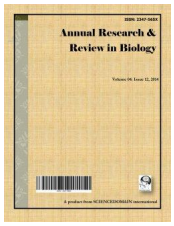

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Characterization of Bacteria Isolated from Dromedary Camels Affected with Pneumonia for the First Time in Sudan

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Of the study were to isolate and identify bacteria from pneumonic lungs and the upper respiratory tracts of camels slaughtered at Tambool Abattoir.
Study Design: A total of 800 samples were collected from 400 camels of different ages and sexes, from different parts of the Sudan. The samples comprised lung specimens and tracheal swabs, from each camel.
Place of Study: The samples collected from Tambool abattoir included congested and hepatized lungs and from abscesses, suppurative and adhered lung tissues then transported immediately on ice to the Veterinary Research Institute, Soba for isolation of bacteria.
Methodology: A total of 713 bacterial isolates were isolated from the samples of which 489 (68.6%) were Gram positive and 224 (31.4%) were Gram negative bacteria. The isolates were characterised by three different techniques: 584 (81.90%), with the conventional, 60(8.42%) with the Api kits and 69 (9.68%) with the automated Vitek 2 Compact system.

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Results: The isolates that were characterized conventionally were: *Actinomyces*, *Aeromonas*, *Bacillus* and *Corynebacterium* spp, *E. coli, Klebsiella pneumoniae (K. pneumoniae), Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* spp.
The isolates characterized by the Api kits were: *Aeromonas* spp, *Burkholderia cepacia, Escherichia coli (E. coli), Enterobacter cloaca, Enterobacter sakazakii, Klebsiella pneumoniae, Pseudomonas, Staphylococcus* and *Streptococcus* spp.
The isolates characterized by the Vitek 2 Compact were: *Acinetobacter*, *Actinomyces* spp, *Aeromonas hydrophilia, Aeromonas salmonicida, Aeromonas viridans, Alloiococcus otitis* (*A. otitis*), *Bacillus* spp*, Bordetella bronciseptica, Burkholderia cepacia, Corynebacterium spp, E. coli*, *Escherichia hermanii*, *Enterobacter cloaca, Enterobacter sakasakii, Facklamia hominis, Gardnerella vaginalis, K. pneumoniae, Leuconostoc pseudomesenteroides, Micrococcus* spp*, Morganella morganii, Pantoe* sp*, Pediococcus* sp*, Providense stuartii, Pseudomonas* spp*, Sphingomonas paucimobilis, Staphylococcus* spp, *Stenotrophomona maltophia* and *Streptococcus* spp included *Streptococcus agalaciae (Str. agalaciae), Streptococcus suis (Str. suis)* and *Streptococcus bovis (Str. bovis).*
Conclusion: *A. otitis* which causes acute otitis media in man was isolated from a camel trachea in this study. *Str. agalaciae* was isolated from a camel for the first time in the Sudan and *Str. suis* and *Str. bovis* were similarly reported.

Keywords: Bacteria; Pneumonia; Camel; A. otitis; *Str. suis; Str. agalaciae; Str. bovis.*

1. INTRODUCTION

Population of camels in the Sudan was more than four million head according to ministry of animal resources [1]. All the camels are *Camelus dromedarius*. Respiratory tract infections are of a common occurrence in various species [2]. Viruses, bacteria, fungi and parasites are incriminating as the main causative agents of pneumonia in mammals [3,4]. These agents may represent risks to camels, other livestock and even human populations [5-8]. Pulmonary diseases in camels were reported to cause considerable losses in production and increased mortalities [9-11]. The clinical signs observed were labored breathing, cough and nasal discharges. The pulmonary lesions observed were different stages of consolidation, hepatization, abscesses and adhesions with pleura [12]. 12 bacterial species were isolated from lymph nodes of dromedary camels in two areas of eastern Sudan [13]. The ecopathology was studied in camels during three seasons in eastern Sudan and different diseases were reported which cause mortalities [14]. Abbas et al. [15] a concurrent infection of invasive aspergillosis and pneumococcos were studied in camels and *Asperigllus fumigatus* isolated from the lungs. *Mycoplasma arginini* (*M. arginini*) was isolated from camels with pneumonia [16]. Different infectious diseases in camel such as pox, mange, enterotoxaemia, pneumonia, mastitis and camel calf diarrhoea were reported [17].

