



Current Research in Bacteriology

ISSN 1994-5426

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Incidence and Antibiotic Susceptibility Pattern of *Staphylococcus* spp. in Urinary Tract Infections (UTI), IRAN, 2013-2014

¹Mohammad Erfani, ²Dariush Ghasemi, ²Reza Mirnejad and ^{1,2}Vahhab Piranfar

¹Department of Biology, Tonekabon Branch, Islamic Azad University of Tonekabon, Tonekabon, Iran

²Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Corresponding Author: Vahhab Piranfar, Department of Biology, Tonekabon Branch, Islamic Azad University of Tonekabon, Tonekabon, Iran Tel: 0098-21-82482554

ABSTRACT

The persistence of this study is to investigate the pattern of antibiotic resistance *Staphylococcus* spp. causing urinary tract infection in 1675 of samples in common pathogens that cause urinary tract infection. This study is the first to evaluate the incidence of antibiotic resistance is such a large number of samples in Iran. The susceptibility of samples obtained from 14332 patients with urinary tract infections admitted to different medical diagnostic laboratories of Iran, was measured using disk diffusion method for 18 common antibiotics and multiplex PCR for *Staphylococcus* identification. Most of the identified *Staphylococcus* spp. were *Staphylococcus saprophyticus* (949 samples) and *Staphylococcus epidermidis* (481 samples). The most resistant antibiotics were identified as methicillin and penicillin, 84.1 and 94.2%, respectively. The findings of this study indicate that *Staphylococcus* spp. were second cause of common infection among outpatients. There are also bacteria with high resistance that is but interfere with prescription of drugs in order to treat urinary tract system infection. Also increasing of resistance to drugs among bacterial pathogens is evolving and requires an inspectoral and research procedure which could provide more information for doctors to treat the infection more efficiently.

Key words: *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, UTI, antibiotics

INTRODUCTION

Bacterial Urinary Tract Infection (UTI) is the most common infection in urinary tract, which causes bladder and renal inflammation (Bien *et al.*, 2012). *Escherichia coli* is the most common agents for this infection, which usually can be found in gastrointestinal tract (Kaper *et al.*, 2004); but today other bacteria, such as *Staphylococcus* spp. can cause such infections, which is gradually increasing (Megged, 2014).

Species, such as *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Staphylococcus epidermidis* cause urinary tract infection mostly in young women and old men and rarely in children (Kaper *et al.*, 2004; Upadhyayula *et al.*, 2012; Sousa *et al.*, 2013; Megged, 2014). *Staphylococcus aureus* is the main cause of UTIs among inpatients and also is the second cause of common infection among outpatients (Onanuga and Awhowho, 2012). These bacteria are also cause of nosocomial pneumonia and are the second reason for septicemia throughout the world (Totsika *et al.*, 2012).

The importance of *Staphylococcus* spp. is not only because of its pathogenicity but these species show high resistance in the treatment of UTIs (Ferreira *et al.*, 2012; Lunacek *et al.*, 2014). Hence, considering the situation of this pathogenic agents and evaluating its risk factors are of great importance.

Considering the great importance of resistant *Staphylococcus* spp., the current study aimed to evaluate and compare antibiotic susceptibility pattern of three species of *Staphylococcus* spp. in a great population of patients with UTIs.

MATERIALS AND METHODS

In a sectional descriptive study of 14322, randomly selected patients suffering from urinary tract infection, referred to medical laboratories in Tehran, from March, 2013 to September, 2014, urine culture test was performed. Culture was prepared using the median urinary of the patients referred to Tehran's laboratories.

Isolation and chemical and molecular identification: *Staphylococcus* species were isolated from blood samples and cultured in blood agar. Plates were incubated in 37°C for 24 h and those containing 10⁵ colonies were reported as positive. Negative plates were re-incubated for more 24 h. Microbiological and biochemical standard tests, such as carbohydrate fermentation and catalase and oxidase tests, were conducted to identify species. Morphological and microscopic examinations also were performed.

Multiplex Polymerase (PCR) Chain Reaction was done using the DNA amplification instrument master cycler gradient (Eppendorf, Germany) for detection of *Staphylococcus* species. Genomic DNA were obtained from *Staphylococcus* species colonies grown overnight on blood agar (Merck Co., Germany) plates by DNA extraction kit (Bioneer Co., Korea) according to manufacturers's but instruction. Specific primer pairs for amplification of these genes are listed in Table 1. This method described by Barry *et al.* (1991) also and Couto *et al.* (2001).

