The incidence of some virulence factors among thebacterial isolates from the patients with prostatitis and their relation with antibiotic susceptibility

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الخلاصة

تتعرض غدة البروستات الى الالتهاب والذي قد يكون جرثوميا او غير جرثومي وذلك لقدرة الجراثيم على انتاج عدد من عوامل الضراوة المساهمة في تطور الاصابة ، جرثومة *Escherichia coli* تعد العامل الرئيسي المسبب لخمج البروستات وقد لوحظ بان السلالات المقاومة للمضادات الحياتية من هذه الجرثومة تكون اقل ضراوة من السلالات الحساسة .

تهدف الدراسة الحالية الى معرفة انتشار ثلاثة من عوامل الضراوة kpsMTII , USP & CNF-1 في الجراثيم المعزولة من المرضى المصابين بخمج البروستات وعلاقتها بالمقاومة للمضادات الحياتية .

استخدمت (11) عزلة جرثومية معزولة من نماذج الادرار الخاصة بمرضى خمج البروستات تتضمن (10) عزلات من جرثومة *E. coli* وعزلة واحدة من جرثومة *Enterobacteraerogenes* ، استخدمت طريقة الانتشار باستخدام اقراص المضادات لتحديد حساسية الجراثيم للمضادات الحياتية ، كما استخدمت طريقة تفاعل انزيم البلمرة التسلسلي لتحديد وجود جينات الضراوة في الجراثيم المعزولة .

اظهرت العزلات حساسية عالية للمضاد اميكاسين (81,81)% فيما كانت العزلات عالية المقاومة للمضادات الاخرى وكالاتي : امبيسيلين و الكوترايموكسازول (100)% لكل منهما ، الازيثرومايسين و السيفوتاكسيم (81,81)% لكل منهما ، السايبروفلوكساسين (72,72)% والجنتامايسين (63,63)% . كان عامل الضراوة kpsMTII الاكثر انتشارا (72,72)% بين العوامل المدروسة يليه USP وبمعدل (18,18)% فيما لم يتم تحديد الجين المسؤول عن انتاج ال CNF-1 في العزلات المدروسة .

ظهر من خلال النتائج اعلاه بان الجراثيم المعزولة من المرضى المصابين بخمج البروستات في الدراسة الحالية كانت قليلة الضراوة مع قدرة عالية على مقاومة المضادات الحياتية.

Abstract

Background: Inflammation is observed in theprostate tissue from patients with prostatitis and the bacteria may involving due to their ability to produce virulence factors. Recent studies have suggested that the decrease in the pathogenicity of *Escherichia coli* is due to acquisitionof resistance to some antibiotics. This study was performed to investigate three virulence factors includingcytotoxic necrotizing factor-1(*cnf*-1),uropathogenic specific protein (*usp*) and group II capsule(*kpsMT*II) in bacterial isolates from the patients with prostatitis and determine their antibiotics susceptibility patterns .

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Methods: The drug sensitivity of (11) bacterial isolates including (10)*E. coli* isolates and (1) *Enterobacteraerogenes* isolate from patients with prostatitiswere evaluated by using a disc diffusion method. The prevalence ofvirulence genes (*cnf*-1, *usp*and*kpsMTII*) was determined by PCR.
Results: The isolates were highly sensitive to amikacin (81.81%).But the isolates showed a high resistant to other antibiotics , ampicillin (100%), Co-trimoxazole (100%) , Azithromycin (81.81%) , Cefotaxime (81.81%) , Ciprofloxacin (72.72%) and Gentamicin (63.63%) . PCR showed that *kpsMTII* (72.72%) was more prevalent than *usp*(18.18%) and the *cnf-1* was not detected in all isolates .
Conclusion: We propose that the isolates from patients with prostatitis were low virulent in combination with high resistance to many antibiotics.

Introduction

The prostate is a male specific genital accessory gland located at the bottom of bladder help in the production of semen . Prostate undergo some disorders like : prostatitis , benign prostatic hyperplasia and prostate cancer [1,2] .Prostatitis is an inflammation of prostate gland, inflammation may be infectious or inflammatory in origin [3,4].

