

Antimicrobial Susceptibility Patterns of Gram-Negative Bacteria Causing Urinary Tract Infections

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الملخص

تقصي أنماط الحساسية للبكتيريا السالبة لصبغة جرام المسببة لالتهابات المسالك البولية تعتبر مشكلة مقاومة البكتيريا للمضادات الحيوانية عقبة رئيسية تواجه الجهود المبذولة لعلاج حالات التهاب المسالك البولية عبر العالم. تم عمل هذه الدراسة الوصفية الراجعة التي اعتمدت على البيانات الموقعة في السجلات الطبية في مختبر مجمع ناصر الطبي من أجل تقصي الأنواع البكتيرية السالبة لصبغة جرام المسببة لالتهابات المسالك البولية، بالإضافة إلى تقصي نماذج الحساسية للعزلات المحصلة لثلاثة عشر نوعاً من المضادات الحيوية الأكثر شيوعاً لمعالجة التهابات المسالك البولية في قطاع غزة.

شملت الدراسة جميع المزارع البكتيرية التي تم عملها من عينات البول المأخوذة من المرضى البالغين وذلك في الفترة الممتدة من يناير 2014 حتى ديسمبر 2014. وقد تم اعتماد المزرعة التي ينمو فيها 100,000 أو أكثر وحدة تكوين مستعمرة/ ملليلتر لنوع واحد فقط من البكتيريا على أنها مزرعة إيجابية. تمت دراسة إجمالي 133 عزلة بكتيرية، حيث تم تحصيل 198 (59.8%) من هذه العزلات من الإناث فيما تم تحصيل 133 (40.2%) عزلة من الذكور.

وجدت الدراسة أن أكثر أنواع البكتيريا السالبة لصبغة جرام شيوعاً بين العزلات كانت بكتيريا الإيشيريشيا كولي (56.9%) تليها عزلات بكتيريا الكلبيسيلا (19.1%) ثم عزلات بكتيريا الإنتروباكتر (10.9%). كما كشفت هذه الدراسة أن أكثر المضادات الحيوية تأثيراً على البكتيريا

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السلالة لصبغة جرام كانت الأمايكاسين (83.7%) والسيروفلوكسوسين (72.7%) والسفتازيديم (69.5%). أما بالنسبة للمضادات الحيوية الأقل تأثيراً فقد كانت الأموكسيلين (10.3%) والكوترايميثوبيريم (31.1%) والسيفالوبيسين (38.1%).

توصي نتائج هذه الدراسة باعتماد السيروفلوكسوسين كعلاج أولي لحالات الإصابة بالتهابات المسالك البولية لحين إصدار نتيجة فحص المزرعة البكتيرية.

الكلمات المفتاحية: التهاب المسالك البولية، الحساسية للمضادات الحيوانية، البكتيريا السالبة لصبغة جرام.

Abstract

Microbial drug resistance is a major problem in the treatment of urinary tract infections worldwide. A hospital-based descriptive retrospective study was implemented to investigate the occurrence of Gram-negative bacteria causing urinary tract infections and to assess their antimicrobial sensitivity patterns in Nasser Medical Complex, Gaza-Strip.

The study included all urine samples collected from adults for culture investigation during the period from January 2014 to December 2014. Positive culture was defined as growth of a single bacterial species with colony count of $\geq 100,000$ cfu/ml. A total of 331 Gram-negative isolates were studied, females constituted the source of 198 isolates (59.8%), whereas 133 isolates (40.2%) were from males.

The common Gram-negative isolates were *E. coli* (56.9%), *Klebsiella* sp. (19.1%) and *Enterobacter* sp. (10.9%). Thirteen commonly locally used antibiotics were implemented in this study. It is found that 83.7% of the isolates showed sensitivity to amikacin, 72.7% to ciprofloxacin and 69.5% to ceftazidime. The lowest sensitivity was shown to amoxicillin (10.3%), co-trimethoprim (31.1%) and cepahlexin (38.1%).

Results of this study may suggest ciprofloxacin as an empirical treatment in our region for urinary tract infections until susceptibility reports are issued.

Keywords: Urinary tract infection, antibiotic susceptibility, Gram-negative bacteria.

