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Detection of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and ESBL Producing *Escherichia coli* Associated with Ovarian Hydrobursitis Syndrome in Female Camels (*Camelus dromedarus*)

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ABSTRACT

Ovarian hydrobursitis syndrome represents a major reproductive problem in dromedary. The aim of the present study was to investigate the causative agents of such syndrome. A total of sixty one non-repetitive isolates (twenty from infection site and 41 from water sources used by camels for drinking) were collected from Al Ahsa province, eastern region of Saudi Arabia. Vitek 2 compact automated system was used to identify the isolates, to determine minimum inhibitory concentration of twenty antibiotics and to explore the antibiogram of certain isolates. Polymerase chain reaction was used to amplify genes encoding extended spectrum β-lactamases (ESBL). *Escherichia coli* (*E. coli*) isolates were the most common where 17 isolates were collected from affected animals while three isolates were collected from the water sources. In addition, two *Klebsiella pneumoniae* and one *Pseudomonas aeruginosa* isolates were collected from infection sites. Moreover, three ESBL producing *E. coli* were identified (two isolates from affected animals and one isolate from the water sources). These three isolates harbored bla_{TEM} while bla_{SHV} and bla_{CTX-M} were not detected. The present study showed that additional infective agents could be associated with ovarian hydrobursitis. In conclusion, strict infection control measures should be applied to prevent spreading of such multidrug resistant pathogens.

Key words: Dromedary, ovarian hydrobursitis, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*

INTRODUCTION

The one hump dromedary (Arabian camel, *Camelus dromedarus*) plays a significant role in the local culture and there is thought to be more than 15 million camels in the Arabian region. Ovarian hydrobursitis syndrome is a major reproductive problem in female camels characterized by

accumulation of fluid inside the bursa (Tibary and Anouassi, 2001). This syndrome can be unilateral or bilateral. The majority of the bilateral cases lead to failure in reproductive system (Al-Eknah and Ali, 2001; Ali *et al*., 2011a).

The pathogenesis of ovarian hydrobursitis syndrome is still unclear as there are little published reports available. Nevertheless, Ali *et al*. (2011b) suggested that such syndrome is initially an inflammatory process. In addition, *Chlamydophila abortus* seems to be responsible for the spreading of such syndrome as recently reported (Ali *et al*., 2012). Moreover, many pathogens were isolated from the bursal fluids of female infected camels e.g., *E. coli* (Ali *et al*., 2011b).

Many multidrug resistant (MDR) pathogens were isolated worldwide (Boucher *et al*., 2009). Production of extended spectrum β-lactamases (ESBLs) is one of the most common mechanisms by which *Enterobacteriaceae* (e.g., *E. coli*) can acquire resistance against different broad spectrum cephalosporins e.g., cefotaxime and ceftazidime (Bradford, 2001; Ramphal and Ambrose, 2006). Such pathogens cause both community and hospital acquired infections leading to a serious problem in clinical medicine and cause great concerns to clinicians (O'Neill, 2008; Vidaillac *et al*., 2009). In addition, enterotoxigenic and enteropathogenic *E. coli* were recently isolated from diarrheal cases from camels (Shabana *et al*., 2013). Furthermore, *E. coli* has been reported in cases with purulent vaginal discharges in female camels (Aly *et al*., 2009).

There are different types of ESBLs e.g., TEM, SHV and CTX-M. Genes encoding enzymes are most common in different members of *Enterobacteriaceae*. As recently published, the prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} in *E. coli* and *Klebsiella pneumoniae* isolates from eastern region of Saudi Arabia (same location at which the current study was performed) was very high (Alsultan *et al*., 2013; Hassan *et al*., 2013).

The aim of the current study was to investigate the causative pathogens of ovarian hydrobursitis syndrome in female camels from eastern region of Saudi Arabia and the resistance pattern of the detected *E. coli* isolates was determined. In addition, the type of ESBL encoding genes was investigated in the ESBL producing *E. coli* isolates.

