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Bacterial infection in camels causing pneumonia with special emphasis on its antibiotic resistance.

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ABSTRACT

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This study aimed to identify some of the bacterial causes of pneumonia in camel.

A total of 100 lung samples from slaughtered imported camel were taken. Samples were subjected to bacteriological examination, antibiotic susceptibility testing and molecular characterization of some antibiotic resistant genes. The results showed that the total prevalence of pneumonia in the examined lung samples were 86%.

In addition, the prevalence of the isolated bacteria were; *S. aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Mycobacterium bovis* with a percentage of 38, 31, 13 and 4; respectively. Antimicrobial susceptibility testing of most isolates indicated the presence of multidrug resistant strains. Molecular characterization of some antibiotic resistance genes indicated the presence of *bla_Z*, *Aac(6')*, *Pbp1A*, *erm B*, *Tet (B)* in the examined different types of bacterial isolates. In addition, *Mpb70* as a specific gene for *M. bovis*. Public health significance and recommendations were discussed.

Keywords:

Antibiogram
Antibiotic resistance genes
Camel
Pneumonia

INTRODUCTION:

The dromedary camels in Africa represent approximately 74% of the global camel population. They have a very high socio-economic value and serve as an essential source of meat and milk for humans (Rhodes et al. 2015). International trade has long been recognized as

a vector for food born infections and has been held responsible for introducing new strains of pathogens into susceptible population; this is why many countries had introduced strict regulations for imported meat so as to save the consumers health. The problem would have a far reaching sequelae when living animals are im-

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ported and disseminate the organisms to the environment until they are slaughtered (Asadi et al. 2023 and Davis et al. 2018).

Multiple stressing factors as rearing systems, climatic changes, unhygienic conditions, and sudden changes in feed with low level herd health status were stated to be risk factors associated with bacterial and viral causes induced pneumonia with camels. Pneumonia outbreaks in camels were usually observed during the change from dry to rainy seasons (Ben Chehida et al. 2021).

However, Camels were formerly assumed to be immune to the majority of livestock illnesses; but new research has proved their susceptibility to a wide range of infections, and camels are regarded to function as a carrier or reservoir for the spread of various animal diseases and zoonoses (Mai-siyama et al. 2014)

Wide varieties of bacteria were isolated from infected lungs of diseased camels including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus* spp., *Streptococcus pyogenes pyogenes*, *Corynebacterium* spp., *Pasteurella* spp., and *Arcanobacterium pyogenes* (Ismael et al. 2014 wareth et al. 2014 Abd El Tawab et al. 2016 Gebru et al. 2018).

In addition, Tuberculosis (TB) is a chronic, reportable granulomatous zoonosis caused by *Mycobacterium tuberculosis* complex and affects many animal species including camels (OIE, 2016).

Clinical bacterial isolates frequently exhibit resistance to these antibiotics through the enzymatic alteration. There are five kinds of aminoglycoside-modifying enzymes (AME) occurring in *Staphylococci* spp., *AAC* (6) and *blaz* genes were studied by Hauschild et al. (2008).

In *Klebsiella pneumoniae* isolates, *TetA* (B) gene was identified to be the most prevalent tetracycline resistance determinant (Bokaeian et al. 2014).

We have investigated the role of *Pbp 1A* in penicillin resistance in and *erm* gene renders *Streptococcus pyogenes* isolates resistant to

most macrolides, lincosamides, and streptomycin B compounds (Uruén et al. 2022).

This study aim to demonstrate different microbial hazards from apparently healthy slaughtered imported camels and antibiogram profile for the isolated bacteria. In addition, detection of antibiotic resistance genes in the most prevalent isolates

MATERIALS and METHODS

Ethical approval: All procedures performed in this study, including the collection of samples, were in accordance with the Egyptian ethical standards of Animal Health Research Institute, and the Animal Rights and Ethical Use Committee of Agriculture Research Center, Animal Health Research Institute, Dokki, Giza, Egypt.

Sampling: Lung samples were collected from one hundred slaughtered camels. PM examination was performed following previously described procedures by Taiwo (2005).

Isolation and identification of Gram positive and Gram negative bacteria according to Quinn et al. (2011).

The surface of collected tissues was burned by hot scalpel blade and inoculum samples were taken from the inner part of the lung using sterile cotton swab, After 24h of incubation in broth, the cultures were mixed; a loopful of the culture was streaked over a 7% sheep blood agar, MacConkey agar, Mannitol salt agar and Edward's media and incubated aerobically at 37°C for 24h. Primary identification of the bacteria was performed using colony morphology, Gram reaction, cellular morphology, catalase and oxidase tests. Final identification was carried out by subjecting pure cultures of single colony type into a series of secondary biochemical tests, namely, methyl-red, indole, citrate utilization, coagulase, motility, TSI, esculin hydrolysis, as well as sugar fermentation tests, as required for each bacterial spp. All media were purchased from Hi media®

Isolation and identification of Mycobacteria according to Marks technique (1972):

The samples were examined for the isolation and identification of *M. bovis* using con-

ventional methods such as direct smear, culture, and biochemistry and molecular methods such as PCR.

Antibiotic susceptibility testing: isolates cultivated into Muller Hinton broth tubes and incubated aerobically at 37° C for 18hr and then cultured into Muller Hinton agar plates and incubated as above for antimicrobial susceptibility testing, which was carried out by the standard disk diffusion method according to **CLSI (2022)**. The following antibiotic discs were used, Ampicillin (AMP, 15µg), Cephalexin (CL, 30µg), Clindamycin (DA, 10µg), Gentamicin (CN, 10µg), Imipenem (IMP, 10µg), Ofloxacin (OFX, 10µg), Rifampicin (RF, 5µg), Streptomycin (S, 10µg) and Tobramycin (TOB, 10µg). Antibiotic sensitivity in relation to zone of inhibition interpreted by the Manufacturing Company MAST® group.

Molecular characterization of isolates:

For DNA isolation: from Gram negative bacteria according to **Wilson (1997)**, 1.5 ml of the bacterial inoculated culture was spun in a microcentrifuge at 10000 rpm for 2mins., or until a compact pellet forms. The supernatant was discarded. The pellet was resuspended in 570µl of TE, SDS (final concentration, 0.5%), and proteinase K (final concentration, 100 mg mL⁻¹), and incubated at 37°C for 1h. To this mixture, 100µl of 0.8 M NaCl and 80µl of CTAB/NaCl (10% CTAB in 0.7 M NaCl) were added, and the microtubes were incubated for 10min. at 65°C. An equal volume of phenol/chloroform/isoamyl alcohol was added, extracted thoroughly, and spun in a microcentrifuge at 10000rpm at 4°C for 5min. The supernatant was transferred to a fresh tube. 0.6vol isopropanol was added to precipitate the nucleic acids and spun in a micro centrifuge at room temperature. The DNA was washed with 70% ethanol to remove residual CTAB and respin at 10000 at room temperature rpm for 5min to re-pellet it. The pellet was re-dissolved in 100µl TE buffer. Due to a high concentration of peptide and cross-bond peptides in the cell wall, Gram positive species are often more resistant to cell lysis. It required addition of lysozymes to the lysis buffer

(**Schindler and Schuhardt 1964 Ezaki and Suzuki 1982 Zschöck et al. 2000 Mason et al. 2001**).