A volume of 1.5 µL of extracted genomic DNA was added to a total volume of 25 µL PCR reaction mixture including 2.5 µL of 10×PCR buffer, 1.5 µL MgCl₂ (50 mM), 0.5 µL dNTPs (10 mM), 1.25 µL of each primer, 0.5 µL of Taq DNA polymerase (5 U µL⁻¹) (Amplicon Co., Denmark) and 16 µL sterile distilled water. The reaction mixture was performed in a thermal gradient cyler (Eppendorf, Germany) with the following PCR protocol: Denaturation at 94°C for 4 min, 30 cycles with denaturation at 94°C for 31 sec, annealing at 52°C for 31 sec, extension at 72°C for 45 sec and final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis in 1.5% agarose gel for 35 min in Tris-acetate buffer and visualized by ethidium bromide staining. *Salmonella enterica* ATCC₉₂₇₀ and Human blood DNA were used as a Negative control for Mutiplex-PCR (Fig. 1).

Table 1: Oligonucleotide sequences for primers used to detect *Staphylococcus* spp.

Markers	Orientation	Primer (5-3)	Amplicon size (bp)	Reference
23S rRNA	Forward	ACGGAGTTACAAAGGACGAC	1250	Straub <i>et al.</i> (1999)
	Reverse	AGCTCAGCCTTAACGAGTAC		
<i>Staphylococcus aureus</i>	Forward	AATTGCGTCAACAGCAGATGCGAG	450	Sunagar <i>et al.</i> (2013)
	Reverse	GGACGTGCACCATATTCGAATGTACC		
<i>Staphylococcus saprophyticus</i>	Forward	AAAAGAAATGTATTGTGCTAAAAGGAA	950	Kuroda <i>et al.</i> (2005)
	Reverse	AAAAATCAGTCATTTGCTACTTAGTAGGT		
<i>Staphylococcus epidermidis</i>	Forward	AGTACAGAACCGTTATGCCTGGCT	720	Sunagar <i>et al.</i> (2013)
	Reverse	TGATGAGTCAATTCGTGCTCCCGT		

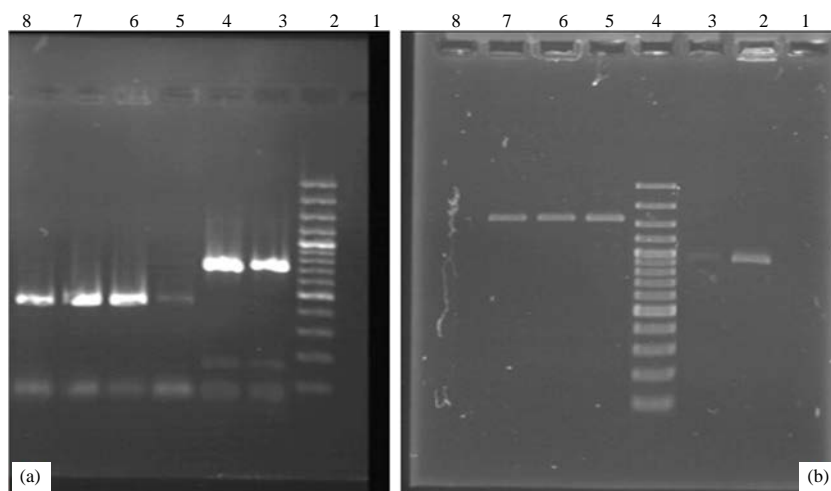


Fig. 1(a-b): Agarose gel electrophoresis of PCR amplified products generated from DNA Samples, A: Lane 2 shows DNA size marker (100 bp DNA ladder), Lane 1 is negative control, Lanes 3 and 4 show amplified *S. epidermidis* 720 bp, Lane 5, 6, 7 and 8 show amplified *S. aureus* 450 bp and B: Lane 2 shows DNA size marker (100 bp DNA ladder), Lane 1 and 8 are negative control, Lanes 2 and 3 show amplified *S. saprophyticus* 950 bp, Lane 5, 6 and 7 show amplified 23S rRNA 1250 bp

Antibiotic susceptibility tests: Drug resistance evaluation was carried out using disk diffusion method and Kirby Bauer method on Mueller-Hinton medium (Merck, Germany) (Lunacek *et al.*, 2014). After inoculating the bacteria on Muller Hinton agar and placing the antibiotic disks, plates were incubated for 24 h in incubator. Then, according to the size of the growth inhibition zone around the disks and international numbers of (CLSI-2011), results were categorized and reported in three groups: Susceptible and sensitive (S), intermediate susceptibility or sensitivity, (I) and resistance (R).