1.1 The Objective

The objective of this study was to investigate bacteria associated with camel Pneumonia in the Sudan.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Eight hundred samples were collected from 400 camels slaughtered at Tambool Abattoir, were of different sexes, ages ranging from six months to 15 years and originated from different states of the Sudan including Kassala, AlGadarif, Kordofan and Darfur states.

From each camel an affected lung specimen and tracheal swab were collected for bacterial isolation and identification. The samples were collected aseptically, each in a sterile plastic bag and transported immediately on ice to the Veterinary Research Institute, Soba for isolation of bacteria.

2.2 Procedures for Isolation of Bacteria

Cultures for isolation of bacteria were made in blood agar and brain heart infusion for the fastidious, mannitol salt agar for staphylococci, MacConkey agar for enterobacteriacae and brilliant green agar for differential diagnosis of *E. coli*. The surface of each lung sample was cauterised with a red hot scalpel blade for decontamination. A deep incision was made in

the cauterised lung surface using a sterile scalpel blade; a sterile swab was dipped into the incised area and streaked onto sheep blood agar (Oxoid CM271) plate. From the incised area a piece of a sample was cut and put in a brain heart infusion broth in a bijou bottle. Each tracheal swab was placed into brain heart infusion broth medium and incubated at 37°C for 24 hr and all were subcultured on a blood agar plates. The cultures were incubated aerobically at 37°C for 24 hr. Any plate that did not show growth within 24 hr was incubated and examined daily for a week to ensure bacterial growth before considering it negative. The isolates recovered were identified at the generic and species levels according to [18,19]. Different procedures were used for the identification of the isolates: the conventional method according to [18]. Api kits method and Vitek 2 Compact automated system (Biomeriouex). A pure culture of each isolate was subcultured onto a blood agar slant and after incubation at 37°C for 24 hr, stored at 4°C till used for identification.

2.3 Identification of the Gram Positive Bacteria with the Vitek 2 Compact

Vitek 2 Compact system is a fully automated system that contains 64 biochemical tests to identify organisms and grades the isolates from acceptable to excellent according to the tests results and gives details of biochemical tests.

2.4 Preparation of Each Bacterial Inoculums

A suspension of each isolate was prepared according to the manufacturer's recommendations by emulsifying the bacterial colonies in 0.45% saline and standerdised with densicheck (Biomerieux) until it was equivalent to 0.5-0.63 McFarland opacity tube [20]. The time between the preparation of the inocula and the card filling was adjusted to be less than 30 minutes [21].

The Gram positive identification card was based on established biochemical methods and newly developed substrates [22-27]. There were 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance.

According to the instruction of Biomerieux manufactures the card for each group of bacteria was automatically filled by a vacuum device, sealed and inserted into the Vitek 2 reader – incubator module (incubation temperature 35.5°C) and subjected to a kinetic colorimetric measurement every 15 min. Data were analyzed using Vitek 2 database version 4.01. All cards used were automatically discarded into a waste container. Final identification results were available in an approximately 8 hours or less in cases of Gram positive bacteria or 10 hours or less in Gram negative bacteria.

3. RESULTS

From the 800 samples, 713 bacterial isolates were obtained from the camel tracheal swabs and pneumonic lungs. The highest incidences 247(61.75%) of pneumonic cases were found in autumn and the lowest 153(38.25%) in summer. The adult camels were more susceptible 307(76.75%) than the younger ones 93(23.25%). The types of bacteria isolated from the different lesions are presented in Table 1 and the frequencies of isolation of the different bacteria are presented in Table 2. Fig 1 shows a suppurative camel lung specimen caused by *Str. agalaciae* and Fig 2 a camel marbled pneumonic lung specimen caused by *Str. suis*.

A total of 584(81.77%), 69(9.68%) and 60(8.42%) isolates were identified by the Conventional, Vitek 2 Compact and Api kits, respectively. From those characterized by the Vitek 2 Compact 2 (0.28) were *S. agalacia,* 2 (0.29) were *S. bovis*, 1 (0.14) was *S. suis* and 1 (0.14) was *A. otitis*.