2.Oligonucleotide Primer:

The primers used were provided by Metabion (Germany), and they are listed in Table (1).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions for conventional PCR.

Bacterial Spp.	Target gene	Primers sequences	Amplified segment (bp)	Initial denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
<i>Mycobacteria</i> Spp.	<i>Mpb70</i>	ACCCTCAACAGCGGTC AGTAC TTACGCCG- GAGGCATTAGCAC	314	95°C 5min	94°C 1min	55 °C 1min	72 °C 1min	72 °C 10min	Zhang et al. 2016
<i>S.aureus</i>	<i>Blaz</i>	GAAGTACGCAGAAGA- GA ACATGGCAA- GCTCTAGGA	173	95°C 5min	94°C 1min	54°C 1min	72 °C 1min	72 °C 10min	Martineau et al. 2000
	<i>Aac (6')</i>	ACTTCAACAC- CTGCTGCTTTC TGACCACTTTATCAG- CAACC	491	95°C 5min	94°C 1min	57 °C 2min	72 °C 30S		Choi et al. 2003
<i>Streptococcus pyogenes</i> Spp.	<i>Pbp 1A</i>	AGGGGTAGTAG- CATTACCAT CAACTATATGACTGG- GATCG	939	95°C 5min	95°C 30S	47°C 30S	72°C 30S	72°C 10min	Kannika et al. 2017
	<i>ermB</i>	GAAAAGGTACTCAAC- CAAATA AGTAACGG- TACTTAAATTGTTTAC	639	95°C 15min	94°C 20S	53°C 20S	72°C 40S		Sutcliffe et al. 1996
<i>Klebsiella pneumoniae</i> Spp.	<i>Tet (B)</i>	CCTCAGCTTCTCAAC- GCGTG GCACCTT- GCTCATGACTCTT	633	95°C 5min	94°C 1min	52°C 30S	72°C 1min	72°C 10min	Walker et al. 2001

RESULTS

Table 2. prevalence of bacterial isolates from the examined lung samples

Total no.	Positive samples		Negative samples	
	No.	%	No.	%
100	86	86%	14	14%

This table showed the prevalence of pneumonia in the examined camel lung samples. Out of examined 100 lung sample, there were 86(86%) positive samples and 14(14%) negative for bacteriological examination

Table 3. Frequency of bacterial isolates from lung lesions of camel (n=100)

Type of infection	Isolated Microorganism	Total Number	Percent
Single infection	<i>Staphylococcus aureus</i>	38	38%
	<i>Streptococcus pyogenes</i>	31	31%
	<i>Klebsiella pneumoniae</i>	13	13%
	<i>Mycobacterium bovis</i>	4	4%
Mixed infections	<i>Staphylococcus aureus</i> & <i>Streptococcus pyogenes</i>	20	20%
	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> and <i>Klebsiella pneumoniae</i>	5	5%

This table showed the Frequency of bacterial isolates from lung lesions of camel. Some lung lesions showed single bacterial infection others showed mixed infections. Out of examined 100 lung sample, there were 38 (38%) *Staphylococcus aureus* isolates; *Streptococcus pyogenes* 31 (31%); *K. pneumoniae* 13(13%) and *M. bovis*

4(4%). Mixed bacterial infection was detected as *Staphylococcus aureus* and *Streptococcus pyogenes* as 20% and *Staphylococcus aureus*, *Streptococcus pyogenes* and *K. pneumoniae* as 5%.

Table 4. Results of antimicrobial susceptibility for the isolates:

Antibiotic classes	Antimicrobial agents	<i>S. aureus</i> (N=10)		<i>Streptococcus pyogenes</i> (N=10)		<i>K. pneumoniae</i> (N=10)	
		S.	R.	S.	R.	S.	R.
<u>β- lactamases</u>							
Penicillin	Ampicillin	4	6	0	10	8	2
Carbapenem	Imipenem	2	8	0	10	7	3
Cephalosporin	Cephalexin	4	6	0	10	9	1
	Gentamicin	9	1	10	0	8	2
<u>Aminoglycosides</u>	Streptomycin	7	3	10	0	7	3
	Tobramycin	4	6	10	0	8	2
<u>Fluoroquinolone</u>	Ofloxacin	9	1	10	0	9	1
	Rifampicin	6	4	0	10	2	8
<u>Macrolids</u>	Clindamycin	5	5	10	0	3	7

Results in table (4) revealed that, out of tested ten *S. aureus* isolates, most isolates were resistant to all used β - Lactamases group of antibiotics. Concerning used Aminoglycosides antibiotics, out of tested ten isolates to Gentamicin, streptomycin and Tobramycin, there were 1, 3 & 6 resistant strains: respectively.

Only one *S. aureus* isolates showed resistance to Ofloxacin. In addition, in testing the sensitivity to Macrolides such as Rifampicin and Clindamycin, there were 4 and 5 resistant strains: respectively.

All isolated *Streptococcus pyogenes* showed resistance to all used β - lactamases group of antibiotics, sensitive to all used Aminoglycosides and Fluoroquinolones.

In addition, all isolates were resistant to Rifampicin and sensitive to Clindamycin. *Klebsiella pneumoniae* isolates showed high sensitivity to Fluoroquinolones and Cephalosporins and high resistance to Macrolides.