Data analysis: Data were analyzed using IBM SPSS Statistics version 20.0.0. Discrete variables were expressed as percentages. Proportions were compared using the Chi-square test. Statistical significant difference was considered at value of $p \leq 0.05$.

RESULTS AND DISCUSSION

In the current study, 1675 (11.69%) *Staphylococcus* spp. were isolated out of 14322 positive samples among which 245 (14.62%) were *S. aureus*, 481 (28.71%) were *S. epidermidis* and 949 (56.65%) were *S. saprophyticus* species (Fig. 2). This rate of prevalence is higher than in the other studies, which accounts for 9.68% (Prakash and Saxena, 2013), 7.8% (Linhares *et al.*, 2013), 6.9% (Farajnia *et al.*, 2009) and lower rate in the other study which accounts for 13% (Khoshbakht *et al.*, 2012) and 12.7% (Yasemi *et al.*, 2014). The prevalence of isolations in patients with UTIs is shown in Fig. 2. The rate of contamination with *S. aureus*, *S. epidermidis* and *S. saprophyticus* in men and women are 24.09, 75.91, 14.76 and 85.24, 16.55, and 83.45%, respectively (Fig. 3). No significant relationship between UTIs and causing agents was observed, except for *S. epidermidis* in the age group lower than 10 years.

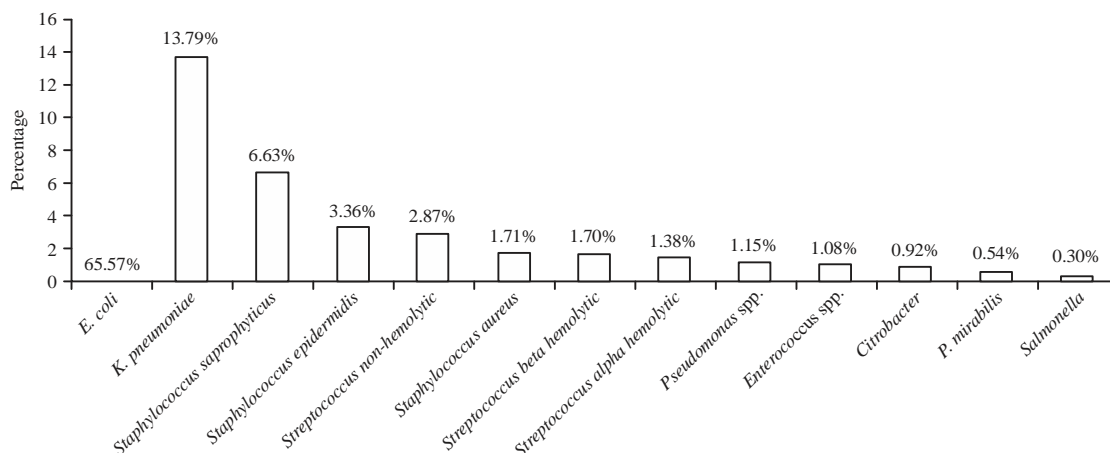


Fig. 2: Prevalence of bacterial urinary tract infections, red line were *Staphylococcus* spp.