INTRODUCTION:

Urine is a sterile body fluids, when infected by bacteria, all the structures of the urinary tract are at risk of being invaded. Urinary tract infections

(UTIs) are defined as the presence of significant bacteriuria along with urinary symptoms and is caused by bacteria ascending into the urinary tract and establish bacteriuria often at levels greater than or equal to 10^5 colony forming units per milliliter (cfu/ ml) of urine. A patient presenting to a clinician with 1 or more UTI symptoms had approximately a 50% chance of having significant bacteriuria (CLSI. 2013, Kibret *et al.* 2014). UTIs are the most common infectious diseases after respiratory tract infection in community practice. It remains a major public health problem in terms of morbidity and financial cost with an estimated 150 million cases per annum worldwide, costing global economy in excess of six billion US dollars (Shivani *et al.* 2014). Furthermore, UTIs are the most common nosocomial infections and account for about 40% of all hospital-acquired infections. Nosocomial UTIs constitute a major source for nosocomial septicemia and related mortality in acute care hospitals. It has been associated with a threefold increased risk for mortality in hospital-based studies, with estimates of more than 50,000 excess deaths occurring per year in the USA as a result of these infections (Burke *et al.* 2009).

Numerous reports have suggested that UTIs can occur in both genders at any age. However, women are especially prone to UTIs than men. The most common medical complaint among women in their reproductive years are UTIs followed by upper respiratory tract infections. One woman in five develops a UTIs during her lifetime. Women are 14-times more likely to suffer from an ascending UTIs than men ([Sibi *et al.* 2014](#)).

UTIs are the most common in patients with diabetes and in those with structural and neurological abnormalities which interfere with urinary outflow. Nosocomial UTIs are also common following catheterization and cystoscopy. The manifestations of UTIs may vary from mild asymptomatic cystitis to pyelonephritis and septicemia (Saligrama *et al.* 2014).

Treatment of UTIs constitutes a great portion of prescription of antibiotics. Antibiotics used in therapy of UTIs are usually able to reach high urinary concentrations, which are likely to be clinically effective. Naledixic acid is supposed to be an appropriate drug in treatment of UTIs caused by Gram-negative bacteria, since studies carried in many countries had demonstrated that many of this bacteria is still sensitive to naledixic

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acid. The prevalence of this sensitivity varies from 73 to 78% (Saligrama *et al.* 2014). Fluoroquinolones are preferred as initial agents for empiric therapy of UTIs in areas where resistance is likely to be of concern. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens (Culleun *et al.* 2013).

With the discovery of newer and powerful antibiotics, the medical society was hopeful to be able to manage microbial infections more effectively. On the other hand, the microbes that continued causing infections despite usage of these newer antibiotics represented a new form of infectious diseases caused by drug resistance microbes. The medical society was hopeful to be able to manage microbial infections more effectively, nevertheless, it is expected that in course of time, microbes will become more resistant because of their new mutants (Saligrama *et al.* 2014).

Empirical antibiotic selection should be based on knowledge of the local prevalence of bacterial species and antibiotic sensitivities (Saligrama *et al.* 2014). This study aimed to detect the incidence of UTIs caused by Gram-negative bacteria among patients attending Nasser Medical Complex (NMC)-Gaza Strip and to describe their antimicrobial susceptibility patterns.

METHODOLOGY:

A retrospective study was done in NMC which is categorized as a second level hospital complex containing 333 beds. The study included analysis of urine culture results for one previous year starting from January 2014 to December 2014. A total of 813 adult patients clinically suspected as having UTIs were involved, including 518 (63.7%) females and 295 (36.3%) males. Outpatients of the total males and females were 530 (65.1%) and inpatients were 283(34.8%).

Mid stream urine samples were collected in sterile wide mouth universal containers. The patients were instructed on how to collect the samples and were awarded about the need for prompt delivery of the collected samples to the laboratory. The samples were properly labeled and given serial numbers.

The samples were cultured on plates of blood agar and MacConkey agar. A 0.01 ml calibrated loop was used to deliver urine sample to the

inoculated plates. After inoculation, the plates were left on the bench for 15 minutes, in order to allow the urine to be absorbed into the agar medium. The plates were then inverted and after 18- 24 hours of incubation at 35 °C, the colonies were counted and samples with colony count $\geq 100,000$ cfu/ml were considered positive cultures (CLSI. 2013).

Initial identification was based on colonial morphology on MacConkey and Blood agar and Gram-stain reaction for isolated bacteria. All 331 Gram-negative isolates were identified by standard appropriate biochemical tests; triple sugar iron test, citrate test, urease test. In some suspected cases API 20E system was used for conformation (Microbeonline. 2013).

