EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
874-879
Year: 2018

SUSCEPTIBILITY PATTERN OF SECOND LINE ANTIBIOTIC COLISTIN AGAINST GRAM NEGATIVE BACTERIA CAUSING URINARY TRACT INFECTION IN SELECTED AREAS OF DHAKA CITY, BANGLADESH

Mohammad Asaduzzaman*[1], Kamalesh Baral[1], Md. Mafizul Islam[1], Md. Abu Nayem[1], Md. Jahangir Alam[1], Farha Matin Juliana[2], Nazmul Hossain[1], Biswajit Das[1], Runa Asma[3] and Sumaiya Jannat Tapati[4]

*[1]Department of Biochemistry, Primeasia University, Banani, Dhaka, Bangladesh.
[2]Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh.
[3]Department of Laboratory Medicine, IBN SINA Diagnostic and Consultancy Center, Badda, Dhaka, Bangladesh.
[4]Department of Biochemistry and Molecular Biology, Jagannath University, Dhaka, Bangladesh.

***Corresponding Author: Mohammad Asaduzzaman**
Department of Biochemistry, Primeasia University, Banani, Dhaka, Bangladesh.

Article Received on 14/01/2018 **Article Revised on 05/02/2018** **Article Accepted on 26/02/2018**

ABSTRACT
In a few decades, infections like Urinary Tract Infection (UTI) caused by multi-drug resistant (MDR) pathogens have become a serious problem, especially in the nosocomial setting. The emergence of multidrug-resistant Gram-negative bacteria that cause nosocomial infections is a growing problem worldwide. The World Health Organization (WHO) has identified antimicrobial resistance as one of the three most important problems for human health. We know that Colistin was first introduced in 1952 and was used until the early 1980s for the treatment of infections caused by gram-negative bacilli. Colistin has identified excellent activity against various gram-negative bacteria in vitro. Our aim was to assess the susceptible pattern of Colistin which also known as polymyxin E, is an antibiotic against gram negative bacteria causing Urinary Tract Infections. A total of 9178 urine samples were collected in 2016 (January-December) and out of which 837 (9.12%) were bacteriologically (Gram Negative Bacteria) positive. Male was found more prone to get UTI under 10 years and between 51-80 years of age and female was more affected in 10 to 50 years and over 80 years of age group. *E. coli* was the most prevalent (89.3%) isolate followed by *Klebsiella* spp. (7.5%), *Pseudomonas* spp. (2.3%) and *Proteus spp.* (0.9%). The most predominant sensitive organisms to Colistin were *Klebsiella* spp. (100%) in both male and female and resistant organisms *Pseudomonas* spp. (33.3%) and *E.coli*. (22.1%) in male and female found respectively.

KEYWORDS: Colistin, Polymyxin, UTI, Gram negative bacteria, Resistance, Uropathogen.

INTRODUCTION
Urinary Tract Infection (UTI) represents as one of the most common diseases encountered in medical practices these days and encompasses a broad range of clinical fields that are associated with a common finding of positive urine cultures.[1] It is serious health problem affecting 150 million people globally in each year.[2] Urinary Tract Infection (UTI) is a very common infection all over the world but it is more prevalent in developing south Asian countries like Bangladesh.[3] They are the second most common types of infection in humans accounting for 8.3 million doctor's visit annually in USA.[4] They are the most common bacterial infection in patients of all ages with high risk in young women resulting in significant morbidity and health care costs.[5]

Urinary tract infection is more common in female than male, because of the short length of the urethra and its proximity to anus. Pregnancy and sexual activity also make female more susceptible to UTI.[6] Different factors like age, sex, immunosuppression and urological instruments may affect prevalence of UTIs.[7] The etiology of UTIs and the antibiotic susceptibility of urinary pathogens, both in community and hospitals, have been changing over the past years and recently, the antibiotic resistance has become a major global problem.[8] UTI can be nosocomially ubiquitous in clinical environment so that prevalence rate of uropathogens is being alarmingly accelerated. To prevent these pathogens, different types of antibiotics and their super generations are used irrespectively with different doses in misused and overused forms. So uropathogens are getting resistant to efficacious drugs adopting different mechanisms of mutations and genetic transformations.[9] Antibiotic resistance is an increasing threat to life and morbidity and mortality.[10]