The first and second categories are infectious caused by bacteria , Bacterial pathogens that causes prostatitis are usually the same of that causes urinary tract infections (UTI) , *E. coli* is a predominant causative agent of bacterial prostatitis , other enteric bacteria such as klebsiella , enterobacter and proteus and *Pseudomonas aeruginosa* are also be documented[5,6]. Uropathogenic*E. coli* is the common cause of bacterial prostatitis , this bacterium has many virulence factors such as hemolysin , cytotoxic necrotizing factor-1, uropathogenic specific protein and kpsMTII capsule which are associated with pathogenicity of bacteria and pathological effects in prostate tissues as illustrated in mouse models [7 ,8,9] .

Cytotoxic necrotizing factor type 1 (CNF1) is a chromosomally encoded toxin constitutively activates small GTPases of the Rho family [10].Its effects involving a variety of cellular functions such as enhancement phagocytosis in epithelial cells [11,12] . Several studies showed that the kpsMTII capsule was high prevalent among *E. coli* strains that causes UTI including prostatitis , The researchers [13] found that the 53.1% of isolates that causes cystitis were have *kpsMTII* gene .*Usp gene* encodes a 346-amino acid protein designated as uropathogenic specific protein (usp). This protein may represent a novel type of *E. coli* bacteriocin, acting against competing *E. coli* strains that occupy the same niche, thereby enhancing their infectivity in the urinary tract environment[14] .

The antibiotics represents the first choice of treatment of bacterial prostatitis ,some antibiotics are poorly penetrate prostatic capsule , others like :

ciprofloxacin , co-trimoxazole and tetracyclines are penetrate well [15] . Most antibiotics are either weak acids or bases that ionize in biological fluids, which inhibits their crossing prostatic epithelium . Only free, non-protein-bound antibiotic molecules enter tissues [16,17] . It's should use bacteriocidal rather than a bacteriostatic antibiotics in acute life threatening infections , sever ill patients require to hospitalization [18].

This study was performed to investigate three virulence factors including cytotoxic necrotizing factor-1 (*cnf*-1), uropathogenic specific protein (*usp*) and group II capsule (*kpsMT*II) in bacterial isolates from the patients with prostatitis and determine their antibiotics susceptibility patterns .

Material and methods

Bacterial isolates

Eleven bacterial isolates involving (10) *E. coli* isolates and (1) isolates of *E. aerogenes*were collected from (60) post massage urine (VB3) specimens of the patients with bacterial prostatitis in Al-Hussein Teaching Hospital in Thi-Qar province through the period from February 2011 to July 2011.

Antibiotic susceptibility test

The drug sensitivity of (11) *E. coli* isolates from patients with prostatitis were evaluated using a disc diffusion method.The antibiotics that including in the current study were : ampicillin (AMP) , co-trimoxazole (SXT) , azithromycin (AZM) , cefotaxime (CTX) , ciprofloxacin (CIP) amikacin (AK) and gentamicin (CN) .

DNA extraction

This performed according to manufacturer instruction of DNA extraction kit (Promega – USA) .

Detection of the virulence genes

The prevalence of virulence genes (*cnf*-1, *usp*and*kpsMTII*) was determined by PCR.DNA primers[13] manufactured by (Bioneer-Korea) that illustrated in table (1) were used in detection of the virulence genes in this study.

Table (1): Primers used in detection of bacterial virulence factors

Gene	Primer sequence (5□-3□)	Size of product bp
cnf-f *cnf*-r	GAA CTT ATT AAG GAT AGT CAT TAT TTA TAA CGC TG	543
usp –f *usp*- r	ATG CTA CTG TTT CCG GGT AGT GTG T CAT CAT GTA GTC GGG GCG TAA CAA T	1000
kpsMTII -f *kpsMTII* –r	GCG CAT TTG CTG ATA CTG TTG CAT CAG ACG ATA AGC ATG AGC A	272

Amplification of DNA for *cnf-1, usp*and*kpsMTII* was carried out in a final volume 20 µlof reaction mixture for each one containing (5) µl of master mix

(Bioneer-Korea) , (5)µl extracted DNA , (2.5) µl from each forward and reverse primer and (5) µl of nuclease free water.
The amplification programs were performed according to [13]in a PCR thermal cycler apparatus (Clever-USA).
The amplification program for detection of *cnf-1*and*kpsMTII* consisted of a preheating cycle at 94ºC after this initial denaturation step, the mixture was subjected to 25 amplification cycles of denaturation at 94 ºC (1 min.) , annealing at 63 ºC(1min) and extension at (68 ºC) for (3) min. the final extension was done at 72ºC for (7 min.).

