Isolation of Common Bacteria Causing Urinary Tract Infections in Pet Animals and Human

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Abstract:

The present study was conducted to investigate bacterial causes of urinary tract infections (UTIs) in diseased and apparently healthy pet animals and human. Bacteriological examination of 106 collected urine samples were classified into 6 groups; HUTI, AHCO, AHDO, CUTI, AHC and AHD; revelaed that there were a positive bacteriuria in 59.4% (63samples). The predominant isolated pathogens were *E. coli, S. aureus* and *Klebsiella spp*. *E. coli* recorded the highest incidence with a percentage of 48.1% followed by *S. aureus* (28.3%) followed by *klebsiella spp* (4.7%). All isolates were sensitive to Amikacin, Azithromycin, and Imipenem; intermedaite sensitivity to Amoxacillin/Clavulanic acid and resistant to Cefepime and Cephradine. By using conventional PCR, the isolated *E. coli*, *S. aureus and Klebsiella spp* were molecularly confirmed for the presence *of phoA, 16S rRNA and gyrA* gene, respectively.

Key words: UTIs, Bacteriuria*, FLUTD, E. coli*, *S. aureus, Klebsiella,* Pet's owners, Pet animals.

Introduction

Feline lower urinary tract disease (FLUTD) is one of the most common reasons for cats to be presented for veterinary care. The most dramatic condition of the lower urinary tract of cats is urethral obstruction with subsequent life-threatening postrenal azotemia. This condition occurs almost in male cats, very rarely in females ***(Hostutler et al, 2005).*** The reported prevalence of bacteruria in FLUTD is variable, depending on the inclusion criteria of investigating studies ***(Lekcharoensuk et al, 2001).*** It was recorded that the most common bacterial species of feline and canine urinary tract infections are *E. coli, Klebsiella* spp., *Staphylococcus* spp., *Enterococcus* spp., *Proteus* spp. and *Pseudomonas* spp. ***(Seguin et al, 2003; Litster et al, 2007).*** Asymptomatic bacteriuria is the presence of bacteria in urine in the absence of other features of infection. It increases the risk of developing symptomatic UTI ***(Hooton et al, 2000).*** Cross-species

transmission may be an important epidemiological factor for UTI in dogs and humans ***(Yuri et al, 1999).*** Sharing of both pathogenic and non-pathogenic bacteria between pet and owner has been reported but it is unknown if transmission of bacteria between pet and owner is of clinical significance ***(Damborg et al, 2009 and Johnson et al, 2008)***. Therefore, the aim of this study was to investigate the most common bacteria present in symptomatic UTIs and asymptomatic (apparently healthy) pet animals and human. Also, biochemical identification, antibiotic sensitivity and molecular confirmation of the isolated bacteria. In addition, illustration of the possibility of zoonotic bacterial UTIs among pet's animals and their owners.

Material and methods

1-Sampling from pet animals and human:

A total of 106 urine samples were collected from diseased and apparently healthy human (54 samples) and pet animals (52). Samples were classified into 6 groups; human with UTIs (HUTI, 46 samples both sex), apparently healthy cat's owners (AHCO, 4 samples from females), apparently healthy dog's owners (AHDO, 4 samples from males), cat with UTIs (CUTI, 12 samples from tom cat), apparently healthy cats (AHC, 19; 9 males and 10 females) and apparently healthy Dogs (AHD, 21 samples, 13 males and 8 females).

A bout 10 ml of normal voided middle stream urine samples were collected from diseased patients or pet's owners who admitted to different hospitals in Ismailia Province according to ***(Foxman, 2002)***. Samples from pet animals were collected after carrying out Physical examination at the department of veterinary internal medicine, Suez Canal University. Catheterization or cystocentesis was used to collect urine samples from cat with urine retention while samples from apparently healthy cat and dog were collected through catching of freely voided urine according to ***Radostits et al (2000) and Kurien et al (2004)***.

The collected urine samples in screw capped sterile tubes were centrifuged for 10 min at 3000 rpm and the supernatant were aseptically discarded while the sediments were used for bacterial isolation according to ***Reine and Langston (2005)***.

2. Bacteriological procedures:

A loopful from the urine sediment was streaked on nutrient agar, MacConkey agar, Eosin Methylene Blue agar (EMB) and mannitol salt agar (MSA) ***(Eggertsdottir et al, 2007 and Helina*** *and* ***Manab, 2014).*** Microscopical examination, motility, oxidase, catalase, IMVC, TSI, urease and coagulase tests for the isolated bacteria were done according to ***Cruickshank et al (1975).*** The antibiotic sensitivity testing using disk diffusion technique was applied for the

isolated bacteria by using Muller-Hinton broth and agar and the inhibition zones were interpreted according to ***NCCLS (2002)***.