Susceptibility of bacterial isolates to thirteen of the most commonly used antimicrobial disks in NMC were tested by the Kirby-Bauer disk diffusion technique on Muller Hinton agar plates following the recommendation of the Clinical Laboratory Standards Institute (CLSI. 2013). Bacterial inoculation were prepared by suspending the freshly grown bacteria in 4-5 ml of 0.85% sterile saline and the turbidity was adjusted to that of a 0.5 McFarland standard. The inocula suspension were spread in three directions on Mueller Hinton agar plate surface with a sterile swab. Five to six filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial HiMedia were placed in each plate (Microbeonline. 2013). The antimicrobial disks used were: amikacin (30 μ g), amoxicillin (10 μ g), amoxicillin/calvulinic acid (10 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefuroxime (30 μ g), cephalexin (10 μ g), ciprofloxacin (5 μ g), co-trimoxazole (30 μ g), gentamicin (10 μ g), naledixic acid (30 μ g) and pipracillin (100 μ g). After 18-24 hours of incubation at 35OC, the inhibition zones' diameters were measured, the inhibition zone size interpretation were as the guidelines stated by the manufacturer.

The variables were encoded numerically to be entered systematically and efficiently. Data was entered and analyzed on SPSS version 20. Statistical analysis using frequencies and cross tabulation between dependant and independent variables was carried out. Chi-square test was used and P-values of ≥ 0.05 was considered statistically significant.

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RESULTS:

Out of 813 urine samples included in this study, 395(48.5%) were positive cultures, of which 331(83.8 %) yielded Gram-negative bacterial isolates, whereas the remaining 64(16.2%) yielded Gram-positive isolates. Among the Gram-negative isolates, females constituted the source of 198 (59.8%) isolate, whereas 133(40.2%) isolates were from males.

E.coli had the highest frequency of isolation, followed by *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp., *Proteus* sp., *Acinetobacter* sp., while *Citrobacter* sp. showed the lowest frequency of isolation. The frequencies of each Gram-negative isolate are shown in table (1).

Sensitivity frequency rates of the microbial agents isolated to the used antibiotics are shown in table (2). The most effective antibiotics generally revealed in this study sensitive mainly to amikacin followed by ciprofloxacin and pipracillin. On the other hand, a high proportion of isolates were resistant to amoxicillin, co-trimoxazole and cephalexin, respectively.

Table (1): Occurrence of Gram-Negative Bacteria Causing UTI in NMC

Isolate	No of isolates	%
<i>E. coli</i>	188	56.9
<i>Klebsiella</i> sp.	82	19.1
<i>Enterobacter</i> sp.	23	10.9
<i>Pseudomonas</i> sp.	17	6.8
<i>Proteus</i> sp.	13	3.8
<i>Acinetobacter</i> sp.	5	1.4
<i>Citrobacter</i> sp.	3	1.0

Table (2): Sensitivity Rates of Gram-Negative Bacteria Causing UTI in NMC

Isolate	Sensitivity to antimicrobial agents (%)												
	Ak	Cip	Pip	Ac	Ca	Ci	Ce	G	Cu	Na	Cp	Co	Am
<i>E. coli</i>	82	85.6	83.5	78.6	81.3	73.9	76.5	72	50.6	56.7	46.7	39.7	15.1
<i>Klebsiella</i> sp.	90	67.9	72.3	48.6	62.3	58.8	57.7	56.1	43.1	46.5	33.3	25.1	9.4
<i>Enterobacter</i> sp.	81.1	75	78.1	69.4	64.6	66.6	58.3	59.1	40.6	48.4	32	29.3	10
<i>Pseudomonas</i> sp.	83.3	66.6	58	39.2	66.6	54.5	46.3	64.7	32.7	19.2	15.3	11.1	8.2
<i>Proteus</i> sp.	80	66.6	70.8	68.8	71.4	71.4	67.1	45.5	63.6	41.3	66.6	35.2	10
<i>Acinetobacter</i> sp	76.6	61	54.1	44.8	60.1	53.3	54.1	54.1	39.3	28.9	13.8	8.1	5.2
<i>Citrobacter</i> sp.	93.1	86.3	81.8	74.5	80.1	67.9	78.8	68.1	70.2	53.3	58.7	45.1	13
OVER ALL SENSITIVITY %	83.7	72.7	71.2	69.5	69.5	63.7	62.7	59.9	49.1	42.0	38.1	31.1	10.1

Ak: Amikacin - Cip: Ciprofloxacin - Pip: Pipracillin - Ac: Amoxycillin / clavulanic acid - Ca: Ceftazidim - Ci: Ceftriaxone - Ce: Cefotaxime - G: Gentamicin- Cu: Cefuroxime - Na: Naledixic acid - Cp: Cephalexin - Co: Co-trimoxazole - Am: Amoxycillin -

DISCUSSION:

In this study 83.8% of the positive cultures gave significant Gram-negative bacterial growth. A significant relation was found between UTIs rate and female patients ($P>0.05$). The occurrence of UTIs among female patients (59.8%) was greater than among male patients (40.2%). These results agree with many frequent reports indicating that UTIs are more frequent in females than males. Astal *et al.* (2002) found in a study carried out in Gaza-Strip that the sex distribution for the positive cultures in patients suffering from UTIs was 75.0% females and 25.0% males. Parallel to our finding, it was reported that females patients were more susceptible to UTIs than males (75.4% vs. 24.6%) (Luke *et al.* 2015).