4. DISCUSSION

The highest incidences 247(61.75%) of pneumonic cases were found in autumn and the lowest 153(38.25%) in summer; probably due to the effects of climatic changes.

The adult camels were more susceptible 307(76.75%) than the younger ones 93(23.25%) probably due to their higher susceptibility.

*Str. agalacia*e was isolated from a suppurative pneumonic lung for the first time in the Sudan (Table 1). It was reported in different camel management systems in Kenya (Somali, Pokot, ranch-camels). It was isolated the from udder infections in 6 of 9 herds and from camels with septic arthritis, skin abscesses, secondary respiratory infections and puerperal infections [28]. The organism was also recently isolated from pneumonic lungs of camels in Ethiopia [29].

Table 1. Bacteria isolated from the tracheal swabs and pneumonic lungs in the camels examined

Lesion of the lung	Bacteria isolated
Congestion	*Staphylococcus* spp, *Corynebacterium* spp, *Pseudomonas* spp, *K. pneumoniae* and *E. coli*
Hepatization	*Staphylococcus* spp, *Streptococcus* spp, *K. pneumoniae, E. coli* and *Corynebacrerium* spp.
Abscesses	*Staphylococcus* spp, *Streptococcus* spp, *Actinomyces* spp, *Micrococcus* spp and *Ps. aeruginosa*
Supprative (mucoid)	*Rhodococcus equi, Corynebacterium* spp, *Ps. aeruginosa, K. pneumoniae, Burkholderia* spp, *S. agalaciae* and *S. epidermidis*
Adhesion	*Aeromonas spp, Streptococcus* spp and *Staphylococcus* spp
Tracheal swabs	*Staphylococcus* spp, *Streptococcus* spp, *Micrococcus* spp, *E. coli*, *Corynebacterium* spp, *Allioicoccus* sp and *B. bronchiseptica*

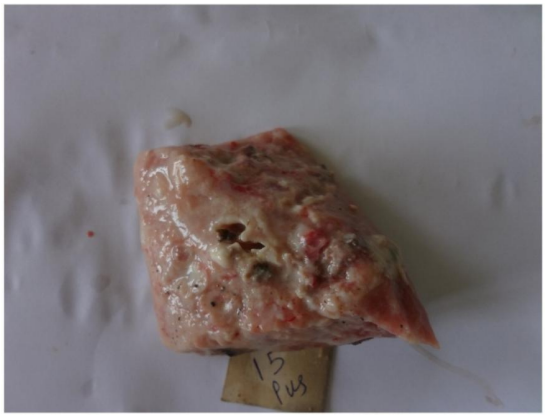

Fig. 1A. suppurative camel lung specimen from which *Str. agalaciae* was isolated

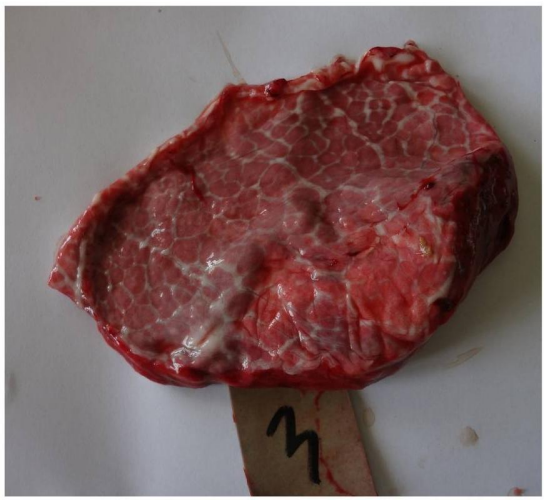

Fig. 2A. camel marbled pneumonic lung specimen from which *Str. suis* was isolated

There were no previous reports about the role of the organism in the respiratory diseases of camels. However, the organism is the cause of neonatal pneumonia, sepsis and meningitis leading to significant morbidity and mortality in humans [30].

