,٤٤%) موجبة لصبغة الجرام و ٦٤ (٤١,٥٦%) سالبة الجرام. وتم عزل كل من الميكروب العنقودي الذهبي وميكروب الكوريني باكتريم الصيدي والإيكولاي ٢٢ (١٨,٦٤%) وكل من الإستربتوكوكس الصيدي وإستافيلوكوكس إيبيرميس والكليبيلا نيموني ١٦ (١٣,٥٥%) والإستربتوكوكس نيموني ١٤ (١١,٨٦%) والباستيرلا ماليتوسيدا ١٠ (٨,٤٧%) والسيدوموناس إيروجينوزا ٨ (٦,٧٨%) والباستيرلا هيموليتيكا ٦ (٥,٠٨%) والبروتيس فالجاريس ٢ (١,٦٩%). وتم إجراء اختبار الأمراض لمعزولات الباستيرلا ماليتوسيدا ووجدت جميع المعزولات من النوع الممرض. وتم عمل اختبار حساسية للمعزولات ووجد أن معظم العترات البكتيرية المعزولة كانت شديدة الحساسية لكل من الإنترفلوكسامين والجنتاميسين والريمكتان ومقاومة لكل من الإستربتومايسين والأمبليين.

SUMMARY

This study was carried out on 85 slaughtered camels in Dakahlia abattoir. 255 samples (85 each of lung, lymph nodes and tracheal swabs) for bacteriological examination. The clinical examination proved that 25

camels have respiratory disorder, the remaining were apparently normal. Bacteriological examination revealed that 118 (46.27%) of the examined samples were positive for bacterial isolates, distributed as 52 (28.88%) and 66 (88%) of apparently normal and diseased animals respectively. 154 bacterial isolates could be detected and classified into 90 (58.44%) Gram-positive and 64 (41.56%) Gram-negative bacteria. The main bacterial isolates were *Staph. aureus*, *Corynebacterium pyogenes* and *E. coli* 22 (18.64%) for each, *Strept. pyogenes*, *Staph. epidermidis* and *Kleb. pneumoniae* 16 (13.55%) for each, *Strept. pneumoniae* 14 (11.86%), *Past. multocida* 10 (8.47%), *Pseudomonas aureginosa* 8 (6.78%), *Past. haemolytica* 6 (5.08%) and *Proteus vulgaris* 2 (1.69%). The pathogenicity test for *Past. multocida* isolates indicated that all isolates were pathogenic. Sensitivity test for the isolated bacteria revealed that most of isolates were highly sensitive to Enrofloxacin, Gentamycin and Rimactan and resistant to Streptomycin and Ampicillin.

Key words: Bacterial causes, respiratory affection, camels.

INTRODUCTION

The camel plays vital socioeconomic roles and supports the survival of millions of people in Asia and Africa. It is being used as a source of protein, milk, hide as well as quite and effective mean of transport.

Respiratory diseases of camels continue to be a major cause of economic loss and adverse on animal. Stress of cold weather, rain, bad hygiene and high humidity rate were incriminated to increase the respiratory infection (bacterial, viral and parasitic). Respiratory affection is the main cause of death among camel calves all over the world (Chowdhary, 1986 and Khanna *et al.*, 1992).

Bacterial infection of the lung is one of the main causes of pneumonia in camels (Rana *et al.*, 1993; Thabet, 1993; Alhendi, 2000 and Seddek, 2002).

Several species of organisms could be isolated from both apparently healthy and affected respiratory tract of camel as *Staphylococci*, *Streptococci*, *Corynebacterium*, *E. coli*, *Pasteurella* and *Klebsiella* (El-Mossalami and Ghawi, 1983; Chauhan *et al.*, 1987; Gobrial *et al.*, 1991; Rana *et al.*, 1993; Fatma *et al.*, 2001 and Seddek, 2002). *Pasteurella* species were responsible for acute form of respiratory infection (Arora and Kalara, 1973), while the recovery of *Pseudomonas*

aeruginosa should be considered as an important finding because this organism was considered to be extremely important in veterinary clinical medicine (Hirsh and Zee, 1999).