Treatment of UTI cases is often started empirically and therapy is based on information determined form the antimicrobial resistance pattern of the urinary pathogen.[11] However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections.[12] Colistin, also known as polymyxin E, is an antibiotic that is used as a second line drugs among these resistance organisms which produced by certain strains of the bacteria *Paenibacillus polymyxa*. It is a mixture of the cyclic polypeptides Colistin A and B and belongs to the class of polypeptide antibiotics known as polymyxins. Colistin is effective against most gram-negative bacilli. Colistin is a decades-old drug that fell out of favor in human medicine due to its kidney toxicity. It remains one of the last-resort antibiotics for multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter*.[13] NDM-1 metallo-β-lactamase multidrug-resistant Enterobacteriaceae have also shown susceptibility to Colistin.[14]

Over a period, Colistin have used as an important class of drugs used primarily to treat urinary tract infections in the world. Unfortunately, Colistin usage is threatened by the rising occurrence of resistance, which has been observed in every species that is treated by this drug. The aim of our study was to see the pattern of Colistin susceptibility against uropathogens in the selected areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh).

MATERIALS AND METHODS

Materials

Study Design

Study Location

This was a retrospective analysis of laboratory data routinely collected from the microbiology department of IBN SINA Diagnostic and Consultation Center, Doyagonj, Dhaka from January 1, 2016 to December 31, 2016. The total sample volumes were 9178.

METHODS

Sample Collection and Bacteriological Assessment

Early morning midstream urine samples were collected aseptically from 9178 (male-2735 and female-6443) patients. The urine samples were collected into sterile wide container (China) with screw cap tops. On the label name, age, sex and time of collection were mentioned. All the patients were instructed on how to collect the urine samples aseptically and taken to the laboratory immediately for culture. In the diagnostic laboratory, each well mixed urine sample (1µL) was inoculated on MacConkey agar (Oxoid) and Blood agar (Oxoid) media plate under class-II Biosafety Cabinet (NUVO Sanaji Malzemelzeni, Imalat Vc Ticaret A.S, Turkey). The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop sterilized by auto loop sterilizer (Germany) following standard procedures. The culture plates were incubated at 37 °C by an incubator (Germany) for 48 hours and observed for the growth of bacteria through formation of colonies. All the bacteria were isolated and identified morphologically using microscopy (Japan) and biochemical tests like TSI (HiMedia), MIU (HiMedia) and Simmons Citrate (HiMedia) agar following standard procedures.[15]

Antibiotic Susceptibility Assessment

The disc diffusion technique was used for antibacterial susceptibility testing of the isolates using commercial antibiotics containing discs. We used the commercial antibiotic disc Colistin (10µg, Oxoid). Interpretation of results was done using zone sizes. Zones of inhibition ≥11 mm were considered sensitive and ≤10 mm resistant. Isolates were classified as either sensitive or resistant based on the definition of the Clinical and Laboratory Standard Institute.[16] Some laboratory stains of known sensitivity of *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619 were used as quality control strains for the antimicrobial discs.

Statistical Analysis

Data were assessed using the Statistical Package for Social Science (IBM SPSS Statistics, version 18, IBM Corporation, SPSS Inc. Chicago, III, USA). The Trend chi square test for statistical comparisons between the groups.

RESULTS

The total 9178 urine samples collected from patients, 837 (9.12%) samples were positive with gram negative organisms and 8341 (90.88%) samples were negative at 2016 (January-December) in selected areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh).

Tabel 1: Distribution table of Urinary Tract Infection (UTI) patients by age groups and gender.

Age (Years)	<10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	>90
Male	34	1	12	9	19	24	54	25	3	0
Female	71	61	115	73	109	84	80	44	12	7
Total	105	62	127	82	128	108	134	69	15	7

Table-1 showed the distribution of patients by age and gender. Highest of the study subjects belonged to the 61-70 years age group (134 patients=80 female + 54 male) and followed by 41-50 years age group (128 patients=109 female + 19 male), 21-30 years age group (127 patients=115 female + 12 male) and 51-60 years age group (108 patients= 84 female + 24 male) respectively.