While , The amplification program for detection of *usp* gene consisted of aninitial denaturation cycle at 94ºC for (5 min.), then the mixture was subjected to 25 amplification cycles of denaturation at 94 ºC (1 min.) , annealing at 66 ºC(1min) and extension at (68 ºC) for (3) min. the final extension was done at 72ºC for (7 min.).
PCR amplification products were determined by 1.5% agarose gel in electrophoresis apparatus (Clever-USA)[19].

Results

Antibiotic susceptibility

The isolates shown high resistance to the tested antibiotics except in case of amikacin where 9 (81.81%) of isolates were sensitive to this antibiotic with high significant differences ($p<0.001$) . While isolates exhibit moderate sensitivity to gentamicin and ciprofloxacin 4,3 (36.37 and 27.28)% of isolates respectively , the low antibiotic susceptibility was shown against cefotaxime and azithromycin where2 isolates (18.19%)were sensitive for each one . In the other hand , all isolated bacteria exhibit completely resistance to ampicillin and co-trimoxazole , two isolates were shown resistance to all tested antibiotics as illustrated in table (2).

Table (2) : Antibiotics Susceptibility test for bacterial isolates that isolated from prostatitis patients.

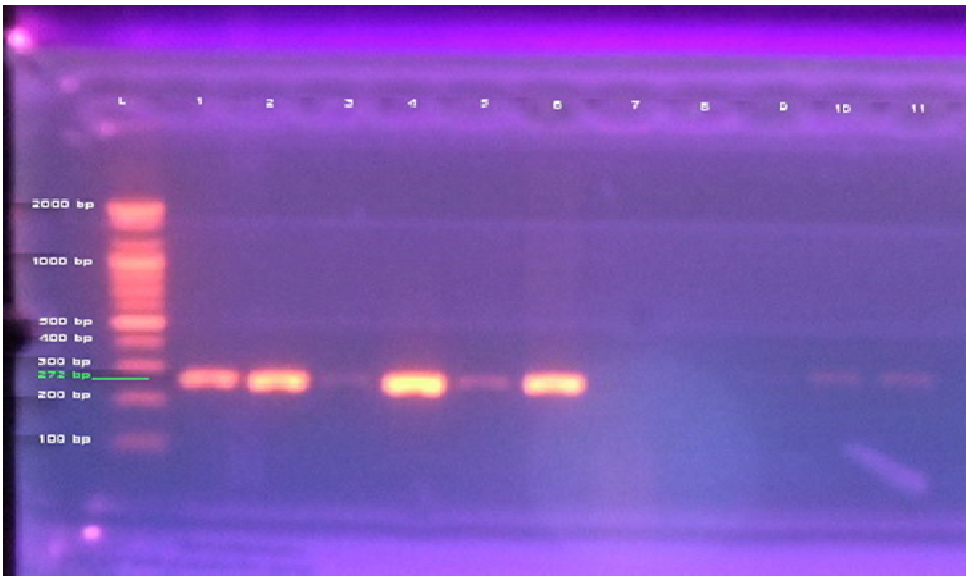
Antibiotics	Susceptible	
	No.	%
Amikacin	9	81.81
Gentamicin	4	36.37
Ciprofloxacin	3	27.28
Cefotaxime	2	18.19
Azithromycin	2	18.19
Co-trimoxazole	0.0	0.0
Ampicillin	0.0	0.0