Hence, the present work aimed to investigate the bacterial cause of respiratory affection in camels in Dakahlia Governorate and In-vitro antibiotic sensitivity against the isolated strains.

MATERIALS and METHODS

Samples:

A total of 255 samples including 85 each of tracheal swabs, lungs and bronchial lymph nodes tissue were collected under aseptic conditions from 85 slaughtered camels (60 apparently healthy and 25 diseased) from different abattoirs in Dakahlia Governorate. All samples were transported as quickly as possible to the laboratory on ice box for bacteriological examination.

Media:

- * Nutrient broth (Oxoid, CM1).
- * Blood agar media (Nutrient agar base Oxoid CM3 + 5 – 10% defibrinated sheep blood).
- * DSA medium (crystal violet- cobalt agar).
- * MacConkey bile salt lactose agar medium (Oxoid, CM7).
- * Mannitol salt agar medium (Oxoid, CM 85).
- * Mueller-Hinton agar (Oxoid, CM 337).

Bacteriological examination:

Each sample was cultured into nutrient broth and aerobically incubated at 37°C for 24 hours. A loopful was taken and cultured onto each of the following solid media, Blood agar; MacConkey agar; Mannitol salt agar and DAS medium. After incubation aerobically at 37°C for 24 – 48 hours, single colonies were picked up, purified onto nutrient agar slants, for identification morphologically, culturally and biochemically according to Koneman *et al.*, (1997); Hirsh and Zee (1999) and Quinn *et al.*, (2002).

Pathogenicity and virulence of isolated *Past. multocida* (Wessman, 1964):

Three Swiss Webster white mice of 18 – 22 grams were used for each isolate, the mice was injected intraperitoneally by 0.1ml of bacterial suspension (1.5×10^8 organism per ml.). All dead mice showed post mortem changes. Reisolation of inoculated strains from heart blood of

dead mice was carried out, the prepared blood films were stained with Leshiman's stain showed the characteristic features of *P. multocida* organisms.

In vitro antibiotic sensitivity test:

The disc diffusion technique was performed on isolated bacteria from infected cases according to Finegold and Martin (1982). Ten chemotherapeutic disks kindly supplied by Oxoid and namely Ampicillin, Enrofloxacin, Gentamycin, Erythromycin, Chloramphenicol, Oxytetracycline, Rimactan, Streptomycin, Penicillin and Trimethoprim-sulphamethoxazole. The degree of sensitivity was determined and interpreted according to Oxoid Manual, (1998).

RESULTS

Table 1: Incidence of positive bacterial isolates from respiratory tract of slaughtered camel samples

Types of samples	Conditions						Total		
	Apparently healthy			Diseased			No.	Positive	
	No.	Positive		No.	Positive			No.	Positive
		No.	%		No.	No.	%		No.
Tracheal swabs	60	24	40.0	25	22	88.0	85	46	54.11
Lung	60	16	26.66	25	24	96.0	85	40	47.06
Lymph nodes	60	12	20.0	25	20	80.0	85	32	37.65
Total	180	52	28.88	75	66	88.0	255	118	46.27

Table 2: Incidence of bacterial isolates culture of respiratory tract affection of slaughtered camel samples

Item	No.	%
Total samples	255	
Positive samples	118	46.27
Samples with single isolates	93	78.81
Samples with Mixed isolates	25	21.19
Gram positive isolates	90	58.44
Gram-negative isolates	64	41.56

Table 3: Incidence percentages and frequency distribution of bacterial isolates in examined respiratory tract of slaughtered camel samples