Table 5. Available review on bacteria isolated from camels in Egypt

Authors	Examined sample type	Most predominant isolated bacteria
Refai et al. (1984)	Lymphnodes	<i>Salmonella</i> spp.
Ali et al. (1987)	Uterine sample	<i>Citrobacter</i> spp. and <i>E. coli</i>
Mostafa et al. (1987)	Raw milk	<i>Staph</i> spp., <i>E. coli</i> and <i>C. perfringens</i>
El- Sayed et al. (1987)	Raw milk	<i>Salmonella</i> spp. and <i>Citrobacter</i> spp.
El Seedy et al. (1990)	Uterine sample	<i>Protus</i> spp
Powers et al. (1990)	Uterine sample	<i>E. coli</i>
Tibary Anouassi (1997)	Uterine sample	<i>Pseudomonas aeruginosa</i>
Tibary et al. (2006)	Uterine sample	<i>Klebsiella pneumoniae pneumonia</i>
Hanan et al. (2010)	Uterine sample	<i>E. coli</i> , <i>Salmonella</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae pneumoniae</i> , <i>Protus</i> and <i>Citrobacter</i> spp.
Abo-El naga and Osman (2012)	Lung samples	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Streptococcus pyogenes</i> , <i>Klebsiella pneumoniae</i> and <i>E. coli</i>
Ismail et al. (2014)	Lung, blood, nasal & tracheal swabs	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp., <i>Klebsiella pneumoniae</i> spp and <i>E. coli</i>
Wareth et al. (2014)	Lung samples	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Streptococcus pyogenes</i> Spp., <i>Klebsiella pneumoniae</i> spp and <i>E. coli</i> and <i>Corynebacterium</i> spp.
Nahed et al. (2017)	Lung tissues, blood samples and nasopharyngeal swabs.	<i>K. Pneumoniae</i> , <i>S. aureus</i> , <i>Shigella</i> spp., <i>E. coli</i> , <i>Proteus</i> spp., <i>P. aeruginosa</i> and <i>Pasteurella</i> spp.
El- Harriri et al. (2017)	Meat samples	<i>Pseudomonas</i> spp.
Al Amery et al. (2019)	Meat samples	<i>Staphylococcus aureus</i>
El- Naker (2019)	Serum samples	<i>Mycobacterium bovis</i>
Shahin et al. (2021)	Diarrheic neonatal camel	<i>E. coli</i>

DISCUSSION:

The respiratory tract of apparently healthy animals acts as a reservoir for many species of microorganisms that reached the nasal cavity through various ways. This study has shown that a wide variety of bacterial species colonize the respiratory passageways of camels in the study area. This is supported by several researchers in Egypt who previously demonstrated diverse bacterial species from various regions of the camel respiratory tract: nasal tracts, tonsil, trachea and lungs (Ismail et al. 2014; Wareth et al. 2014; Ahmed and Musa 2015 and Nahed et al., 2017).

The consistent isolation of these organisms from the pneumonic lungs of various species of animals might indicate their role in causing

different respiratory infections especially when the immune system of the animal is compromised by some other external factors. The normal bacterial flora of a healthy individual can be altered by several factors such as changes in the hygienic condition, environmental and climatic conditions, and nutritional and immunological status of the animal. Such factors could lower the resistance of the lung tissue and the existing organism most probable would get the upper hand, leading to the presentation of a variety of pathologies (Bosch et al. 2013).

Tables (2) and (3) showed the prevalence of pneumonia in the examined camel lung samples. Out of examined 100 lung sample, there were 86(86%) positive samples and 14(14%) negative for bacteriological examination.

In the examined lung samples, there were single bacterial infection; *S. aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *M. bovis* as well as mixed bacterial infections. Similar bacterial isolates were detected by **Bani Ismail (2017)**.

Staphylococcus aureus is known to occur as a commensal on the skin, the nose, and mucous membranes of healthy humans and animals and also an opportunistic pathogen in multiple infectious diseases (**Lozano et al. 2016**).

In this study, *S. aureus* was the commonest bacteria in the pneumonic lungs, 38%, which is higher when compared to the report of **Azizollah et al. (2009)**, **Wareth et al. (2014)**, **Ismail et al. (2014)**, **Hussain et al. (2017)** and **Al-Amery et al. (2019)** as 14, 14.5, 37.1, 24.8 and 14.5 percentages; respectively, from lungs of apparently healthy camels. Higher results were obtained by **Ben Chehida et al. (2021)** who isolated *S. aureus* from (95.6%) examined samples. The present and previous data suggest that the bacteria reside as a normal inhabitant of upper respiratory tract and possibly as a causative agent of secondary pneumonia.

Most species of the genus *Streptococcus* are considered potential pathogens, occur in nature, and some are commensal in the respiratory, genital, and alimentary tracts and skin of animals and man (**Parks et al. 2015**). Our results showed that, out of examined 100 lung sample, *Streptococcus pyogenes* were isolated from 31(31%). *Streptococcus pyogenes* have been isolated from clinically healthy camels although they were not definitely identified and characterized (**Azizollah et al. 2009**). Lower results were obtained by **Wareth et al. (2014)** as 10% isolation rate. On the contrary, higher isolation rate were detected by **Ahmed et al. (2015)** as 94% from examined pneumonic lung.

Klebsiella pneumoniae was recovered at an enormously comparable frequency from the pneumonic and healthy lungs. Our results showed that, out of examined 100 lung sample, *Klebsiella pneumoniae* was recovered from 13 (13%). **Al-Doughaym et al. (1999)** recorded

similar results from the lungs of pneumonic camels 10.9%. Higher isolation rates were reported by **Wareth et al. (2014)** and **Nahed et al. (2017)** as 26.7 and 44.0%; respectively. Lower figures have also been reported by **Abubakar et al. (2010)** in Nigerian and **Ismail et al. (2014)** and **Ahmed and Musa (2015)** in Egypt as 6.3, 1.8 and 0.1% percentages; respectively from apparently healthy camels.

Several studies on camel TB have been conducted in several countries, including Egypt, confirming the occurrence of TB in camel populations (**Koni et al. 2016**).

A high prevalence of camel TB is usually found among farmed camels and those in close proximity to cattle, which are mainly affected by *Mycobacterium bovis* (**Bennet et al. 2014**). The transmission of *M. bovis* between animals primarily occurs through aerosols, direct contact, sharing the same food and water and suckling (**El-Sayed et al. 2016**).

The prevalence of TB in camels based on bacteriological examination was 4% (Table3). Higher TB prevalence rate were obtained by **Beyi et al. (2014)**, **Narnaware et al. (2015)**, **Jibril et al. (2016)**, **Ahmad et al. (2019)**, and **Elnaker et al. (2019)** who reported a prevalence rate of 8.3, 19.56, 9.82, 33.4 and 60.87 percentages; respectively. On the other hand, a lower TB rate in Egypt than that obtained in this study was reported by **Manal and Gobran (2008)**, who concluded that the prevalence of TB in camels was 0.7%.

Concerning the mixed bacterial infection which dominated by *S. aureus* and *Streptococcus pyogenes* then *S. aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae* were detected as 20% and 5% of examined lungs; respectively. These findings were explained by **Mostafa (2004)** who stated that, the pulmonary mixed infection is commonly detected because the respiratory air passages act as a reservoir for potential pathogenic microorganisms which develop pneumonia on the onset under stress factors, poor sanitation, and climatic conditions.

Pneumonic mixed pathogens demonstrated the complexity of the disease where *S. aureus* may predispose infection by other pathogens. These results agree with **Taha et al. (2007)**, **Sayed and Zaitoun (2009)**, **Abo El naga and Osman (2012)** and **Gebru et al. (2018)**.