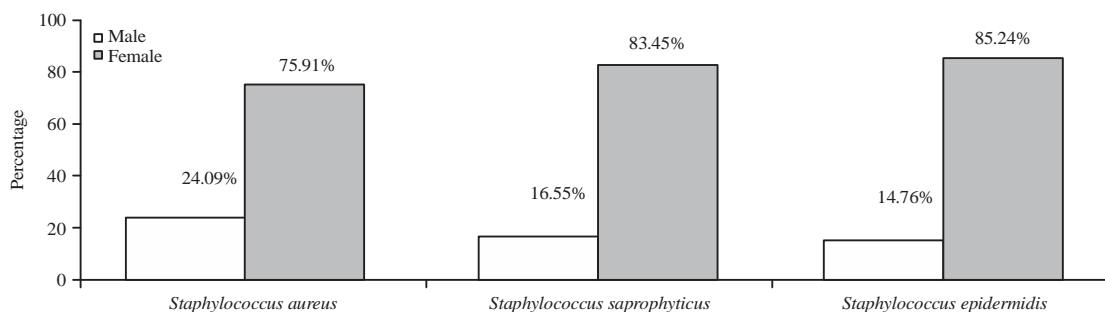


Fig. 3: Prevalence of bacterial urinary tract infections between genders

Antibiotic susceptibility testing results: In the current study, different routine antibiotics used against these bacteria in Iran were examined and the results is shown in Fig. 4a-c. Accordingly, *S. aureus* and *S. epidermidis* showed resistance to methicillin and penicillin, 84.1 and 94.2%, respectively (Fig. 4a). Significant resistance to penicillin was also observed in *S. epidermidis* as 93.3% (Fig. 4b).

Twenty-four different antibiotics were used to evaluate the susceptibility pattern of *S. saprophyticus*, which among them the highest rates of resistance were observed to methicillin and cefixime with 95.4 and 91.1%, respectively.

In similar study, Khoshtakht *et al.* (2012) shown that *S. saprophyticus* isolates, as the most frequent Gram positive bacteria in UTIs, exhibited high resistance to ampicillin, tetracycline and erythromycin (92.31%) and high susceptibility to nitrofurantoin and vancomycin (92.3%) (Khoshtakht *et al.*, 2012).

Staphylococcus spp. can be isolated from various environments (Sunagar *et al.*, 2013). They are the most common medical microorganisms and are one of the most important human pathogenic agents. These bacteria are one of the skin normal flora. Kuroda *et al.* (2005) but it has not prevented wide resistance of these bacteria against antibiotics yet (Davies and Davies, 2010). In addition to huge advances and innovation of new antibiotics, significant resistance against *Staphylococcus* spp. was emerged. The current study was conducted on the numerous staphylococcal species isolated from patients who referred to medical laboratories in Tehran, Iran. Results of the current study showed increasing resistance of these bacteria against routine antibiotics.