Types of isolates	Total No. of isolates		Tracheal swabs samples						Lung samples						Lymph nodes samples						Overall percentages (255 samples)		
			App. Normal (60 samples)			Diseased (25 samples)			App. Normal (60 samples)			Diseased (25 samples)			App. Normal (60 samples)			Diseased (25 samples)			Total No.	Total Inc. %	Total freq. %
	No.	%*	No. of isol.	Inc. %	Freq. %	No. of isol.	Inc. %	Freq. %	No. of isol.	Inc. %	Freq. %	No. of isol.	Inc. %	Freq. %	No. of isol.	Inc. %	Freq. %	No. of isol.	Inc. %	Freq. %			
<i>Streptococcus pyogenes</i>	16	13.55	2	8.33	3.33	2	9.09	8	1	6.25	1.66	1	4.16	4	3	25	5	3	15	12	12	10.17	4.70
<i>Streptococcus pneumoniae</i>	14	11.86	2	8.33	3.33	2	9.09	8	1	6.25	1.66	1	4.16	4	1	8.33	1.66	1	5	4	8	6.78	3.14
<i>Staphylococcus aureus</i>	22	18.64	1	4.16	1.66	2	9.09	8	1	6.25	1.66	2	8.33	8	-	-	-	1	5	4	7	5.93	2.75
<i>Staphylococcus epidermidis</i>	16	13.55	8	33.33	13.33	1	4.55	4	4	25.0	6.66	-	-	-	3	25	5	-	-	-	16	13.55	6.27
<i>Corynebacterium pyogenes</i>	22	18.64	2	8.33	3.33	4	18.19	16	-	-	-	2	8.33	8	-	-	-	3	15	12	11	9.33	4.31
<i>Pasteurella multocida</i>	10	8.47	-	-	-	1	4.55	4	2	12.5	3.33	2	8.33	8	2	16.66	3.33	3	15	12	10	8.47	3.92
<i>Pasteurella haemolytica</i>	6	5.08	-	-	-	-	-	-	-	-	-	-	-	-	1	8.33	1.66	-	-	-	1	0.85	0.39
<i>E. coli</i>	22	18.64	4	16.67	6.66	1	4.55	4	2	12.5	3.33	1	4.16	4	2	16.66	3.33	1	5	4	11	9.33	4.31
<i>Klebsiella pneumoniae</i>	16	13.55	3	12.5	5.0	3	13.65	12	4	25.0	6.66	-	-	-	-	-	-	1	5	4	11	9.33	4.31
<i>Pseudomonas aeruginosa</i>	8	6.78	1	4.17	1.66	2	9.09	8	-	-	-	1	4.16	4	-	-	-	-	-	-	4	3.39	1.56
<i>Proteus vulgaris</i>	2	1.69	1	4.17	1.66	-	-	-	1	6.25	1.66	-	-	-	-	-	-	-	-	-	2	1.69	0.78
<i>Staph. aureus + C. pyogenes</i>	-	-	-	-	-	1	4.55	4	-	-	-	2	8.33	8	-	-	-	2	10	8	5	4.23	1.96
<i>Staph. aureus + Strept. Pyogenes</i>	-	-	-	-	-	1	4.55	4	-	-	-	3	12.5	12	-	-	-	-	-	-	4	3.39	1.56
<i>Staph. aureus + K. pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	-	1	4.16	4	-	-	-	-	-	-	1	0.85	0.39
<i>Strept. Pneumoniae + C. pyogenes</i>	-	-	-	-	-	1	4.55	4	-	-	-	2	8.33	8	-	-	-	1	5	4	4	3.39	1.56
<i>E.coli + K. pneumoniae + P. aureginosa</i>	-	-	-	-	-	1	4.55	4	-	-	-	2	8.33	8	-	-	-	1	5	4	4	3.39	1.56
<i>E.coli + Past. haemolytica +Staph. Aureus</i>	-	-	-	-	-	-	-	-	-	-	-	3	12.5	12	-	-	-	2	10	8	5	4.23	1.96
<i>E.coli + C. pyogenes +Strept. Pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	-	1	4.16	4	-	-	-	1	5	4	2	1.69	0.78
Total	154		24			22			16			24			12			20			118		

* The percentage was calculated according to the number of positive samples (118).