Incidence of virulence genes

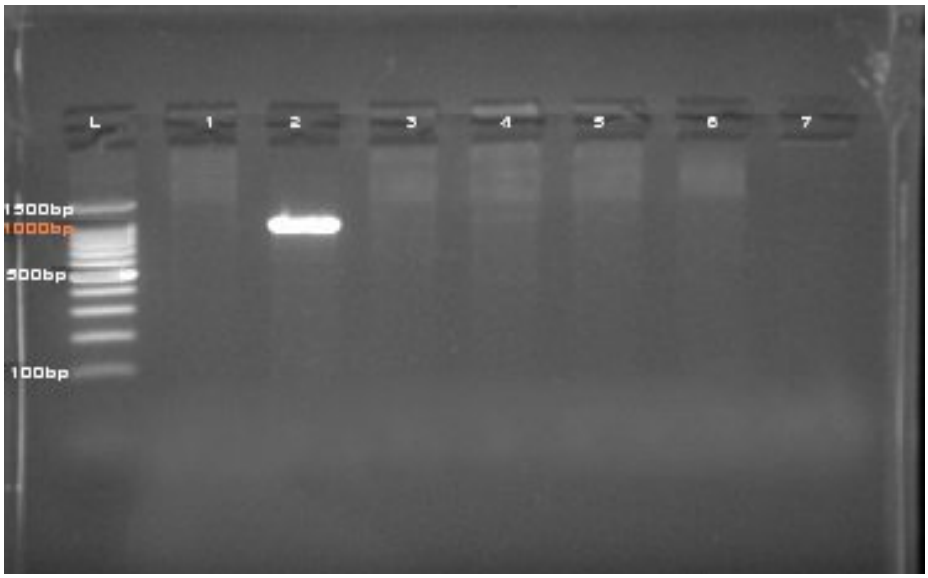
After the gel-electrophoresis , the *cnf-1* gene was not detected in all isolates (0%) , the *kpsMTII*(figure 1)was more prevalent among the isolated bacteria that causes prostatitis where it detected in 8 isolates (72.72 %) with the high significant differences ($p < 0.001$), *usp*genes (figure 2) was identified in 2 isolates (18.18%) as shown in table (3) .

Table (3) : The virulence factors that expressed by bacterial isolates

Virulence factors	Positive		Negative	
	No.	%	No.	%
Cnf-1	0.0	0.0	11	100
Usp	2	18.18	9	81.82
kpsMTII	8	72.72	3	27.27



Fig(1):-Agarose gel electrophoresis of PCR of *kpsMTII* gene product of (272) bp . L: ladder; other lane represents the no. of isolate 1,2,3,4,5,6,7,8,9,10,11 from prostatitis patients . The lanes 1 , 2 , 3 , 4 , 5 , 6 , 10 , 11 were gave a positive (distinct band) results for a PCR product of tested gene



Fig(2):-Agrose gel electrophoresis of PCR of *usp* primer that give a PCR products of (1000 bp) . L: ladder; other lane represents the no. of isolate 1,2,3,4,5,6,7 from prostatitis patients , where lane 2 gave a *usp* gene product (positive PCR result)

Discusion

Depending on these findings , the isolates shown high resistance to several classes of antibiotics even though the members of aminoglycosides (amikacin and gentamicin) were the most active antibiotics against isolated bacteria which may because of these antibiotics are used under precautions by a doctors due to their side effects (nephrotoxic) [20] and these antibiotics are less useful by people for takes randomly due to their administration is difficult which done by injection which help in decrease the rate of bacterial adaptation and antibiotic resistance to aminoglycosides . In the other hand , the wide spread using of β-lactams antibiotics , ciprofloxacin , azithromycin and co-trimoxazole and their safety and easily of administration of these antibiotics (usually orally) help in increase the dependency on these antibiotics and increase the resistance of bacteria which occur mainly by horizontal gene transfer .

Concurrent resistance to antimicrobials of different structural classes has arisen in a large number of bacterial species and may confuse the therapeutic management of infections, including infection of the genito-urinary tract , the result of current study was well matched to the findings that obtained by many researchers[21,22,23,24] , which shown that the multidrug-resistant isolates comprised an important percent of *E. coli* isolates from different genito-urinary tract specimens including the prostate with high resistance to

ampicillin, trimethoprim-sulfamethoxazole , gentamicin and ciprofloxacin , also they shown that the multi-drug resistance was more among men than in women and they found that the predominant phenotype of resistance among the MDR *E. coli* isolates was ampicillin, TMP/SMX and tetracycline resistance.

the data resemble to those of some researches [25,26] which mentioned that amikacin-resistant *Escherichia coli* strains were isolated rarely from clinical samples and the prior UTI is a common risk factor for resistance to the different antibiotics tested including aminoglycosides and penicillins , this risk factor is predominant among prostatitis patients which suffer from recurrent UTI and chronic infections , the consequences of this problem on prostatitis patients is there are few antibiotics are able to penetrate well to prostate tissue to reaches to the site of infections .