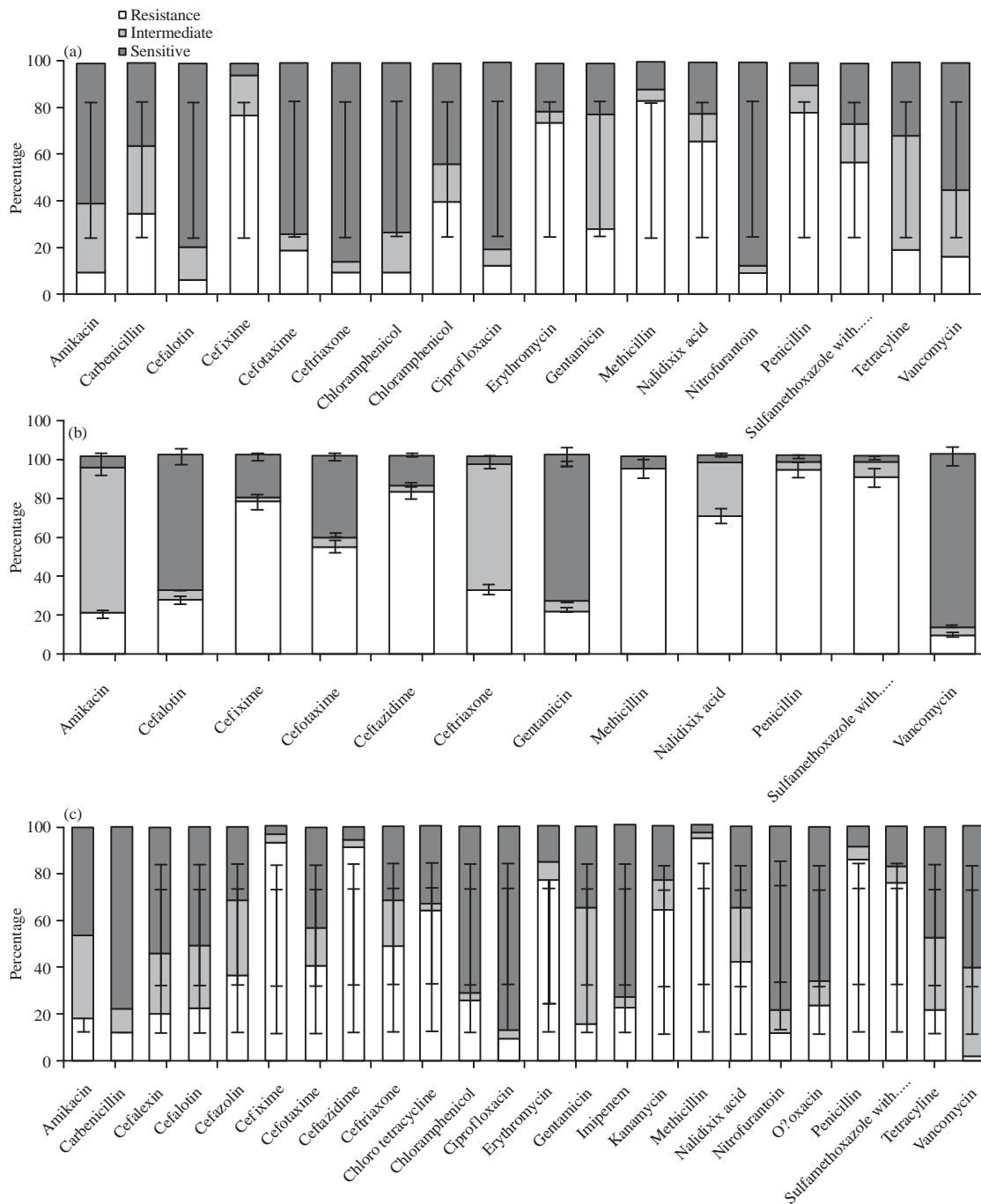


Fig. 4(a-c): (a) Antibiotic susceptibility pattern of *Staphylococcus aureus*, (b) *Staphylococcus epidermidis* and (c) *Staphylococcus saprophyticus*

Former studies showed that the rate of contamination with *S. aureus* in women is higher than men and also in summers is significantly higher than other seasons (Kuroda *et al.*, 2005; Sood and Gupta, 2012). This is due to women short urinary tract and lack of hygiene during hot season.

Transmission of contamination from anus to vagina may be considered as another risk factor for these diseases. Sexual intercourse may also transfer infection to vagina and cervix. Since, these commensal agents are usually localized around urinary tract, observing personal hygiene, especially during hot season, can prevent probable infections.

Results of the current study showed no significant relationship between UTIs and causing agents in age groups over 10 years; *S. saprophyticus* was the most prevalent species among all age groups and genders and *S. epidermidis* was not observed in the age group lower than 10 years. This results confirmed with Farajnia *et al.* (2009).

Resistance to methicillin has been reported frequently; therefore, it does not recommend treating UTIs. Also, considering the prevalent resistance pattern of bacteria against antibiotics, using penicillin, nalidixic acid, cefixime, erythromycin and sulfamethoxazole are not recommended in single-drug therapies. In the case of improper administration of mentioned antibiotics, bacteria will gradually show complete resistance and will become inapplicable, like methicillin, in further studies.

Considering the moderate resistance of *S. saprophyticus* against gentamicin and vancomycin, further wide resistance against these antibiotics is estimated (Fig. 4c). However, vancomycin (86.1%) and gentamicin (74.0%) are the most effective antibiotics against *S. epidermidis* and it is estimated that amikacin and ceftriaxone lose their efficiency on these bacteria soon. Antibiotics such as nitrofurantoin, ceftriaxone and ciprofloxacin are the most effective ones against *S. aureus* and it is estimated that tetracycline and gentamicin lose their efficiency on these bacteria soon.