In addition, the failure to isolate bacteria from some examined lung tissues with lesions might be due to the involvement of other pathogens such as anaerobic bacteria, virus, Mycoplasma, fungi and may be parasites (**Lopéz, 2001**).

Furthermore, this study assessed the antibiotic susceptibility profiles of the bacterial isolates in order to choose the most effective antimicrobial agents that could be used to treat camels with respiratory problems as shown in table (4) which revealed that, out of tested ten *S. aureus* isolates, most isolates were resistant to all used β -Lactamases group of antibiotics. Concerning used Aminoglycosides antibiotics, out of tested ten isolates to Gentamicin, streptomycin and Tobramycin, there were 1, 3 & 6 resistant strains: respectively. Only one *S. aureus* isolates showed resistance to Ofloxacin. In addition, in testing the sensitivity to Macrolides such as Rifampicin and Clindamycin, there were 4 and 5 resistant strains: respectively.

All isolated *Streptococcus pyogenes* showed resistance to all used β -lactamases group of antibiotics, sensitive to all used Aminoglycosides and Flouroquinolones. In addition, all isolates were resistant to Rifampicin and sensitive to Clindamycin. *Klebsiella pneumoniae* isolates showed high sensitivity to Flouroquinolones and Cephalosporins and high resistance to Macrolides.

Findings in table (5) were similar to **Al Amery et al. (2019)** and **Ben Chehida et al. (2021)** who find similar antibiogram profile for isolated *S. aureus*. Similar anti-biogram profile of *Streptococcus pyogenes* was detected by **Ahmed et al. (2015)** and **Mutua et al. (2017)**. Accordingly, increased level of resistance among the respiratory pathogens against the commonly used antimicrobials in respiratory tract infections was observed. There was agreement in

presence of multidrug resistant strains with the results of **Elhariri et al. (2017)**. The high rate of resistance observed in many of the isolates could be either because they are frequently and unnecessarily prescribed or sold over the counter in the open market and private veterinary drug shops without prescription. Therefore, there is a need for practitioners and researchers to be aware of the bacterial flora of the camels and of their antibiotic sensitivities to be informed of the appropriate antibiotics to be used in the course of respiratory infections and control programs (**Shryock and Richwine, 2010** and **Ding and He, 2010**).

Transfer of resistance in bacteria has been documented to occur between different animal species, within humans, from animals to humans, and from humans to animals (**Mutu et al., 2017**).

In studying the resistance pattern of the isolated bacteria Fig. (1), (2), (3), (4) and (5) showed the different amplified fragments of different detected antibiotic resistance genes. In addition, Fig. (6) Showed the amplified fragment of *M. bovis* specific gene.

Clinical bacterial isolates frequently exhibit resistance to these antibiotics through the enzymatic alteration of aminoglycosides. *Staphylococci*, *streptococci*, and *enterococci* are gram-positive cocci. *AAC (6')* has a particular significance because it modifies aminoglycosides of therapeutic importance, including kanamycin, tobramycin, and gentamicin, respectively (**Abo-State et al. 2018**).

Penicillin resistance in *staphylococci* is caused by several methods. The most important method is the resistance due to a penicillin-binding protein, *PBP2a*, encoded by *me-cA*, and is primarily connected to human isolates. Investigations into *blaZ*-encoded penicillin resistance have been considerable. Additionally, *blaZ* has been linked to penicillin resistance in coagulase-negative *staphylococci* (CoNS).

(**Liao et al. 2017**).

Enterobacteriaceae family members such as *Klebsiella pneumoniae* often produce ESBLs; however, other genera of the *Enterobacteri-*

aceae family have recently been reported to contain some other enzymes.

Tetracycline has been used regularly to treat various diseases, but regrettably this has led to the emergence of resistance forms. In prior clinical surveys, *tetA (B)* gene was identified as the most prevalent tetracycline resistance determinant with a wide host range since it resides on highly mobile genetic elements that readily transfer between different bacterial genera (Bokaeian et al. 2014).

Penicillin inhibits the growth of streptococci by the inactivation of penicillin-binding proteins (PBPs). Streptococcal resistance to penicillin is due to the production of altered PBPs which have a decreased affinity for the antibiotic. We have investigated the role of *PBP 1A* in penicillin resistance and confirm that that alteration of *PBP 1A* plays a vital role in full penicillin resistance development.

In addition, Target modification occurs at the level of the ribosomes via an *erm* gene encoding a 23S rRNA methylase. There are currently at least eight classes of *erm* genes distinguishable by hybridization criteria. Erm methylases add either one or two methyl residues to a highly conserved adenine residue in domain V, the peptidyl transferase center, of 23S rRNA. Uruén et al. (2022).

In *Streptococcus pyogenes*, two specific primers were used, *ermB* and *Pbp1A*. *ermB* product was 639bp which was compatible with Uruén et al. (2022). Like Kannika et al. (2017) the amplified fragment of *PbPIA* gene was 939. Penicillin inhibits the growth of pneumococci by the inactivation of penicillin-binding proteins (PBPs).

Concerning *S.aureus* isolates, *blaZ* and *AAC6'* genes were detected. The obtained PCR product was 173bp and 491bp; respectively. Our results agreed with Martineau et al. (2000) and Choi et al. (2003); respectively.

All tested *Klebsiella pneumoniae* isolates harbored *Tet B* gene. The amplified fragment was 633bp and these results agreed with Bokaeian et al. (2014). *Mpb70 gene of Mycobac-*

teria bovis was detected in all tested isolates. The amplified fragment was 314bp which was like Zhang et al. (2017).

Table (5) showed the available literature for several studies on camel in Egypt. From 1967 to 2022. These studies discussed either live animal samples such as raw milk samples, diarrheic samples, Genital tract washes, nasal and tracheal swabs or slaughtered camel samples from abattoir including meat samples, lymphnodes, lung samples and genital tract samples.

In conclusion, the present study in this area pointed out that respiratory infection is considered as the major cause of morbidity and mortality in camels. It is a multifactorial process among which a variety of bacterial species have been associated with respiratory problems. Furthermore, our isolates have shown considerable resistance to commonly prescribed antimicrobials in the country calling for the need to conduct susceptibility testing for control of camel respiratory infections in the area.

RECOMMENDATIONS:

controlling respiratory diseases of the camel's should give due attention in alleviating stress during different managemental practices including transportation, lairaging, feeding, watering, etc. and on those measures that has to be taken during stressful conditions.

Transborder camel movements should be controlled.

Abattoir workers should be educated to avoid infecting themselves or spreading the pathogens.

Proper abattoir records can serve as indicators for field disease conditions and consequently aid in planning, prevention and control programs by relevant authorities.

Restrictions for abuse of antibiotics in livestock production to avoid the emerging of antibiotic resistant pathogens.