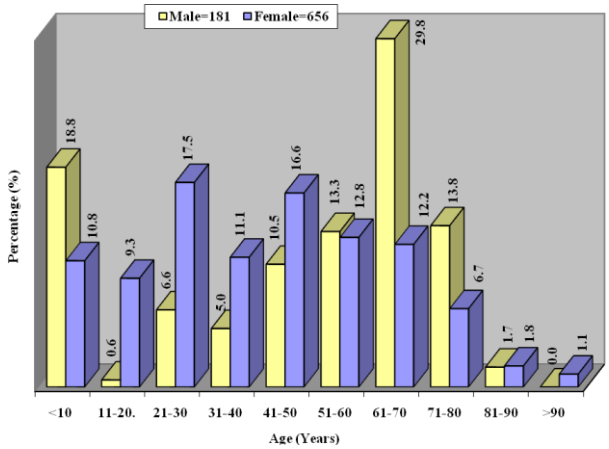

Fig.-1: UTI percentage among different age groups of male (N=181) and female (N=656).

In this study, percentage of male patients were more porne to female patients (18.80% > 10.80%) under 10 years age groups. In between 11-20, 21-30, 31-40 and 41-50 years of age group female UTI infection (9.3%, 17.5%, 11.1% and 16.6% respectively) is higher than male (0.60%, 6.6%, 5.0% and 10.5% respectively). In between 51-60, 61-70 and 71-80 years age male infection (13.3%, 29.8% and 13.8% respectively) is higher than female (12.8%, 12.2% and 6.7% respectively). In between 81-90 and above 90 years age female infection (1.8% and 1.1% respectively) is higher than male (1.7% and 0.0% respectively) but here number of patients were very few.

Table 2: Distribution of specific uropathogen mediated UTI among UTI patients (n=837).

Organisms	Male	Female	Total
E. coli	153(18.3%)	594(71.0%)	747(89.3%)
Proteus spp.	3(0.4%)	4(0.5%)	7(0.9%)
Pseudomonas spp.	9(1.1%)	11(1.2%)	20(2.3%)
Klebsiella spp.	16(1.9%)	47(5.6%)	63(7.5%)
Total	181(21.7)	656(78.3)	837(100.0%)

Table-2 showed that the most predominant organism *E. coli* 747(male 153 and female 594) found in UTI patients. As per number distribution, the second prevalent organism was *Klebsiella* spp. 63 (male 16 and female 47). Other isolated organism found as follows *Pseudomonas* spp. 20 (male 9 and female 11) and *Proteus* spp. 7 (male 3 and female 4). 181 samples (21.7%) were obtained from male subjects while the remaining 656 (78.3%) were from female.

Table-3: Prevalence of different uropathogens among male and female patients.

Organisms	Male (n=181)		Female (n=656)	
	Number	Percentage	Number	Percentage
E. coli	153	84.5%	594	90.5%
Proteus spp.	3	1.7%	4	0.6%
Pseudomonas spp.	9	5.0%	11	1.7%
Klebsiella spp.	16	8.8%	47	7.2%
Total	181	100.0%	656	100.0%

In our study Table-3 showed that the prevalence of the uropathogens among male and female UTI patients. Among the male and female patients the most prone uropathogen *E. coli* was found (84.5%) and (90.5%) followed by *Klebsiella* spp. (8.8%) and (7.2%), *Pseudomonas* spp. (5.0%) and (1.7%), and *Proteus* spp. (1.7%) and (0.6 %) respectively.

Table-4: Susceptibility pattern of Colistin against uropathogens among male UTI patients.