Molecular confirmation of the isolated bacteria using conventional polymerase chain reaction (PCR) was performed for the isolated bacteria from each group through using specific primer sequence (Table 1). A total of 6 isolates of *E. coli* representative for all group were tested for detection of alkaline phosphatase gene (*phoA*); five representative isolates of *S. aureus* were tested for detection of *16S rRNA* gene and 3 representative isolates of *Kilbsiella spp*.were selected for detection of *gyrA*. DNA extraction was performrd by using commercial extraction kits (QIAamp DNA Mini Kit) and agarose gel electrophoresis was done according to ***Sambrook et al (1989)***.

Results

The bacteriological examination of 106 collected samples revelaed a positive bacteriuria in 59.4% (63/106) and negative bacteriuria in 40.6% (43/106)**.** The prevalence of bacteriuria was 76.1% (35/46), 50%(2/4), 25%(1/4), 66.7%(8/12), 47.4%(9/19) and 38.1%(8/21); respectively in HUTI, AHCO, AHDO, CUTI, AHC and AHD (Table 2).

The predominant pathogens causing bacteriuria in the collected samples were *E. coli, S. aureus* and *Klebsiella spp*. (Table 3). The prevalence of *E. coli* in urine samples was 63% (29), 25% (1), 25% (1), 66.7 (8), 31.6% (6), 28.6% (6); while the prevalence of *S. aureus* was 26.1% (12), 25% (1), 0%, 50 (6), 26.3% (5), 28.6% (6); meanwhile the prevalence of *Klebsiella spp* was 6.5% (3), 0%, 0%, 0%, 5.3% (1), 4.8% (1) respectively according to the corresponding group. *E. coli* recorded the highest incidence with a percentage of 48.1% (51/106) followed by *S. aureus* which recorded 28.3% (30/106) followed by *Klebsiella spp* which recorded 4.7% (5/106).

A total of 86 isolates were recovered from the tested samples. *E. coli* isolates recorded the highest incidence with a percentage of 59.3% (51/86) followed by *S. aureus* which recorded 34.9% (30/86) followed by *Klebsiella spp* which recorded 5.8% (5/86). *E. coli* were recovered with higher incidence from groups (AHDO, HUTI and CUTI) (100%, 65.9% and 57.1%) respactivelly; *S. aureus* were recovered with higher incidence from groups (AHCO, AHD and CUTI) (50%, 46.2% and 42.9%) respectively; while *Klebsiella spp.* were obtained with higher incidences from groups (AHC, AHD and HUTI) (8.3%, 7.7% and 6.8%) respectively as shown in Table (4).

The antibiotic sensitivity for the isolated bacteria showed that all isolates were sensitive to Amikacin**,** Azithromycin, and Imipenem. *E.*

coli and *S. aureus* were sensitive to Ciprofloxacin, and Norofloxacin. Also, *E. coli and Klebsiella spp* were sensitive to Tobramycin and Gentamicin. Moreover, all isolates showed intermedaite sensitivity to Amoxacillin/Clavulanic acid. In addition, resistance to Cefepime and Cephradine were observed in all isolates. Also*, E. coli and Klebsiella spp*. were resistant to Cefotaxime, Cefaclor and Cefuroxime while *S. aureus* and *Klebsiella spp.* were resistant to Vancomycin (Table 5).

Concerning the molecular confirmation of the isolated bacteria, the obtained PCR results revealed that all selected isolates from diseased and apparently healthy human and pet animals were carried species-specific genes, *phoA* gene at 720 bp which is specific for *E. coli* as shown in Figure (1) while *S. aureus* isolates were positive for the presence of *16S rRNA* gene at 791 bp as shown in Figure (2). Also, *Kilbsiella spp.* isolates were positive for the presence of *gyrA* gene at 441 bp as shown in Figure (3).