E. coli express several virulence factors for adherence , colonization ,initiation of infections and tissue lesions such as siderophores, toxins, capsules and fimbriae [27,28]. The result of the present study was differ from those obtained by some researchers [29,30,31] that detected *cnf-1* in 64% and 63% of *E. coli* isolates from prostatitis patients , respectively . The reasons for these differences may be due to distinct population analyzed or from the presence of different bacterial strains among the patients that were analyzed in these studies or the differences in the number of isolates under the studies and the origin of isolates . .

The findings of current study were agreed to those of some reaearchers in the prevalence of *kpsMTII*and*usp* genes and different in the prevalence of *cnf1* gene this may be because of the differences in the size of samples (previous study used 162 *E.coli* isolates compared with 11 isolates in existing study, the site of infections and the virulence features among the isolates[13].

Several studies revealed that the quinolone resistant UPEC strains were low virulent and invade immune-compromised patients .While susceptible strains were virulent and affects non immune-compromised patients [32].The ciprofloxacin resistance and multidrug resistance are associated with low virulence strains [32,33] .

The isolates in the current study were highly resistant to antibiotics used and less virulent which compatible to previous studies , it possible that this phenomena occur because of a low presence of certain virulence factors precedes resistance to antibiotics [32,34] or it due to the low presence of virulence is occur after the acquisition of resistance genes [35,36] . The coexistence of these two mechanisms is possible , recently , a specific chromosomal background only partially corresponding to the phylogenetic background could precede mutation to antibiotic resistance [37,33] . Some strains (CP1) of the UPEC from CP patients had a less virulence factors such as *usp* and *cnf-1* genes than UPEC isolates from cystitis patients . Also, the

characteristics of both host and pathogens was determined the developments of symptoms[38] .

It is well known that quinolones induce the SOS system response (DNA repairing mechanism), which could favor the splitting of bacteriophages or related sequences such as PAIs from the bacterial chromosome [39] . Investigators reported that sub-inhibitory concentrations of quinolones could induce the partial or total in vitro loss of PAIs in UPEC strains which could be considered as cause of decreased virulence in the isolates in the current study. They suggested that this partial or total loss of PAIs induced by quinolones can be observed to occur by a SOS dependent or independent pathway, respectively [6].

Conclusion

In brief, the bacterial isolates from patients with prostatitis were low virulent in combination with high resistance to many antibiotics .

References

1 . Krieger, J.N.,Riley, D.E., Cheah, P.Y., Liong, M.L. and Yuen, K.H. (2003) .Epidemiology of prostatitis: new evidence for a world-wide problem . World J. Urol. 21(2):70-74. (abstract)

2 . Rhee, J.J., Piesman, M. and Costabile, R.A. (2009) . Acute bacterial prostatitis and prostatic abscess . www.Medscape.com .

3 . Schaeffer, A. J. (1999). Prostatitis: US perspective . Int. J. Antimicrob. Agents . 11: 205–211. (abstract)

4 . Nickel, J.C., Nyberg, L.M. and Hennenfent, M. (1999) . Research guidelines for chronic prostatitis: consensus report from the first National Institutes of Health . International Prostatitis Collaborative Network. Urol. 54(2):229. (abstract)

5 . Kumon, H. (1992) . Detection of a local prostatic immune response to bacterial prostatitis. Infect. 20 :236-238.

6 . Soto, S. M., Smithson, A., Martinez, J. A., Horcajada, J. P., Mensa, J. and Vila, J. (2007). Biofilm formation in uropathogenic*Escherichia coli* strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. J. Urol. 177: 365–368.

7 . Karisik, E., Ellington, M.J., Livermore, D.M. and Woodford, N. (2008) .Virulence factors in *Escherichia coli* with CTX-M-15 and other extended-spectrum β-lactamases in the UK . *J. Antimicrob. Chemother.*61 (1): 54-58.

8 . Rippere-Lampe, K.E., Lang, M.,Ceri, H., Olson, M.,Lockman, H.A. and O'Brien, A.D. (2001) . Cytotoxic Necrotizing Factor Type 1-Positive *Escherichia coli* Causes Increased Inflammation and Tissue Damage to the Prostate in a Rat Prostatitis Model . Infect. Immun. 69(10): 6515–6519.

9 . Bergh, J., Marklund, I., Thellenberg-Karlsson, C., Gro¨nberg, H., Elgh, F. and Alexeyev, O.A. (2007) .Detection of Escherichia coli 16S RNA and Cytotoxic Necrotizing Factor 1 Gene in Benign Prostate Hyperplasia . Euro. Urol. 51 : 457–463 .