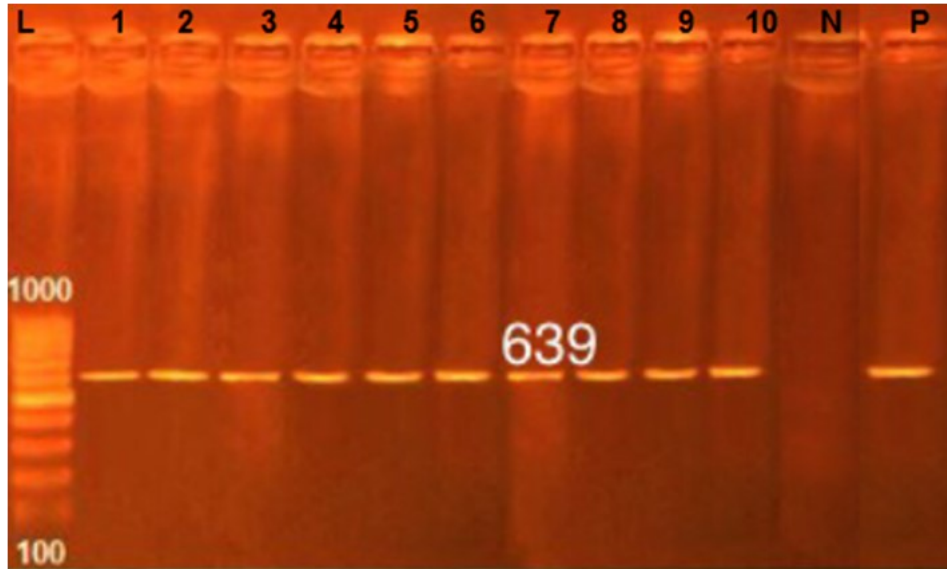


Fig. 1 Agarose gel electrophoresis of PCR products showing amplification of *Streptococcus pyogenes* *ermB* gene products at 639 bp. MWM-molecular weight marker (100-1000 bp DNA ladder) + control (positive, negative), all Ten isolates were positive for *ermB* gene.

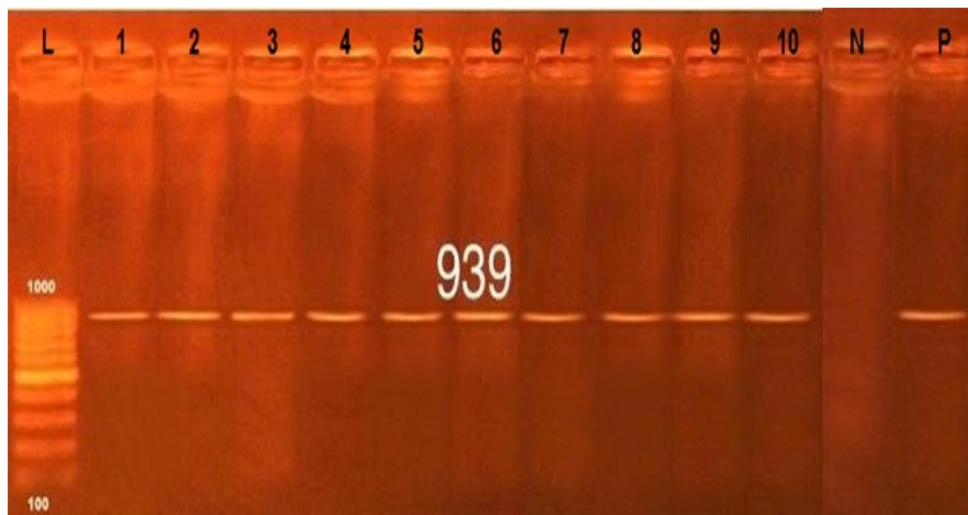


Fig. 2 Agarose gel electrophoresis of PCR products showing amplification of *Streptococcus pyogenes* *Pbp1A* gene products at 939 bp. MWM-molecular weight marker (100-1000 bp DNA ladder) + control (positive, negative), all Ten isolates were positive for *pbp1A* gene.

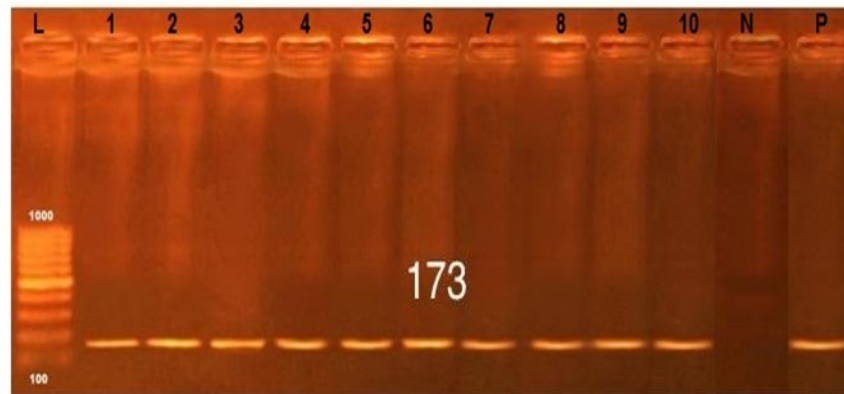


Fig.3 Agarose gel electrophoresis of PCR products showing amplification of *S. aureus* *blaZ* gene products at 173 bp. MWM-molecular weight marker (100-1000 bp DNA ladder) + control (positive, negative), all Ten isolates were positive for *blaZ* gene.

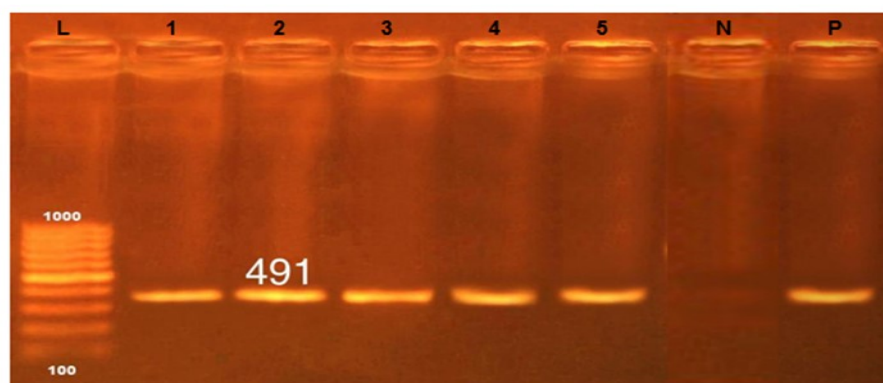


Fig.4 Agarose gel electrophoresis of PCR products showing amplification of *S. aureus* *aac(6)* gene products at 491 bp. MWM-molecular weight marker (100-1000 bp DNA ladder) + control (positive, negative), all Five isolates were positive for *aac(6)* gene.