MATERIALS AND METHODS

Bacterial isolates: A total of twenty non-repetitive isolates were collected from deep ovarian cysts of female camels (*Camelus dromedary*) aged 9-12 years old in the slaughter houses. In addition, forty one isolates were obtained from different water sources used by camels for drinking in Al-Ahsa province, eastern region of Saudi Arabia. The clinical appearance of all females (n = 20) were characterized by vaginal adhesions with muco-purulent vaginal discharge. Isolates were collected from deep site infection from the fluid of an encapsulated ovary, immediately after slaughtering and hanging of the carcass. Ovarian hydrobursitis was detected within ovarian cysts larger than 25-30 mm. Isolates were preliminarily identified by conventional microbiological techniques and identification was confirmed by Vitek 2 compact automated system (BioMerieux, Marcy L'Etoile, France).

Molecular identification of *E. coli* isolates by Polymerase Chain Reaction (PCR): The chromosomal DNA from each *E. coli* isolate was obtained by emulsifying a single colony after an overnight growth on MacConkey agar (Oxoid, Basingstoke) in 25 μL sterilized distilled water in a micro centrifuge tube and then boiled for 10 min. A specific coding region (147 bp) of the *uid*A structural gene of *E. coli* was amplified by PCR using two primers (UAL-754 and UAL-900) previously described by Tsai *et al*. (1993). The nucleotide sequences of the two primers were depicted in Table 1.

Table 1: Nucleotide sequence of primers used in this study

Primer name	Nucleotide Sequence (5′- 3′)	Reference
UAL-754	AAAACGGCAAGAAAAAGCAG	Tsai *et al.* (1993)
UAL-900	ACGCGTGGTTACAGTCTTGCG	Tsai *et al.* (1993)
TEM-F	AGATCAGTTGGGTGCACGAG	Yazdi *et al.* (2011)
TEM-R	CAGTGCTGCAATGATACCG	Yazdi *et al.* (2011)
SHV-F	CGCCTGTGTATTATCTCCC	Alsultan *et al.* (2013)
SHV-R	GGCGATTTGCTGATTTCGC	Alsultan *et al.* (2013)
CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	Mulvey *et al.* (2004)
CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG	Mulvey *et al.* (2004)

Determination of antibiotic resistance pattern and minimum inhibitory concentration: The antimicrobial susceptibility tests (antibiogram and minimum inhibitory concentration) were performed by Vitek 2 compact automated system using AST-N116 cards according to the instructions of the manufacturer. The twenty tested antimicrobial agents were; ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefazolin, cefuroxime, cefuroxime axetil, cefoxitin, cefpodoxime, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, tigecycline and sulphamethoxazole/trimethoprim.

Detection of ESBL producing isolates: Preliminary phenotypic detection of ESBL production was initially reported by Vitek 2 compact automated system depending on the resistance pattern of the tested isolates. Confirmation of ESBL production was carried out using cefepime/cefepime plus clavulanic acid (PM/PML) Etest strips (AB Biodisk, Solna, Sweden) as described by the manufacturer. Minimum Inhibitory Concentration (MIC) ratio of ≥8 for the two reagent sides, a phantom zone or deformation of either ellipse is indicative of ESBL production.

Detection of β-lactamase encoding genes by PCR: The PCR was used to detect the presence of genes coding for TEM, SHV and CTX-M enzymes. The primers used to amplify bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were shown in Table 1. All primers were purchased from Eurofins, mwg/operon, Ebersberg, Germany.

RESULTS

Twenty pathogens (17 *E. coli*, 2 *Klebsiella pneumoniae* and one *Pseudomonas aeruginosa*) were isolated from deep ovarian cysts of female camels suffering from ovarian hydrobursitis syndrome as shown in Table 2. For tracing the potential source of detected pathogens causing such syndrome, additional forty one isolates were collected from different water sources used by camels for drinking. The forty one isolates were identified as follows: *Kocuria rosea* (10), *Aeromonas hydrophila* (9), *Burkholderia cepacia* group (8), *E. coli* (3), *Klebsiella pneumoniae* (2), *Pseudomonas alcaligenes* (2), *Acinetobacter lwoffii* (2), *Pseudomonas putida* (1), *Acinetobacter junii* (1), *Enterococcus faecium* (1), *Kluyvera intermedia* (1) and *Leclercia adecarboxylata* (1). For genotypic conformation of the twenty *E. coli* isolates, a 147 bp of *uid*A gene was amplified and the results were depicted in Fig. 1.