REFERENCES

- Barry, T., G. Colleran, M. Glennon, L.K. Dunican and F. Gannon, 1991. The 16s/23s ribosomal spacer region as a target for DNA probes to identify eubacteria. *PCR Methods Appl.*, 1: 51-56.
- Bien, J., O. Sokolova and P. Bozko, 2012. Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *Int. J. Nephrol.* 10.1155/2012/681473
- Couto, I., S. Pereira, M. Miragaia, I.S. Sanches and H. de Lencastre, 2001. Identification of clinical staphylococcal isolates from humans by internal transcribed spacer PCR. *J. Clin. Microbiol.*, 39: 3099-3103.
- Davies, J and D. Davies, 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.*, 74: 417-433.
- Farajnia, S., M.Y. Alikhani, R. Ghotaslou, B. Naghili and A. Nakhband, 2009. Causative agents and antimicrobial susceptibilities of urinary tract infections in the Northwest of Iran. *Int. J. Infect. Dis.*, 13: 140-144.
- Ferreira, A.M., M.F. Bonesso, A.L. Mondelli, C.H. Camargo and M.L.R.S. Cunha, 2012. Oxacillin resistance and antimicrobial susceptibility profile of *Staphylococcus saprophyticus* and other staphylococci isolated from patients with urinary tract infection. *Chemotherapy*, 58: 482-491.
- Kaper, J.B., J.P. Nataro and H.L.T. Mobley, 2004. Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.*, 2: 123-140.
- Khoshbakht, R., A. Salimi, H.S. Aski and H. Keshavarzi, 2012. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Karaj, Iran. *Jundishapur J. Microbiol.*, 6: 86-90.
- Kuroda, M., A. Yamashita, H. Hirakawa, M. Kumano and K. Morikawa *et al.*, 2005. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc. Nat. Acad. Sci. USA.*, 102: 13272-13277.

- Linhares, I., T. Raposo, A. Rodrigues and A. Almeida, 2013. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: A ten-year surveillance study (2000-2009). *BMC Infect. Diseases*, Vol. 13, No. 1. 10.1186/1471-2334-13-19
- Lunacek, A., U. Koenig, C. Mrstik, C. Radmayr, W. Horninger and E. Plas, 2014. Unexpected multidrug resistance of methicillin-resistant *Staphylococcus aureus* in urine samples: A single-center study. *Korean J. Urol.*, 55: 349-353.
- Megged, O., 2014. *Staphylococcus aureus* urinary tract infections in children are associated with urinary tract abnormalities and vesico-ureteral reflux. *Pediatr. Nephrol.*, 29: 269-272.
- Onanuga, A. and G.O. Awhowho, 2012. Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract infections in Yenagoa, Nigeria. *J. Pharm. Bioallied Sci.*, 4: 226-230.
- Prakash, D. and R.S. Saxena, 2013. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut city, India. *ISRN Microbiol.* 10.1155/2013/749629
- Sood, S. and R. Gupta, 2012. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. *Indian J. Community Med.*, 37: 39-44.
- Sousa, V.S.D., R.F. Rabello, R.C. da Silva Dias, I.S. Martins and L.B.G. da Silva dos Santos *et al.*, 2013. Time-based distribution of *Staphylococcus saprophyticus* pulsed field gel-electrophoresis clusters in community-acquired urinary tract infections. *Memorias Instituto Oswaldo Cruz*, 108: 73-76.
- Straub, J.A., C. Hertel and W.P. Hammes, 1999. A 23S rDNA-targeted polymerase chain reaction-based system for detection of *Staphylococcus aureus* in meat starter cultures and dairy products. *J. Food Prot.*, 62: 1150-1156.
- Sunagar, R., S.N. Deore, P.V. Deshpande, A. Rizwan and A.D. Sannejal *et al.*, 2013. Differentiation of *Staphylococcus aureus* and *Staphylococcus epidermidis* by PCR for the fibrinogen binding protein gene. *J. Dairy Sci.*, 96: 2857-2865.
- Totsika, M., D.G. Moriel, A. Idris, B.A. Rogers and D.J. Wurpel *et al.*, 2012. Uropathogenic *Escherichia coli* mediated urinary tract infection. *Curr. Drug Targets*, 13: 1386-1399.
- Upadhyayula, S., M. Kambalapalli and B.I. Asmar, 2012. *Staphylococcus epidermidis* urinary tract infection in an infant. *Case Rep. Infect. Dis.* 10.1155/2012/983153
- Yasemi, M., H. Peyman, K. Asadollahi, A. Feizi and S. Soroush *et al.*, 2014. Frequency of bacteria causing urinary tract infections and their antimicrobial resistance patterns among pediatric patients in Western Iran from 2007-2009. *J. Biol. Regul. Homeost. Agents*, 28: 443-448.