Table 1: *Oligonucleotide primers sequences source: Midland Certified Reagent Company_oilgos (USA).*

M.O	Gene	Primer Sequence 5'-3'	Amplified product	Reference
E. coli	*phoA*	F: CGATTCTGGAAATGGCAAAAG	720 bp	**Hu *et al.* (2011)**
		R:CGTGATCAGCGGTGACTATGAC		
S. aureus	*16S rRNA*	F:CCTATAAGACTGGGATAACTTCGGG	791 bp	**Mason *et al.* (2001)**
		R:CTTTGAGTTTCAACCTTGCGGTCG		
Klebsiella spp.	*gyrA*	F: CGC GTA CTA TAC GCC ATG AAC GTA	441 bp	**Brisse and Verhoef (2001)**
		R: ACC GTT GAT CAC TTC GGT CAG G		

Table 2: *Prevelance of bacteriuria in urine samples:*

Group	No of samples	Positive samples	
		No.	%
HUTI	46	35	76.1
AHCO	4	2	50.0
AHDO	4	1	25.0
CUTI	12	8	66.7
AHC	19	9	47.4
AHD	21	8	38.1
Total	106	63	59.4

Table 3: *prevalence of isolated bacteria from different groups:*

Group	No of samples	*E. coli*		*S. aureus*		*Klebsiella spp.*	
		No.	%	No.	%	No.	%
HUTI	46	29	63.0	12	26.1	3	6.5
AHCO	4	1	25.0	1	25.0	0	0.0
AHDO	4	1	25.0	0	0.0	0	0.0
CUTI	12	8	66.7	6	50.0	0	0.0
AHC	19	6	31.6	5	26.3	1	5.3
AHD	21	6	28.6	6	28.6	1	4.8
Total	106	51	48.1	30	28.3	5	4.7

Table 4: *Percentage of different isolated bacteria in different groups:*

Group	No of isolates	*E. coli*		*S.aureus*		*Klebsiell spp*	
		No.	%	No.	%	No.	%
HUTI	44	29	65.9	12	27.3	3	6.8
AHCO	2	1	50.0	1	50.0	0	0.0
AHDO	1	1	100	0	0.0	0	0.0
CUTI	14	8	57.1	6	42.9	0	0.0
AHC	12	6	50	5	41.7	1	8.3
AHD	13	6	46.2	6	46.2	1	7.7
Total	86	51	59.3	30	34.9	5	5.8

Table 5: *Antibiotic sensitivity test for identified bacteria from bacteriuria samples*

Antibiotic	*E. coli*			*S. aureus*			*Klebsiella spp*		
	S	I	R	S	I	R	S	I	R
Amikacin	+++			+++			+++		
Amoxacillin/ Clavulanic acid		+			+			+	
Azithromycin	+++			+++			+++		
Ciprofloxacin	+++			+++					-ve
Cefotaxime			-ve		+				-ve
Cefepime			-ve			-ve			-ve
Cephradine			-ve			-ve			-ve
Cefaclor			-ve		+				-ve
Cefuroxime			-ve		+				-ve
Gentamycin	+++					-ve	+++		
Imipenem	+++			+++			+++		
Levofloxacin	+++				+				-ve
Norofloxacin	+++			+++					-ve
Tobramycin	+++					-ve	+++		
Vancomycin		+				-ve			-ve

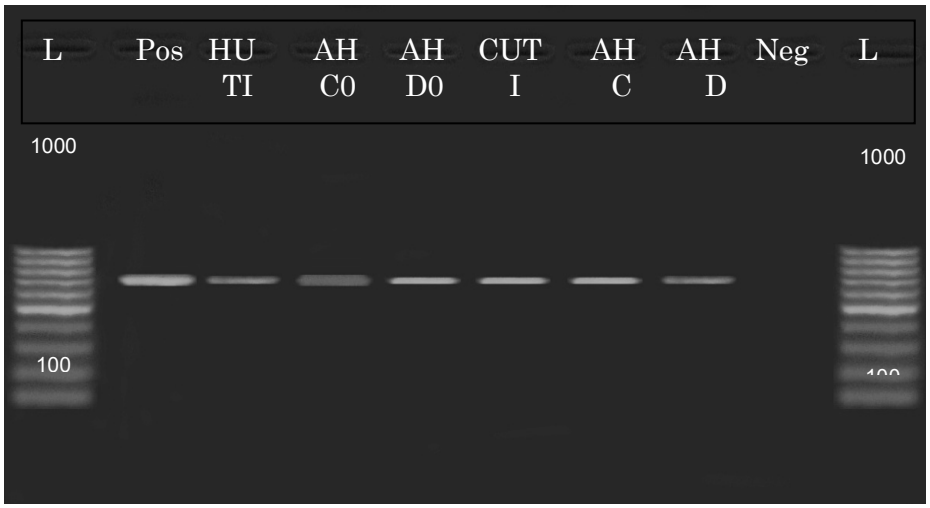

Figure 1: *Agarose gel electrophoresis of amplified phoA gene of E. coli at 720 bp. Lane 1: Marker (m.w 100-1000), Lane 2: Positive control, Lane 3-8: Tested isolates. Lane 9: Negative control.*