Table 4: Pathogenicity of isolated *Pasteurella multocida* in mice

No. of isolates	No. of inoculated mice	Time of death			
		Less than 24hr.	24 hours	48 hours	72 hours
10	30	10	16	4	0.0

Table 5: Antibiogram of the isolated microorganisms recovered from examined samples

Antimicrobial agent and its potency	Organisms	<i>Strept. Species</i> (N = 30)		<i>Staph. Species</i> (N = 38)		<i>C. pyogenes</i> (N = 22)		<i>Pasteurella species</i> (N = 16)		<i>E. coli</i> (N = 22)		<i>K. pneumoniae</i> (N = 16)		<i>Ps. aeruginosa</i> (N = 8)	
		Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %
Ampicillin	10ug	18	60	12	31.58	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0
Enrofloxacin	5ug	28	93.33	35	92.10	16	72.73	14	87.50	22	100	16	100	6	75.0
Erythromycin	15ug	5	16.66	34	89.47	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0
Gentamycin	10ug	24	80.0	36	94.74	20	90.90	2	12.50	20	90.90	14	87.50	4	50.0
Oxytetracyclin	30ug	3	10.0	6	15.79	6	27.27	8	50.00	-	0.0	2	12.50	4	50.0
Streptomycin	10ug	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0	-	0.0
Rimactan	30ug	26	86.66	30	78.95	16	72.73	12	75.00	18	81.81	6	37.50	6	75.0
Penicillin	10ug	-	0.0	12	31.58	12	54.54	-	0.0	-	0.0	-	0.0	2	25.0
Chloramphenicol	30ug	-	0.0	30	78.95	12	54.54	8	50.00	16	72.73	-	0.0	2	25.0
Trimethoprim-sulph-methoxazole	1.25, 23.75ug	-	0.0	4	10.53	4	18.18	-	0.0	18	81.81	3	18.75	-	0.0

DISCUSSION

Bacterial infection of the respiratory tract of camels represent important problems confronting animal production. The present study deals with the pathogenic bacteria present in the respiratory tract of slaughtered camels.

Bacteriological investigation of the respiratory tract samples (tracheal swabs, lung, bronchial lymph node), Table (1) revealed that 52 (28.88%) of 180 samples and 66 (88%) of 75 samples collected from apparently normal and diseased slaughtered camels respectively. Out of 118 positive samples, 93 (78.81%) were found having single infection while 25 (21.19%) cases having mixed infection.

Finding of bacteriological investigation of 85 tracheal swabs revealed that 46 (46.27%) samples were positive for bacterial infection, Table (1) showed that 60 of tracheal swabs collected from apparently healthy slaughtered camels including 24 cases were positive to bacteriological examination with the percentage of 40%, while 22 from 25 diseased camels were positive with the percentage of 88%.

The data present in Table (1) showed that the positive bacteriological examination of lung samples were 40 (47.06%), which were distributed in apparently normal and diseased slaughtered camels as 16 (26.66%) and 24 (96%) respectively. Also lymph nodes samples revealed that 32 out of 85 samples were positive with the percentage of 37.65%, where it was distributed as 12 (20%) and 20 (80%) in apparently normal and diseased samples respectively.

These results indicated that the respiratory tract of apparently normal animals acted as a reservoir for many species of pathogenic and potential pathogenic microorganisms. Stress factors such as changes in the hygienic, environmental and climatic conditions play a role in the onset of pneumonia (Buxton and Fraser, 1977). This concept was supported in the present study by the fact that a number of bacteria was isolated from 52 cases which showed no pathological lesions (Table 1).

A total number of 154 bacterial isolates recovered from examined samples, were identified as 90 (58.44%) Gram-positive organisms and 64 (41.56%) Gram-negative, (Table 2). These results nearly similar with those reported by Fatma *et al.*, (2001) and Seddek, (2002), while in disagreement with Al-Doughamyl *et al.*, (1999) who recorded 82.4% Gram-positive, 15% Gram-negative and 2.6% mixed.