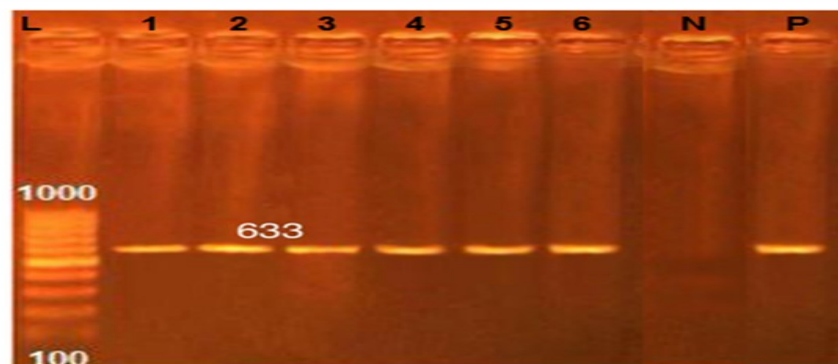


Fig. 7 Agarose gel electrophoresis of PCR products showing amplification of *K. pneumoniae* *TetA(B)* gene products at 633 bp. MWM-molecular weight marker (100-1000 bp DNA ladder) + control (positive, negative), all six isolates were positive for *TetA(B)* gene.

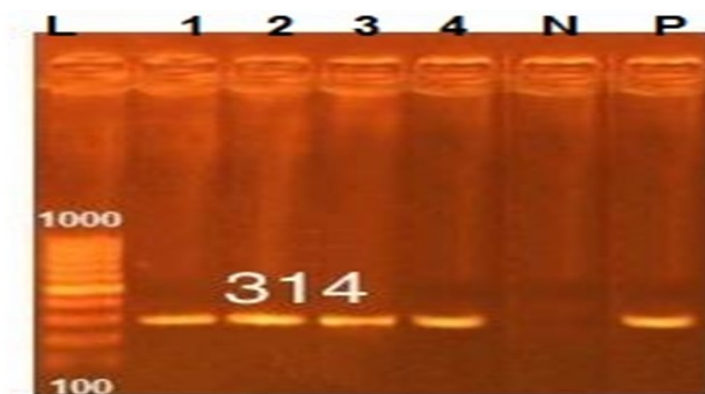


Fig.8 Agarose gel electrophoresis of PCR products showing amplification of *M. bovis* specific mpb70 gene products at 314bp. MWM-molecular weight marker (100-1000bp DNA ladder) + control (positive, negative), all four isolates were positive for mpb70 gene.

REFERENCES:

- Abdel Tawab AA, El Hofy F, Al-Jeddawy A, Abo-Hamad E 2016. *Pasteurella multocida* in camels: incidence, capsular and virulence genes characterization. *BVMJ*. 31(2):171-175.
- Abo-Elnaga TR and Osman WA 2012. Detection of Pathogens of Condemned Lungs of One Humped Camels (*Camelus dromedarius*) Slaughtered in Matrouh Abattoirs, Egypt. *Global Veterinaria* 9 (3): 290-296. DOI: 10.5829/idosi.gv.2012.9.3.6581
- Abubakar MS, Fatihu MY, Ibrahim NDG, Oladele SB and Abubakar MB 2010. "Camel pneumonia in Nigeria: Epidemiology and bacterial flora in normal and diseased lung," *African Journal of Microbiology Research*, vol. 4, no. 23, pp. 2479–2483.
- Ahmad I, Kudi CA, Babashani M, Chafe UM, Yakubu YA and Shittu A. 2019. Tuberculosis in dromedary camels slaughtered in Nigeria: A documentation of lesions at postmortem. *Trop. Anim. Health Prod.*, 51(1): 73-78.
- Abo-State Mervat, Saleh YE, Ghareeb HM 2018. Prevalence and sequence of aminoglycosides modifying enzymes genes among *E.coli* and *Klebsiella pneumoniae* species isolated from Egyptian hospitals. *11(4)*: 408-415. <https://doi.org/10.1016/j.jrras.2018.08.005>
- Ahmed ME and Musa MT 2015. Characterisation of bacteria isolated from dromedary camels affected with pneumonia for the first time in Sudan. *Annual Research and Review in Biology* (7):61-67.
- Ahmed ME, Musa MT and Mohammed AE 2015. Bacteria Associated with Pneumonia in Camels (*Camelus Dromedarius*) in the Sudan and Sensitivity of Some Isolates to Antibiotics using Vitek 2 Compact. *Global Journal of Science Frontier Biological Science*. 15 (5)
- Al-Amery Kh, Elhariri M, Elsayed A, El-Moghazy G, Rehab E, Heba M, El Hariri M and Dalia H 2019. Vancomycin-resistant *Staphylococcus aureus* isolated from camel meat and slaughterhouse workers in Egypt. *Antimicrobial Resistance and Infection Control*. 8:129. <https://doi.org/10.1186/s13756-019-0585-4>
- Al-Doughaym AM, Mustafa KM and Mohamed GE 1999. "Aetiological study on pneumonia in camel (*Camelus dromedaries*) and in vitro antibacterial sensitivity pattern of the isolates," *Pakistan Journal of Biological Science*, vol. 2, no. 4, pp. 1102–1105.
- Ali M, Shalaby SIA, Shalash MR, Nawito MF and Afify MM 1987. Bacterial status of abnormal genitalia of the camels. *Egyptian J. Vet. Sci.* (24):41-42.
- Asadi B, Seyedasgari F, Ashrafi Tamai I, Yarmohammadi M, Ebadi R, Kim E, Barin A. 2023. Isolated Bacteria from the Uteri of Camels with Different Reproductive Backgrounds: A Study on Sampling Methodology, Prevalence, and Clinical Significance. *Vet. Sci.* 10, 39. <https://doi.org/10.3390/>