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E. coli	115	75.2%	38	24.8%
Proteus spp.	3	100.0%	0	0.0%
Pseudomonas spp.	6	66.7%	3	33.3%
Klebsiella spp.	13	81.3%	3	18.8%
Total	137	75.7%	44	24.3%

Table-4 showed that Colistin was sensitive against isolated uropathogenic bacteria in male (75.7%) and rest of resistant (24.3%). The most sensitive organism to colistin was *Proteus* spp. (100%) found but here numbers were very few. On the other hand the most prevalent resistant organisms *Pseudomonas* spp. (33.3%) were found but here the number was very few. In contrast of frequency, *E. coli* was the most significant organism which was (75.2%) sensitive and (24.8%) resistant to Colistin. The other sensitivity patterns were *Klebsiella*

spp. (81.3%), and *Pseudomonas* spp. (66.7%) and resistant *Klebsiella* spp. (18.8%).

Table-5: Susceptibility pattern of Colistin against uropathogens among female UTI patients

Name of organisms	Sensitive		Resistant	
	Number	Percentage	Number	Percentage
E. coli	463	77.9%	131	22.1%
Proteus spp.	4	100.0%	0	.0%
Pseudomonas spp.	9	81.8%	2	18.2%
Klebsiella spp.	37	78.7%	10	21.3%
Total	513	78.2%	143	21.8%

Table-5 showed that Colistin was sensitive against isolated uropathogenic bacteria in female (78.2%) and rest of resistant (21.8%). The most sensitive organism to Colistin was *Proteus* spp. (100%) found but here numbers were very few. On the other hand the most prevalent resistant organisms *E. coli* (22.1%) were found. The other sensitivity patterns were *E. coli* (77.9%) *Klebsiella* spp. (78.7%) and *Pseudomonas* spp. (81.8%) and resistant *Klebsiella* spp. (21.8%) and *Pseudomonas* spp. (18.2%) respectively.

DISCUSSION

The bacterial resistance rates are increasing due to misuse of antibiotics. This study aimed to evaluate the antimicrobial agent Colistin's susceptibility pattern against gram negative bacteria isolated from patients with UTI seen at the IBN SINA Diagnostic Center, Doyagonj, Dhaka, Bangladesh. In this study, we tested total 9178 urine samples and 837 (9.12%) were bacteriological positive and 8341 (90.88%) were bacteriological negative found. Table-1 showed the distribution of patients by age and gender. Highest of the study subjects belonged to the 61-70 years age group (134 patients=80 female + 54 male) and followed by 41-50 years age group (128 patients=109 female + 19 male), 21-30 years age group (127 patients=115 female + 12 male) and 51-60 years age group (108 patients= 84 female + 24 male) respectively. In our study we saw that mostly female patients are affected by uropathogens in all the age groups in contrast male patients. Most prevalent frequency of female patients affected by uropathogens was found in 61-70 years age group. There is a significant difference between gender and age group at 5% ($P<0.05$).

In addition Fig.-1 also demonstrated the UTI percentage among different age groups of male (N=181) and female (N=656). The, percentage of male patients were more porne to female patients (18.80% > 10.80%) under 10 years age groups. In between 11-20, 21-30, 31-40 and 41-50 years of age group female UTI infection (9.3%, 17.5%, 11.1% and 16.6% respectively) is higher than male (0.60%, 6.6%, 5.0% and 10.5% respectively). In between 51-60, 61-70 and 71-80 years age male infection (13.3%, 29.8% and 13.8% respectively) is higher than female (12.8%, 12.2% and 6.7% respectively). In between 81-90 and above 90 years age female infection (1.8% and 1.1% respectively) is higher than male (1.7% and 0.0% respectively) but here number of patients were very few. Female were more suffered with UTI and it caused by *E. coli* (83.9%). This may explain the highest frequency of UTIs observed in women when compared to men, which is often attributed to a shorter urethra that facilitates colonization by these microorganisms. Furthermore, another mechanism that could explain the lower frequency of UTI in men would be the prostatic fluid, which has antibacterial substances.[3] There is a significant difference between gender and age group at 5% ($P<0.05$).