The obtained results (Table 3) revealed that a wide variety of pathogenic and potentially pathogenic bacterial isolates from examined

samples with variable incidence and frequency percentages of major animal and public health significance. These organisms were as follows: *Streptococcus pyogenes* 16 (13.56%); *Streptococcus pneumoniae* 14 (11.86%), *Staphylococcus aureus* 22 (18.63%), *Staphylococcus epidermidis* 16 (13.56%); *Corynebacterium pyogenes* 22 (18.63%); *Pasteurella multocida* 10 (8.47%); *Past. haemolytica* 6 (5.08%); *E. coli* 22 (18.63%); *Klebsiella pneumoniae* 16 (13.56%); *Pseudomonas aeruginosa* 8 (6.78%) and *Proteus vulgaris* 2 (1.69%). These organisms were distributed as single isolate with incidence 12 (10.17%), 8 (6.78%), 7 (5.93%), 16 (13.56%), 11 (9.33%), 10 (8.47%), 1 (0.85%), 11 (9.33%), 11 (9.33%), 4 (3.39%) and 2 (1.69%) respectively. These results nearly similar with those reported by Mahmoud *et al.*, (1988); Rana *et al.*, (1993) and Seddek, (2002).

The obtained results (Table 3) revealed that the mixed isolates were the most predominate in the diseased examined samples. In mixed infection *E. coli* was isolated with *K. pneumoniae* and *Ps. aeruginosa* from 4 samples with incidence 3.39%, *E. coli* with *Past. haemolytica* and *Staph. aureus* from 5 samples with incidence 4.23% and *E. coli* with *C. pyogenes* and *Strept. pneumoniae* from 2 samples with incidence 1.69%. Another combination between *Staph. aureus* and each of *C. pyogenes*, *Strept. Pyogenes* and *Klebsiella pneumoniae* with an incidence 5 (4.23%), 4 (3.39%) and 1 (0.85%) respectively. While *Strept. pneumoniae* and *C. pyogenes* could be detected in 4 samples with an incidence 3.39% (Table 3). These result nearly similar with Rana *et al.*, (1993); Fatma *et al.*, (2001) and Seddek, (2002). It was clear that the mixed infection recorded only from examined diseased samples.

Pasteurella multocida and *P. haemolytica* have an etiological association with pneumonic pasteurellosis. The pathogenicity of isolates of *Pasteurella multocida* to white mice (Table 4) revealed that all isolates were highly pathogenic to mice after intraperitoneal injection with 1.5×10^8 viable organisms, producing acute septicemia and death within 48 hours post inoculation. This agrees with the result obtained by Aliaa, (2002).

In vitro, the susceptibility distribution of each isolated pathogen to different antibiotic is represented in Table (5), most of the isolates were highly sensitive to Enrofloxacin, Gentamycin and Rimactan, moderately sensitive to Chloramphenicol, Oxytetracycline and Trimethoprim-sulphamethoxazol and resistant to Ampicillin and Streptomycin, those findings are partially agreement with those mentioned by Raid (1989); Abd El-Kader, (1992); Thabet, (1993); Ahmed, (1994); Amany, (2000)

and Seddek, (2002). The resistance of bacterial isolates to some antibiotics may be attributed to wrong dosage, duration of treatment and route of administration (Amstutz, *et al.*, 1982).

Respiratory disorders is still serious problem due to its special property that multifactors are responsible and the difficulty to determine the definite cause; so more efforts must be done to overcome that problem, such efforts as periodical clinical and bacteriological examination of apparently healthy animals to avoid misuse of antibiotics.

Finally, it must be strongly stressed that the recovered pathogenic and potentially pathogenic isolates have an important role in the respiratory affection, hence adequate hygienic measures as well as proper management of animals would reduce the degree of exposure of animals to disease producing agent.

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