- vetsci10010039
- Azizollah E, Bentol-hoda M and Raziah K 2009. "The aerobic bacterial population of the respiratory passageway of healthy Dromedarius in Najaf-Abbad abattoir central Iran," *Journal of Camelid Science*, pp. 26–29.
- Bani Ismail Z 2017. Pneumonia in Dromedary Camels (*Camelus dromedarius*): A Review of Clinico-Pathological and Etiological Characteristics. *J. Camel Pract. Res.* (24): 49–54.
- Bokaeian M, Saeidi S, Shahi Zahra , Kadaei V 2014. *TetA* and *tetB* Genes in *Klebsiella pneumoniae* Pneumoniae Isolated From Clinical Samples. *Gene, Cell, and Tissue*: 1, (2): e18152. DOI: 10.17795/gct-18152.
- Ben Chehida F, Gharsa H, Tombari W, Selmi R, Khaldi S, Daaloul M, Ben Slama K, Messadi L. 2021. First Report of Antimicrobial Susceptibility and Virulence Gene Characterization Associated with *Staphylococcus aureus* Carriage in Healthy Camels from Tunisia. *Animals*. 11, 2754. <https://doi.org/10.3390/ani11092754>
- Bennett JE, Dolin R and Blaser MJ. 2014. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Vol. 2. Elsevier Health Sciences, US.
- Beyi AF, Gezahegne KZ, Mussa A, Ameni G and Ali MS. 2014. Prevalence of bovine tuberculosis in dromedary camels and awareness of pastoralists about its zoonotic importance in Eastern Ethiopia. *J. Vet. Med. Anim. Health*, 6(4): 109-115.
- Bosch A, Biesbroek G, Trzcinski K, Sanders EAM, Bogaert D. 2013. Viral and Bacterial Interactions in the Upper Respiratory Tract, *PLoS Pathog*, 9(1), e1003057
- Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH. 2003. Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among *Staphylococcus* species. *J Korean Med Sci.*(18): 631-6.
- CLSI 2022. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; M100-32nd Edition. Wayne, PA: Clinical and Laboratory Standards Institute.
- Davis PA, McDowell LR, Wilkinson NS, Buergelt CD, Van Alstyne R, Weldon RN and Marshall TT. (2006). Effects of selenium levels in ewe diet on selenium in milk and the plasma and tissue selenium concentrations of lambs. *Small Rumin. Res.*, (65):14-23.
- Ding C, He J. 2010. "Effect of antibiotics in the environment on microbial populations," *Applied Microbiology and Biotechnology*, vol. 87, no. 3, pp. 925–941, 2010.
- Elhariri M, Dalia H, Rehab E and Sohad MD 2017. Extended-spectrum beta-lactamase-producing *Pseudomonas aeruginosa* in camel in Egypt: potential human hazard. *Clin Microbiol Antimicrob.*16:21. DOI 10.1186/s12941-017-0197-x
- Elnaker YF, Diab MS, Ibrahim NA, El-Gedawy A, Zaki RS, Radwan A. 2019. Seroprevalence and molecular characterization of *Mycobacterium bovis* infection in camels (*Camelus dromedarius*) in the Delta region, Egypt, *Veterinary World*, 12(8):1180-1187. doi: 10.14202/vetworld.2019.1180-1187
- El-Sayed A, El-Shannat S, Kamel M, Castañeda- Vazquez M, Castañeda-Vazquez, H. 2016. Molecular epidemiology of *Mycobacterium bovis* in humans and cattle. *Zoonoses Public Health*, 63(4): 251-264.
- EL- Sayed MA, Safwat EE, Serur BH, El-Sayed Z and Abdel Rhaman M. 1987. The bacterial flora of the cervicovaginal area and prepuce in camels. *J. Egypt Vet Med Ass.* (47): 703-712.
- El-Seedy FR, Ismail M, El-Sayed Z, Enany ME and Abdel Ghany M. 1990. Bacterial species implicated in fistulus wither affecting one humped camels in Egypt. *J Egypt Med. Ass.* (50): 81-92.
- Ezaki T and Suzuki S. 1982. Achromopeptidase for lysis of anaerobic gram-positive cocci. *J. Clin. Microbiol.* (16): 844-846.
- Gebru M, Tefera G, Dawo F, Tessema TS. 2018. Aerobic bacteriological studies on the respiratory tracts of apparently healthy and pneumonic camels (*Camelus dromedaries*) in selected districts of Afar Region, Ethiopia. *Trop Anim Health Prod* 50:603–611 <https://doi.org/10.1007/s11250-017-1476-4>
- Hanan MI, Hala IS and Safwat EE 2010. Microbiological studies on gram negative bacteria isolated from uterine samples of slaughtered she camels. *Egypt J of Appl Sci.* 25 (4B).
- Hussain MH, Habasha FG, Mansour KA.

2017. Clinical, microbial, histopathological and molecular investigation of interstitial pneumonia in camels in Iraq. *Vaol.* 16 No. (3) 6th Conference (1st international) 27-28.
- Ismail M, El-Deen NE, El-Hariri M. 2014. Bacteriological examination of respiratory tract of apparently healthy camels in Egypt. *Int J.* 5(1):65–8.
- Jibril Y, Mamo G, Hanur I, Zewude A and Ameni G. 2016. Prevalence of camel tuberculosis and associated risk factors in camels slaughtered at Akaki Abattoir, Ethiopia. *Ethi-op. Vet. J.*, 20(1): 23-38.
- Kannika K, Pisuttharachai D, Srisapoom P, Wongtavatchai J, Kondo H, Hirono I, et al. 2017. Molecular serotyping, virulence gene pro-filing and pathogenicity of *Streptococcus pyogenes agalactiae* isolated from Tilapia farms in Thailand by multiplex PCR. *J Appl Microbiol* 2017; 122:1497–507; <https://doi.org/10.1111/jam.13447>
- Koni A, Juma A, Morini M, Nardelli S, Connor R and Koleci X. 2016. Assessment of an ELISA method to support surveillance of bovine tuberculosis in Albania. *Ir. Vet. J.*, 69 (1): 11.
- Liao K, Chen Y, Wang M, Guo P, Yang Q, Ni Y, Yu Y, Hu B, Sun Z, Huang W. 2017. Molecular characteristics of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae pneumoniae* causing intra-abdominal infections from 9 tertiary hospitals in China. *Diagn Microbiol Infect Dis.* 87(1):45-48 <https://doi.org/10.1016/j.diagmicrobio.2016.10.007>
- López A. 2001. Respiratory system, Thoracic cavity, and pleura. In: M.D. McGavin, W.W. Carlton, J.F. Zachary, (eds.), Thomson's Special Veterinary pathology, 3rd Edition, (Mosby, A Harcourt health Sciences company, London), 125–191
- Lozano C, Gharsa H, Slama BK, Zarazaga M, Torres C 2016. *Staphylococcus aureus* in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent, *Microorganisms*; 4(1) <https://doi.org/10.3390/microorganisms4010012>
- Mai-siyama IB. 2014. Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization rate among ruminant animals slaughtered for human consumption and contact persons in Maiduguri, Nigeria. *Afr J Microbiol Res.*2014; (8):2643–9.
- Manal MY and Gobran R (2008). Some studies on tuber-culosis in camel. *Egypt. J. Comp. Pathol. Clin. Pathol.*, 21(4): 58-74.
- Marks J. 1972. Ending the routine guinea-pig test. *Tubercle* 53: 31-34 [https://doi.org/10.1016/0041-3879\(72\)90043-8](https://doi.org/10.1016/0041-3879(72)90043-8)
- Martineau F, Picard FJ, Lansac N, Menard C, Roy PH, Ouellette M. 2004. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother.* (44): 231-8
- Mason WJ, Blevins JS, Beenken K, Wibowo N, Ojha N, Smeltze MS. 2001. Multiplex PCR protocol for the diagnostics of staphylococcal infection. *J. Clin. Microbiol.* (39): 3332-3338
- Mostafa AS, Ragab AM, Safwat EE, El-Sayed Z, Abdel Rhahman M, El Danaf NA and Shouman MT. 1987. Examination of raw she camel milk for detection of subclinical mastitis. *J Egypt Vet Med Ass.* (47):117-128.
- Moustafa AH. 2004. Study of some aerobic bacterial causes of respiratory affection in slaughtered camels in Dakahlia Governorate. *Assuit Vet. Med. J.*, (50): 95-105.
- Mutua JM, Gitao CG, Bebora LC, FK, Mutua 2017. Antimicrobial Resistance Profiles of Bacteria Isolated from the Nasal Cavity of Camels in Samburu, Nakuru, and Isiolo Counties of Kenya. *Journal of Veterinary Medicine.*2017, Article ID 1216283. <https://doi.org/10.1155/2017/1216283>
- Nahed SS, Tarek R AE, Amani AH, Iman AEE, Asmaa AD 2017. Clinicopathological and Bacteriological Studies on Pneumonia in Camel (*Camelus dromedarius*). *J Vet Adv* 2016, 6(4): 1228-1236 .DOI: 10.5455/jva.20160409123446
- Narnaware SD, Dahiya SS, Tuteja FC, Nagarajan G, Nath K and Patil NV. 2015. Pathology and diagnosis of *Mycobacterium bovis* in naturally infected dromedary camels (*Camelus dromedarius*) in India. *Trop. Anim. Health Prod.*, 47(8): 1633-1636.
- OIE 2016. Bovine Tuberculosis in OIE Terrestrial Animal Health Manual. Ch. 11, 5. OIE, France. p586-589
- Parks T, Barrett L, Jones N. 2015. Invasive