10 . Davis, J.M., Carvalho, H.M., Rasmussen, S.B. and O'Brien, A.D. (2006) .Cytotoxic necrotizing factor type 1 delivered by outer membrane vesicles of uropathogenic*Escherichia coli* attenuates polymorphonuclear leukocyte antimicrobial activity and chemotaxis . Infect. Immun. 74: 4401–4408.

11 . Gerhard, R., Schmidt, G., Hofmann, F. and Aktories, K. (1998) . Activation of Rho GTPases by *Escherichia coli* cytotoxic necrotizing factor 1 increases intestinal permeability in Caco-2 cells . Infect. Immun. 66: 5125–5131.

12 . Fiorentini, C., Falzano, L., Fabbri, A., Stringaro, A., Logozzi,M., Travaglione,S., Contamin, S., Arancia, G., Malorni, W. and Fais, S. (2001) . Activation of Rho GTPases by cytotoxic necrotizing factor 1 induces macropinocytosis and scavenging activity in epithelial cells . Mol. Biol. Cell. 12: 2061–2073.

13 . Tiba, M.R., Yano, T. and Leite, D.S. (2008) . Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis . *Rev. Inst. Med. trop. S. Paulo 50: 255-260* .

14 .Parret, A. H. and De Mot, R.(2002).*Escherichia coli's uropathogenic-specific protein: a bacteriocin promoting infectivity?* Microbiol.148:1604–*1606* .

15 . Naber, K. G. and Madsen, P. O. (1999). Antibiotics: basic concepts. In *Textbook of Prostatitis.* (Nickel J. C., Ed.) pp. 83–94. Isis Medical Media, Cambridge.

16 . Nickel, J.C. and Moon, T. (2005) . Chronic bacterial prostatitis: an evolving clinical enigma. Urol. 66:2-8.

17 . Charalabopoulos, K., Karachalios, G., Baltogiannis, D., Charalabopoulos, A., Giannakopoulos, X. and Sofikitis, N. (2003) . Penetration of antimicrobial agents into the prostate. Chemother. 49:269-279. (Abstract)

18 . Hua, V.N. and Schaeffer, A.J. (2004) . Acute and Chronic Prostatitis. In: Resnick MI, editor. The Medical Clinics of North America. Philadelphia: W. B. Saunders 483-494.

19 . Sambrook, J. and Rusell, D.W. (2001). Molecular cloning: a laboratory manual. Cold spring Harbor, NY: Cold spring Harbor laboratory press.

20 . Brooks, G.F., Butel, J.S. and Morse, S.A. (2004) . Jawetz, Melnick, &Adelberg's Medical Microbiology, 23rd Edition McGraw-Hill Companies .

21 . Sahm, D.F., Thornsberry, C., Mayfield, D.C., Jones, M.E. and Karlowsky, J.A. (2001). Multidrug-resistant urinary tract isolates of Escherichia coli: prevalence and patient demographics in the United States in 2000. Antimicrob. Agents Chemother. 45:1402-1406.