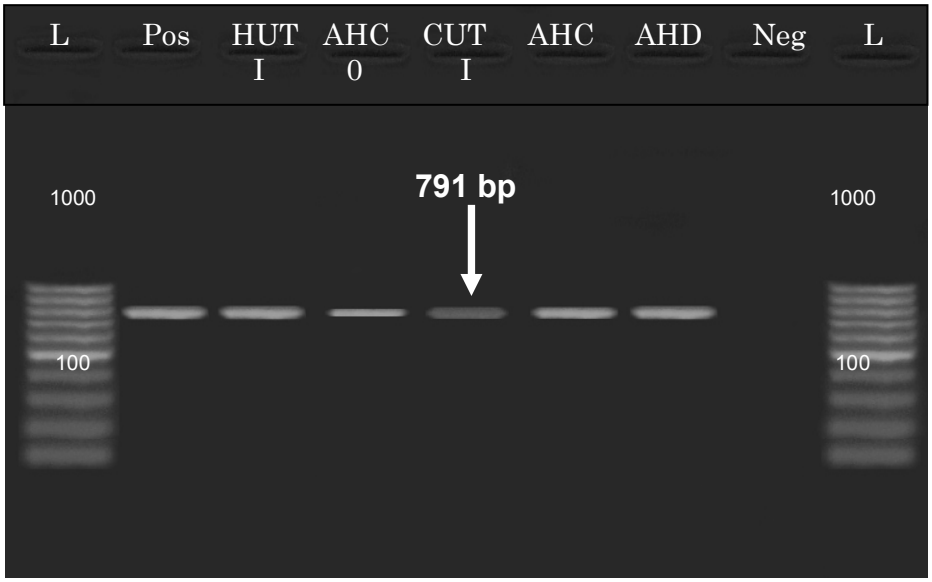

Figure 2: *Agarose gel electrophoresis of amplified 16S rRNA gene of S. aureus at 791bp. Lane 1: Marker (m.w 100-1000), Lane 2: Positive control, Lane 3-7: Tested isolates. Lane 8: Negative control.*

Figure 3: *Agarose gel electrophoresis of amplified (gyrA) gene of Klelbsiella spp. Lane 1: Marker (m.w 100-600), Lane 2: Positive control, Lane 3-5: Tested isolates. Lane 6: Negative control.*

Discussion

In the present study, the incidence of bacteriuria was 76.1%, 50%, 25%, 66.7%, 47.4% and 38.1%, repectively. In HUTI, the reported incidene (76.1%) was near the percentage reported by ***Kahlmeter (2003)*** who mentioned that the prevalence of bacteriuria in patients suffered from UTIs was 69.2%. The incidence of asymptomatic bacteriuria in AHCO and AHDO was (50%) and (25%) that was in agreement with ***Jakob et al (2012)*** who mentioned that women who had cat or dog in the home had a different vaginal flora, in particular with increased *E. coli* colonization**.** Concerning CUTI, the incidence of bacteriuria was 66.7%. This was in agreement with the study of ***Eggertsdóttir et al (2007)*** who mentioned that 33% of cats suffered from FLUTD showed positive bacteriuria.

The incidence of bacteriuria in asymptomatic cats was 47.4% that was in accordance with ***Litster et al (2009)*** who reported incidence of bacteriuria in urine specimens collected from healthy cats was 28.9% some completely asymptomatic cats presented for routine geriatric checkups had culture positive urine. Also, the prevalence of subclinical bacteriuria in healthy dogs was 38.1%,

That was inagreement with ***(Stephanie et al, 2014)*** who reported a prevalence of 8.9% positive bacteriuria in asymptomatic dogs. Also, up to 95% of UTIs in dogs are clinically silent as mentioned by ***McGuire et al (2002)*** *and* ***Seguin et al (2003).***

E. coli was the most common bacteria isolated from bacteriuria cases. The total prevalence of *E. coli* was 48.1% (51/106 samples) meanwhile, *E. coli* isolates recorded the highest incidence with a percentage of 59.3% (51/86) (Table 3&4). These findings were in agreement with, ***Shalini et al (2011)*** who found that the most common organisms isolated from patients with UTIs were *E. coli* which represented by 64.3% of the isolates. In addition, *E. coli* was accounted to 77.0% of isolates according to ***Kahlmeter (2003)*** and 80% to 85% of the UTIs infections according to ***Vasudevan (2014).*** The faecal flora of humans and animals are considered a reservoir of UPEC ***(Johnson et al, 2003)*** that colonize the intestine before causing an ascending UTI ***(Yamamoto, 2007).***