The resistance pattern of the twenty *E. coli* isolates and the Minimum Inhibitory Concentration (MIC) values against twenty different antimicrobial agents was determined by Vitek 2 compact system and the results were shown in Table 3. The results revealed that three *E. coli* isolates (two isolates from deep ovarian cysts and one isolate from water sources)

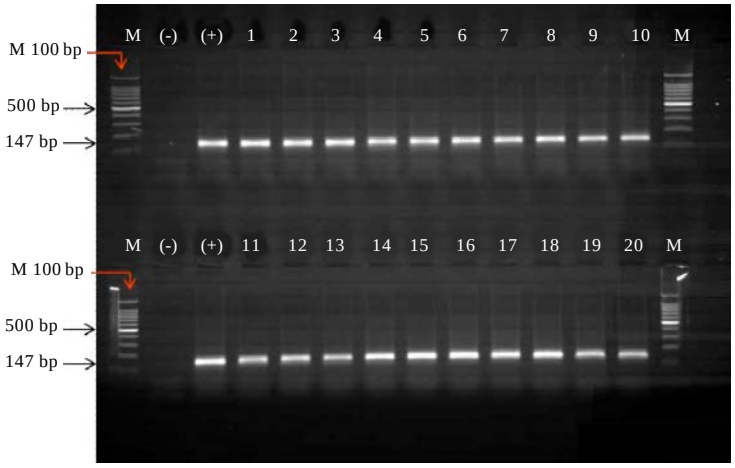

Fig. 1: Genotypic identification of twenty *E. coli* isolates

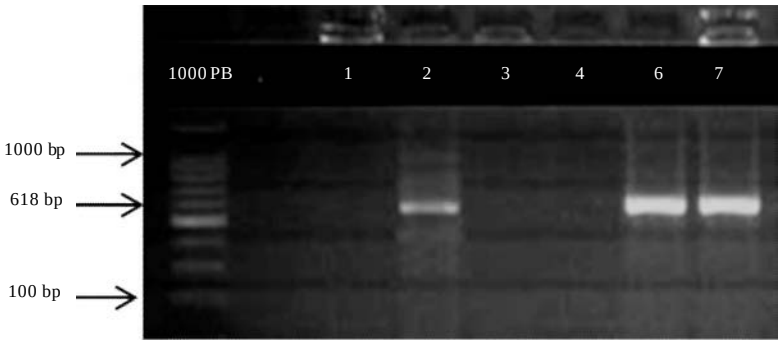

Fig. 2: Amplicons of bla_{TEM} from three ESBL producing *E. coli* isolates

Table 2: Microorganisms associated with ovarian hydrobursitis

	n = 20	
Microorganism	No.	%
Escherichia coli	17	85
Klebsiella pneumoniae	2	10
Pseudomonas aeruginosa	1	5

were ESBL producers. This result was confirmed by Etest-ESBL strips cefepime/cefepime plus clavulanic acid. On the other hand, all tested isolates showed susceptibility to piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin and levofloxacin while, one ESBL-producing isolate showed resistance to tigecycline.

To explore the type of β-lactamase encoding genes harbored by the three ESBL-producing *E. coli*, PCR was used to amplify a specific fragment of bla_{TEM}, bla_{SHV} and bla_{CTX-M}. As shown in Fig. 2, the three isolates harbored bla_{TEM} while, none of the other two genes (bla_{SHV} and bla_{CTX-M}) were detected.