22 .Rafay, A.M. and Nsanze, H.N. (2003) . Multi-drug resistance of Escherichia coli from the urinary tract. Saudi Med. J. 24:261-264.(Abstract)
23 .Karlowsky, J.A., Hoban, D.J., Decorby, M.R., Laing, N.M. and Zhane, G.G. (2006) . Fluoroquinolone-resistant urinary isolates of Escherichia coli from outpatients are frequently multidrug resistant: results from the North American Urinary Tract Infection Collaborative Alliance-Quinolone Resistance study. Antimicrob. Agents Chemother. 50 :2251-2254.
24 .Santo, E., Salvador, M.M. and Marin, J.M. (2007) . Multidrug-resistant urinary tract isolates of Escherichia coli from RibeirãoPreto, São Paulo, Brazil. Braz. J. Infect. Dis. 11:575-578.
25 . Sotto, A., De Boever, C.M., Fabbro-Peray, P., Gouby, A. Sirot, D. and Jourdan, J. (2001) . Risk Factors for Antibiotic-Resistant *Escherichia coli* Isolated from Hospitalized Patients with Urinary Tract Infections: a Prospective Study . J. Clin. Microbiol. 39: 438–444.
26 . Ruiz, J., Bertran, S., Sauca, G., Julià, A., Vila, X., Gómez, E., Jiménez de Anta, M.T. and Vila, J. (2005) . Isolation of an amikacin-resistant *Escherichia coli* strain after tobramycin treatment of previous recurrent episodes of respiratory tract infections caused by *Pseudomonas aeruginosa* . Clin. Microbiol. Infect. 11:71-73. (Abstract)
27 . Yamamoto, S., Tsukamoto, T., Terai, A., Kurazono, H., Takeda, Y. and Yoshida, O. (1995) . Distribution of virulence factors in *Escherichia coli* isolated from urine of cystitis patients. Microbiol. Immunol. 39:401-404.
28 . Mulvey, M.A.(2002) . Adhesion and entry of uropathogenic*Escherichia coli*. Cell. Microbiol. 4: 257-271.
29 . Andreu, A., Stapleton, A.E., Fennel, C., Lockman, H.A., Xercavins, M. Fernandez, F. and Stamm. W.E. (1997) . Urovirulence determinants in *Escherichia coli* strains causing prostatitis. J. Infect. Dis. 176:464-469.
30 . Mitsumori, K., Terai, A., Yamamoto, S., Ishitoya, S. and Yoshida, O. (1999) . Virulence characteristics of *Escherichia coli* in acute bacterial prostatitis. J. Infect. Dis. 180:1378–1381.
31 . Kurazono, H., Yamamoto, S., Nakano, M., Nair, G.B., Terai, A., Chaicumpa, W. and Hayashi, H. (2000) . Characterization of a putative virulence island in the chromosome of uropathogenic*Escherichia coli*possessing a gene encoding a uropathogenic-specific protein. Microb. Pathog. 28:183-189.
32 . Moreno, E., Planells, I., Prats, G., Planes,A.M., Moreno, G. andAndreu, A. (2005) . Comparative study ofEscherichia coli virulence determinants in strains causing urinary tract bacteremia versus strains causing pyelonephritis and other sources of bacteremia. Diagn. Microbiol. Infect. Dis.53:93-99 .
33 . Houdouin, V., Bonacorsi, S., Bidet, P., Bingen-Bidois, M., Barraud, D. and Bingen, E. (2006). Phylogenetic background and carriage of pathogenicity island-domains in relation to antibiotic resistance profiles

among *Escherichia coli*urosepsis isolates. J. Antimicrob. Chemother. 58: 748–751. (Abstract)

34 . Johnson, J.R., Johnston, B., Kuskowski, M.A., Colodner, R. And Raz, R. (2005) . Spontaneous conversion to quinolone and fluoroquinolone resistance among wild-type *Escherichia coli* isolates in relation to phylogenetic background and virulence genotype. Antimicrob Agents Chemother.49: 4739–4744.

35 . Vila, J., Simon, K., Ruiz, J., Horcajada, J.P., Velasco, M., Barranco, M., Moreno, A. and Mensa, J. (2002) . Are quinolone-resistant uropathogenic Escherichia coli less virulent? J. Infect. Dis. 186:1039-1042.

36 . Piatti, G., Mannini, A., Balistreri, M. and Schito, A. M. (2008). Virulence factors in urinary *Escherichia coli* strains: phylogenetic background and quinolone and fluoroquinolone resistance. J. Clin. Microbiol. 46: 480–487

37 . Drews, S. J., Poutanen, S. M., Mazzulli, T., McGreer, A. J., Sarabia, A., Pong-Porter, S., Rzayev, Y., Willey, B., Green, K. and Low, D. F. (2005). Decreased prevalence of virulence factors among ciprofloxacin-resistant uropathogenic*Escherichia coli* isolates. J. Clin. Microbiol. 43: 4218–4220.

38 . Rudick, C.N., Berry, R.E., Johnson, J.R., Johnston, B., Klumpp, D.J., Schaeffer, A.J. and Thumbikat, P. (2011) . Uropathogenic*Escherichia coli* induces chronic pelvic pain . Infect. Immun. 79 : 628–635 .

39 . Phillips, I., Culebras, E., Moreno, F. and Baquero. F. (1987) . Induction of the SOS response by new 4-quinolones. J. Antimicrob. Chemother.20:631-638.(Abstract)