Concerning the prevalence of *S. aureus*, it recorded 28.3% (30/106 samples). Moreover, the isolates of *S. aureus* were recorded 34.9% (30/86) (Table 3&4). These findings were in agreement with those previously reported by ***Vasudevan (2014)*** who mentioned that Staphylococcus species are one of the major pathogens of UTIs that constitutes to 10% to 15%; 0.5% ***(Barrett et al, 1999),*** 1.3% ***(Goldstein, 2000)*** and 6.3%

(Shalini et al, 2011) of total isolates**.**

The prevalence of *klebsiella spp.* was 4.7% (5/106 samples). Also, *klebsiella spp* isolates recorded 5.8% (5/86) (Table 3&4). The urinary tract is the most common site of infection for *Klebsiella*. *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) ***(Lye et al, 1992).***

The antibiotic sensitivity of isolated bacteria revealed that all isolates were sensitive to Amikacin**,** Azithromycin, and Imipenom; intermedaite sensitive to Amoxacillin/ Clavulonic acid and resistant to Cefepime and Cephradine were observed in all isolates (Table 5). The obtained results were in agreement with those previously reported by ***Shalini et al (2011)*** who reported that 80% of *E. coli, klebsiella* and *S. aureus* that isolated from patients with UTIs were sensitive to amikacin, while more than 70% were sensitive to norfloxacin, ciprofloxacin and levofloxacin and very high rate of resistant was reported in amoxicillin and amoxi-clav. There is an increasing trend of resistance to many antimicrobials including the fluoroquinolones ***(Kahlmeter, 2003).*** The increase in bacterial resistance to fluoroquinolone is multifactorial especially in patients who have received fluoroquinolone prophylaxis ***(Tabibian et al, 2008).***

Concerning the PCR results of *E. coli,* alkaline phosphatase *(phoA)* gene at 720 bp was confirmed in the selected isolates from all groups (Figure 1). These results were inagreement with those previously reported by ***Yu and Thong (2009)*** who mentioned that *phoA* gene is a housekeeping gene present in all *E. coli* strains. Concerning *16S rRNA* gene of *S. aureus*, the selected isolates from the defiened groups were positive for the presence of this gene at 791 bp which is specific for *S. aureus* (Figure 2). These results were inagreement with previously reported by ***Clarridge (2004)*** who mentioned that *16S rRNA* of *S. aureus* is characterized by genetically homogeneous.

In regard to *gyrA* of *Kilbsiella spp.*, the selected isolates were positive for this gene at 441 bp (Figure 3). These results were in accordance with those previously reported by ***Brisse and Verhoef (2001)*** who classify *Klebsiella pneumonie* according to nucleotide variations of the *gyrA*.

Conclusion

The obtained PCR results as well as cultural methods for isolation of bacteria confirmed that there were common bacterial pathogens incriminated in UTIs in human and pet animals. Zoonotic transmission of these bacteria may increase the risk of UTIs in human in contact with these animals.

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الملخص العربى
عزل البكتريا المسببة لعدوى الجهاز البولى فى الحيوانات المنزلية والإنسان

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أجريت هذه الدراسة لعمل استبيان على البكتريا المسببة لعدوى الجهاز البولى فى الحيوانات المنزلية والانسان حيث تم تجميع عدد ١٠٦ عينة بول من كل من الانسان والحيوانات المنزلية تم تقسيمهم الى ٦ مجموعات. تم فحص عينات البول بكتريولوجيا وأوضحت النتائج ان نسبة العينات الموجبة لوجود البكتريا كانت ٥٩,٤%.وقد أظهرت نتائج العزل والتصنيف عزل عدد (٥١ من ١٠٦) من بكتريا الايشريشيا كولاى *E. coli* بنسبة (٤٨,١%) وعدد (٣٠ من ١٠٦) من المكورات العنقودية الذهبية بنسبة (٢٨,٣ %). و عدد (٥ من ١٠٦) من بكتريا الكلبسيلا بنسبة (٤,٧ %). كما أوضحت اختبارات الحساسية للمضادات الحيوية للعترات المعزولة حساسية الميكروبات لكل من الأميكاسين والايزيثرومايسين والاميبينيم وكانت مقاومة لكلا من السيفبيم والسيفادرين.هذا وقد اوضح تحليل انزيم البلمرة المتسلسل ايجابية العينات المعزولة من المجموعات المختلفة لوجود جين *phoA* الخاص ببكتريا الايشريشيا كولاى *E. coli* و*16 S rRNA* الخاص بالمكورات العنقودية الذهبية و **gyrA** الخاص بالكلبسيلا.