- streptococcal disease: a review for clinicians, *British Medical Bulletin*, 115 (1): 77–89
- Powers BE, Johnson LW, Linton LB, Garry F and Smith J. 1990. Endometrial biopsy technique and uterine pathologic findings in llamas. *J. Am. Vet. Med. Assoc.* (197): 1157-1162.
- Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S and Fitz Patrick ES 2011. *Veterinary Microbiology and Microbial Disease*. 2nd Edition, Wiley-Blackwell, Chichester. Wiley, Hoboken.
- Refai M, El-Saidy WG, Osman K, Lotfi Z, Safwat EE and Elias S 1984. Salmonella in slaughtered camels in Egypt. *Zagazig vet J.* (9):266-276.
- Rhodes S, Crawshaw T, de la Rúa-Domenech R, Bradford S, Lyashchenko KP, Mamo G, Summers D, Wernery U and Zanolari P 2015. *Mycobacterial Infections in Camelids*. CABI, Oxfordshire. p216-234.
- Sayed SM and Zaitoun A. 2009. Aerobic bacterial triangle pathogen of pneumonic feedlot Buffalo-calves, Assuit Governorate, Egypt. *Ass. Univ. Bull. Environ.*(12): 55-60.
- Schindler CA and Schuhardt VT. 1964. Lyso-staphin: a new bacteriolytic agent for the staphylococcus. *Proc. Natl. Acad. Sci. USA* (51):414-421.
- Shahein MA, Dapgh AN, Kamel E, Ali SF, Khairy EA, Abuelhag HA, Hakim AS. 2021. Advanced molecular characterization of enteropathogenic *Escherichia coli* isolated from diarrheic camel neonates in Egypt, *Veterinary World*. 14(1): 85-91. doi: [www.doi.org/10.14202/vetworld.2021.85-91](https://doi.org/10.14202/vetworld.2021.85-91)
- Shryock TR, Richwine A. 2010. “The interface between veterinary and human antibiotic use,” *Annals of the New York Academy of Sciences*, vol. 1213, no. 1, pp. 92–105.
- Sutcliffe J, T, Grebe A. Tait-Kamradt L, Wondrack 1996. Detection of erythromycin-resistant determinants by PCR. *Antimicrob. Agents Chemother.* (40):2562–2566.
- Taha K, Shlaby A, Sami MB, Deeb S. 2007. Pathological studies on the association of pneumonia and kidney affection in camels (*Camelus dromedarius*). *Egypt. J. Path. Clin. Path.*, (20): 235-262.
- Taiwo VO. 2005. *A Manual for Necropsy Procedure for Veterinary Students and Clinician*. Dabfol Print and pack Limited, Dugbe, Ibadan, Nigeria, pp. 2-21.
- Tibary A and Anouassi A. 1997. Reproductive disorders of the female camelidae – In: *Theriogenology in camelidae: Anatomy, physiology, BSE, pathology and Artificial breeding* Ed. Actes Edition, Institute Agronomique et Veterinaire Hassan 11, pp. 317-368.
- Tibary A, Anouassi A and Sghiri A. 2006. Infectious causes of reproductive loss in camelids. *Theriogenology* August. 66(3):633-47.
- Uruén C, García C, Fraile L, Tommassen J and Arenas J. 2022. How *Streptococcus pyogenes suis* escapes antibiotic treatments. *Veterinary Research*. 53:91 <https://doi.org/10.1186/s13567-022-01111-3>.
- Walker RA, Lindsay E, Woodward MJ. 2001. Variation in clonality and antibiotic-resistance genes among multi-resistant *Salmonella enterica* serotype Typhimurium phage-type U302 (MR U302) from humans, animals and foods. *Microb Drug Resist*, 7 (1):13- 21.
- Wareth G, Murugaiyan J, Dalia F, Khater DF, Moustafa SA. 2014. Subclinical pulmonary pathogenic infection in camels slaughtered in Cairo, Egypt. *J. Infect. Dev. Ctries.*, 8(7): 909-913.
- Wilson K. 1997. Preparation of genomic DNA from Bacteria. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith
- Zhang H, Wang Z, Cao X, Wang Z, Sheng J, Wang Y, Zhang J, Li Z, Gu X and Chen C (2016). Loop-mediated isothermal amplification assay targeting the mpb70 gene for rapid differential detection of *Mycobacterium bovis*. *Archives of microbiology* 198(9): 905-911. <https://doi.org/10.1007/s00203-016-1232-6>
- Zschöck M, Botzler D, Blöcher S, Sommerhäuser J, Hamann HP 2000. Detection of genes for enterotoxins (ent) and toxic shock syndrome toxin-1 in mammary isolates of *Staphylococcus aureus* by polymerase chain reaction. *Int. Dairy J.* (10):569-574.