Table-2 showed that the most predominant organism *E. coli* 747(male 153 and female 594) found in UTI patients. As per number distribution, the second prevalent organism was *Klebsiella* spp. 63 (male 16 and female 47). Other isolated organism found as follows *Pseudomonas* spp. 20 (male 9 and female 11) and *Proteus* spp. 7 (male 3 and female 4). 181 samples (21.7%) were obtained from male subjects while the remaining 656 (78.3%) were from female. In contrast of frequency, female UTI patients were higher than male in all the causative agent of UTI. There is significant difference between gender and causative agent of UTI at 5% ($P<0.05$).

In our study Table-3 showed that the prevalence of the uropathogens among male and female UTI patients. Among the male and female patients the most prone uropathogens *E. coli* was found (84.5%) and (90.5%) followed by *Klebsiella* spp. (8.8%) and (7.2%), *Pseudomonas* spp. (5.0%) and (1.7%), and *Proteus* spp. (1.7%) and (0.6%) respectively. The study noted that male were more infected by the entire organism (*Klebsiella* spp., *Pseudomonas* spp, and *Proteus* spp.) except *E. coli*, which was more for female than male. There is a significant difference between gender and causative agent of UTI at 5% ($P<0.05$).

Moreover, the early introduction of effective drugs against bacterial infections in the last century has changed the medical behavior and has significantly reduced the mortality rates due to these agents. However, the widespread use of antibiotics has induced different mechanisms of bacteria resistance to these drugs.[17] Table-4 showed that Colistin was sensitive against isolated uropathogenic bacteria in male (75.7%) and rest of resistant (24.3%). The most sensitive organism to

Colistin was *Proteus* spp. (100%) found but here numbers were very few. On the other hand the most prevalent resistant organisms *Pseudomonas* spp.(33.3%) were found but here the number was very few. In contrast of frequency, *E. coli* was the most significant organism which was (75.2%) sensitive and (24.8%) resistant to colistin. The other sensitivity patterns were *Klebsiella* spp. 81.3%, and *Pseudomonas* spp. (66.7%) and resistant *Klebsiella* spp. (18.8%). There is no significant difference among gender, causative agent and susceptibility of Colistin at 5% (P>0.05).

However, Table-5 showed that Colistin was sensitive against isolated uropathogenic bacteria in female (78.2%) and rest of resistant (21.8%). The most sensitive organism to Colistin was *Proteus* spp. (100%) found but here numbers were very few. On the other hand the most prevalent resistant organisms *E. coli* (22.1%) were found. The other sensitivity patterns were *E. coli* (77.9%), *Klebsiella* spp. 78.7%, and *Pseudomonas* spp. (81.8%) and resistant *Klebsiella* spp. (21.8%) and *Pseudomonas* spp. (18.2%). There is no significant difference among gender, causeting agent and susceptibility of Colistin at 5% (P>0.05).

Treatment of urinary tract infections is becoming more complicated with an increase of the number of resistant strains to antibiotics and prevalence of antibiotic resistance mechanisms. It had observed that horizontal gene transfer is a factor in the emergence and spread of antimicrobial resistance in clinical isolates. Consequently, it has been suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption in a given environment.[18] It is important that clinicians are aware of the regional antibiotic resistance rates before initiating experimental antimicrobial therapy for UTI treatment, as it is well-described that urinary infection with a resistant pathogen is more likely to lead to bacteriological/clinical failures.[19]

CONCLUSION

In a conclusion, the results showed that there was a high prevalence of occurrence of urinary tract infection among patients of areas (Doyagonj, Gandaria, Jatrabari, Sayedabad, Dhaka, Bangladesh). Most of the bacteria were sensitive to Colistin. We see in our study that day by day Colistin is being make resistant which is very alarming message for human being. Awareness is needed of both the population and health professionals about the importance for the correct use of antibiotics. The Colistin use should be performed only after the microbial susceptibility confirmation, and it is necessary to find other alternatives for the empirical treatment. The bacterial resistance prevention can be performed through control measures that limit the spread of resistant bacteria and the rational use of antimicrobial policy.