Other new isolates in this study were *Str. suis* and *Str. bovis*. Both species occur in swine and cattle, respectively. In the Sudan, camel nomads migrate to wildlife areas in summer months, where camel come into contacts by wild pigs and cattle and that might have been the reason for their infections. *Str. suis* is a major porcine pathogen worldwide and can be transmitted to humans by close contact with sick or carrier pigs. A case of ocular infection that progressed rapidly to a corneal ulcer was reported due to the bacterium [31]. It also causes meningitis, septicaemia, endocarditis, arthritis, and septic shock in both pigs and humans, and mortality is high. Human infection with *Str. suis* occurs mainly among certain risk groups that have frequent exposure to pigs or pork. Outbreaks of *Str. suis* infections in human are uncommon, although several outbreaks occurred in recent years in China. In July, 2005, the largest outbreak of *Str. suis* infections occurred in Sichuan province, China, where 204 people were infected and 38 of them died. A total of 409 cases of human infection due to *Str. suis* occurred worldwide, most of which were in China, Thailand, and the Netherlands that led to 73 deaths [32]. It was also isolated in a rare case from blood and pleural fluid of a man with pneumonia in south Korea [33]. It was found that there was a high incidence of colorectal cancer associated with *Str. bovis* infections in Malaysia [34].

Table 2. The frequency of the bacterial isolates identified by the different methods

Isolate	Conventional method	Api kits	Vitek 2 compact	Total no.	Average/ STDEV
Staphylococcus spp	174(24.40%)	25(3.51%)	17(2.38%)	216 (30.40%)	72 / 88.43
Streptococcus spp	94(13.18%)	20(2.81%)	11(1.54%)	125 (17.60%)	41.67 / 45.54
Enterobacteria spp	148(20.76%)	10(1.40%)	12(1.68%)	170 (23.90%)	56.67 / 79.10
Micrococcus spp	49(6.87%)	_	4(0.56%)	53 (7.50%)	17.67/ 27.21
Corynebacteria spp	27(3.79%)	_	2(0.30%)	29 (4.00%)	9.67 / 15.04
Pseudomonas spp	17(2.39%)	1(0.14%)	3(0.42%)	21 (3.00%)	7 / 8.71
Actinomyces spp	15(2.10%)	_	2(0.30%)	17 (2.40%)	6.67 / 8.14
Aeromonas spp	14(1.97%)	20.28%)	6(0.84%)	22 (3.10%)	13.33/ 7.02
Alloiococcus otitis	_	_	2(0.30%)	2 (0.30%)	0.67 / 1.15
Facklamia hominis	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Bordetella broncoseptica	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Leuconostoc pseudomesenteroides	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Pediococcus sp	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Stenotrophomona maltophia	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Sphingomonas paucimobilis	_	_	3(0.42%)	3 (0.42%)	1 / 1.73
Burkholderia cepacia	_	2(0.28%)	2(0.30%)	4 (o.60%)	1.33 /1.15
Bacillus spp	45(6.31%)	_	_	45 (6.30%)	15 / 25.98
Gardnerella vaginalis	_	_	1(0.10%)	1 (0.10%)	0.33 / 0.58
Total no. of bacteria	584(81.91)	60(8.42%)	69(9.67%)	713 (100%)	237.67/299.97

A. otitis is a newly recognized species of Gram positive bacterium which was recently discovered as a pathogen associated with acute otitis media in Japanese children. The organism is a major pathogen among the other isolates from otitis media: (*Str. pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*). It needs rich media for growth and grows slowly [35]. Limited studies were conducted on this organism and this is the first report of its isolation in camels the Sudan.

5. CONCLUSION

The conventional tests resulted in identification of fewer bacterial species from camels in this study.

More bacteria were isolated by the Vitek 2 Compact, followed by Api kits. Vitek 2 Compact system enables to isolate bacteria of public health importance.

Camels were found to be reservoirs of eight bacterial species pathogenic to man.

A newly recognized species of Gram positive bacterium *A. otitis* which was recently discovered as a pathogen associated with acute otitis media was isolated from tracheal swabs of camel affected with pneumonia in Sudan.

Str. agalaciae was isolated from camel for the first time in the Sudan.

Str. suis and *Str. bovis* were reported for the first time in pneumonic lungs in camels.

Identification of bacterial isolates should be carried by Vitek 2 Compact or Api kits for more accurate and reliable results, to save time for identification and overcome the problems of contamination, scarcity of the substrates used in the conventional tests and inconclusive results.

6. RECOMMENDATIONS

Camels are important food, agriculture packed and export animal and control of their diseases is deem essential.

Bacteria of public health importance should be monitored by isolation and treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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