Table 3: Resistance pattern and Minimum Inhibitory Concentration (MIC) range of twenty *E. coli* isolates

	No. of resistant isolates		
Antimicrobial agents	n = 20	%	MIC range (mg L^{-1})
Ampicillin	3	15	≤2-≥32
Ampicillin/sulbactam	2	10	≤2-≥32
Piperacillin	2	10	≤2-≥128
Piperacillin/tazobactam	0	0	≤4
Cefazolin	1	5	≤4-≥64
Cefuroxime	2	10	4-->64
Cefuroxime axetil	2	10	4-≥64
Cefoxitin	1	5	≤4-≥64
Cefpodoxime	1	5	≤2-≥8
Cefotaxime	1	5	≤1-16
Ceftazidime	0	0	≤1
Cefepime	0	0	≤1
Imipenem	0	0	≤1-4
Meropenem	0	0	≤0.25-1
Gentamicin	0	0	≤1
Tobramycin	0	0	≤1
Ciprofloxacin	0	0	≤0.25-0.5
Levofloxacin	0	0	≤0.12-1
Tigecycline	1	5	<0.5-≥8
Trimethoprim+sulfamethoxazole	3	15	<20-≥320

DISCUSSION

Ovarian hydrobursitis is a malformation responsible for reduced reproductive performance of dromedary camel (Tibary and Anouassi, 2001) which may lead to undesirable cultural, social and economical consequences. The current study investigated an additional risk factor in female camels (*Camelus dromedarus*) that may be associated with ovarian hydrobursitis and threaten camel production.

In the current study, the prevalence of *E. coli* isolated from deep infected site of the animal was significantly high (17 out of 20, 85%) which may be considered as a potential new risk factor involved in the ovarian hydrobursitis in the eastern region of Saudi Arabia. This result is in accordance with previous published report where *E. coli* and other microorganisms were detected in 46.7% of bursal fluids (Ali *et al*., 2011b). In contrast, Ali *et al*. (2012) showed that antibodies against *Chlamydophila abortus* were detected in 86.3% of female camels affected with ovarian hydrobursitis (Ali *et al*., 2012). Such discrepancy may be due to the long distance between the regions at which the two studies were conducted.

The majority of the *E. coli* isolates showed susceptibility to most tested antimicrobial agents specially carbapenems, fluoroquinolones and aminoglycosides. Interestingly, one of the ESBL producing *E. coli* showed resistance to tigecycline which is considered as an effective therapeutic option beside carbapenems for treatment of infections caused by such pathogens (Souli *et al*., 2006; Cha, 2008). Therefore, it is highly recommended to determine the resistance pattern of the infective pathogens before starting the therapeutic regimen.

In addition to *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (1 and 2 isolates out of 20, respectively) were isolated from the affected female camels. To the best

of the authors' knowledge and after search in the PubMed, the association of these two pathogens with ovarian hydrobursitis in camels was not previously reported. Therefore, these two pathogens could be considered as additional risk factors and infective agents associated with such syndrome. After carrying out antibiotic sensitivity testing, these three isolates showed susceptibility to most antimicrobial agents tested.

Emergence of EBSL producing *Enterobacteriaceae* particularly *E. coli* has been recognized globally as a source of serious infection associated with high rate of mortality (Pitout and Laupland, 2008). The prevalence of EBSL-producing *E. coli* in the Al Ahsa province, eastern region of Saudi Arabia is significantly high as recently reported (Alsultan *et al*., 2013). Detection of such pathogens in the infected site of the affected animals and in the water sources should be considered as an alarm for the infection control authorities to apply effective measures to prevent spreading of these pathogens. On the other hand, although other microorganisms such as *Kocuria rosea*, *Aeromonas hydrophila* and *Burkholderia cepacia* group were isolated from water sources, they were never detected in the infected organs. In contrast, *Pseudomonas aeruginosa* was isolated from deep infected site but never isolated from water sources.

In the present study, bla_{TEM} was detected in the three ESBL producing *E. coli* isolates while bla_{SHV} and bla_{CTX-M} were completely absent. This result is in agreement with recently published data from our lab where the prevalence of bla_{TEM} in 60 ESBL-producing *E. coli* isolates was 73.3% (Alsultan *et al*., 2013).

CONCLUSION

E. coli is more likely to be the causative agent leading to ovarian hydrobursitis syndrome in female camels (*Camelus dromedarus*) in the eastern region of Saudi Arabia. The high prevalence of ESBL producing *E. coli* is mainly due to dissemination of bla_{TEM} in this region. Infection control measure should be strictly applied to prevent the spread of such pathogens. In addition, antibiotic susceptibility testing is highly recommended before treating ovarian hydrobursitis disease in female camels.

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