REFERENCES

1. Castro-Orozco R, Barreto-Maya AC, Guzman-Alvarez H, Ortega-Quiroz RJ and Benitez-Pena L (2010). Antimicrobial resistance pattern for gram-negative uropathogens isolated from hospitalized patients and outpatients in Cartagena, Rev Salud Publica (Bogota). 2005-2008; 12(6): 1010-1019.
2. Orenstein R and Wong ES Urinary tract infections in adults. Am Fam Physician., 1999; 59: 1225-1234.
3. Jahangir Alam, Farha Matin Juliana, Md Rahimgir, Mohammad Nazir Hossain, Babry Fatema and Mohammad Asaduzzaman "Resistance Pattern of Ciprofloxacin against common Uropathogens in Selected Area of Dhaka city, Bangladesh." IOSR Journal of Nursing and Health Science (IOSR-JHNS)., 2017; 6(50: 52-57.
4. Annabelle TD and Jennifer AC Surveillance of pathogens and resistance patterns in urinary tract infection. Phil J Microbial Infect Dis., 1999; 28: 11-4.
5. Stamm WE and Norrby SR Urinary tract infections: disease panorama and challenges. J Infect Dis., 2001; 183:(Suppl 1): S1-S4.
6. Ramesh N, Sumathi CS, Balasubramanian V, Ravichandran KP, Kannan VR Urinary tract infection and antimicrobial susceptibility pattern of extended spectrum of beta lactamase producing clinical isolates. Advan Biol Res., 2008; 2(5-6): 78-82.
7. Iqbal T, Naqvi R and Akhter SF Frequency of urinary tract infection in renal transplant recipients and effect on graft function. J Pak Med Assoc., 2010; 60(10): 826-829.
8. Akram M, Shahid M, Khan AU Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob., 2007; 6: 4.
9. Laisa Ahmed Lisa, Dipak Kumar Paul, Sudhangshu Kumar Biswas, Nirmal Chandra barman, Shital Kumar Barman Drug Resistance Profiles of Potential Gram Negative Rods Isolated from Urinary Tract Infected (UTI) Patients of Bangladesh with Four South Asian Countries. Int J Pharma Sciences, 2015; 5(4): 1160-1166.
10. AM El-Mahmood, AT Tirmidhi and A Mohammed Antimicrobial susceptibility of some quinolone antibiotics against some urinary tract pathogens in a tertiary hospital, Yola, Adamawa State, Nigeria. J Cli Med and Research., 2009; 1(2): 26-34.
11. Wilson ML and Gaido L Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. Clin Infect Dis., 2004; 38: 1150-1158.
12. National Committee for Clinical Laboratory Standards Performance standards for antimicrobial disc susceptibility tests. 7th editon. Wayne, Pennsylvania, USA: NCCLS, M2-A7., 2000.
13. Falagas ME, Grammatikos AP, Michalopoulos A "Potential of old-generation antibiotics to address current need for new antibiotics". Expert review of anti-infective therapy.,

doi:10.1586/14787210.6.5.593. PMID 18847400. October 2008; 6(5): 593–600.

14. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study". The Lancet Infectious Diseases. doi:10.1016/S1473-3099(10)70143-2. PMC 2933358 . PMID 20705517. 2010; 10(9): 597–602.
15. Cheesborough M District Laboratory practice in Tropical Countries, Cambridge United Press, UK, part, 2006; 2: 7-106.
16. Clinical and Laboratory Standard Institute Methods for the Dilution Antimicrobial Susceptibility Tests for Bacteria., 2006.
17. Silveira GP, Nome F, Gesser JC, Sá MM, Terenzi H Estratégias utilizadas no combate a resistência bacteriana. Quím Nova., 2006; 29: 844-855.
18. Brown JR, Daniel G, Julie A, Ingraham BK, David JH, Stanhope MJ Horizontal transfer of drug-resistant amino-acyl-transfer-RNA synthetases of anthrax and Gram-positive pathogens. EMBO Rep., 2003; 4(7): 692-698.
19. Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, Johnson J, Noreddin A, Don E.Low DE, Karlowsky JA, Hoban DJ Antibiotic resistance in Escherichia coli outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents., 2006; 27(6): 468-475.