This finding was anatomically proposed to be a result of the shorter and wider urethra in females ([Sibi et al. 2014](#)). Furthermore, the antibiotic use changes the vaginal flora and promotes colonization of the genital tract with *E. coli* resulting in subsequent increased risk of UTIs (Jackson. 2010).

The most predominant organisms isolated among the patients attended NMC during the study period were *E. coli* followed by *Klebsiella* sp. This

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finding agrees with many other reports indicating that Gram-negative bacteria, mostly *E. coli* and *Klebsiella* sp., are the commonest pathogens isolated in patients with UTIs (Nathiswan *et al.* 2010, Hudson *et al.* 2014, Themphachana *et al.* 2014). In India, Patel *et al.* (2010), showed that *E. coli* was the most common isolated Gram-negative organism followed by *Klebsiella* sp. and *Proteus* sp. In Iran, it was revealed that UTIs predominant agents were *E. coli* (65.2%), *Klebsiella* sp. (26%) and *Pseudomonas aeruginosa* (3.6%) (Seyed 2014). Also, Sibi *et al.* (2014) observed that among the Gram-negative isolated uropathogens, *E. coli* was the predominant bacteria (43.9%) followed by *K. oxytoca* (19.4%) and *K. pneumoniae* (13.3%).

The most effective antibiotics in this study were amikacin (83.7%), ciprofloxacin (72.7%) and ceftazidime (69.5%). These results are partially parallel to two local studies previously carried out in Gaza-Strip. The first was reported by Astal *et al.* (2002), in which they demonstrated that the most effective antibiotics against all the isolates were ciprofloxacin (95.9%), amikacin (95.0%) and ceftazidime (94.2%). The second study was conducted by Astal (2005), in which he observed that the highest sensitivity rates were to amikacin (90.0%) followed by ciprofloxacin (85.0%).

Recently Sibi *et al.* (2014) revealed relatively similar findings to ours. Amikacin had the highest overall sensitivity (76.7%) and the subsequent highest sensitivity was observed with ciprofloxacin (73.3%), cefotaxime (65%) and nalidixic acid (63.9%). Another supporting outputs were revealed by Akhtar *et al.* (2014). They reported amikacin as the most effective antibiotic (63%) followed by Cefotaxime (55%), Amoxicillin and Ciprofloxacin (49%) each.

The achieved sensitivity rate for ciprofloxacin in this study was 72.7%. This result contradicts what was revealed by El Bouamri *et al.* (2014), who reported 18% sensitivity rate result for ciprofloxacin. Nevertheless, two earlier studies revealed lower but closer sensitivity rate results to our findings regarding ciprofloxacin: 41.3%, (Ullah *et al.* 2009) and 42% (Alemu *et al.* 2012).

The least effective antibiotics tested in this study were amoxicillin (10.1%) followed by co-trimoxazole (31.1%).

Our findings regarding co-trimoxazole, which is the most commonly used antibiotic to treat UTIs, agree with what was reported recently by El Bouamri *et al.* (2014) and Themphachana *et al.* (2014). They recorded 34% and 35% sensitivity rate to co-trimoxazole among the isolates respectively. On the other hand, Ullah *et al.* (2009) reported a clear drop in the sensitivity rate to co-trimoxazole (6.52%) in their study.

Unfortunately, it is obvious that antibiotic resistance has become an increasingly pressing clinical issue in many countries. This high rate of resistance to antibiotics could be due to several causes. Inappropriate prescribing of antibiotics is one important cause of this alarming phenomenon. In patients with suspected UTIs, antibiotic treatment is usually started empirically before urine culture results are available. This administration of treatment without considering the microbiologic and antibiotic sensitivity data gives the microorganisms the opportunity to develop mechanisms to circumvent their mode of action. In addition, poor infection control strategies and antibiotic treatment of duration shorter than required result in more resistance in bacterial strains (Alsammani *et al.* 2014, Somashekara *et al.* 2014).

The survey results suggest that antimicrobial agents should no longer be prescribed for treatment of suspected UTIs until susceptibility reports are revealed. However, the decision to use a particular antibiotic depends on its toxicity, cost and attainable level. Among adults, ciprofloxacin is preferred as initial agent for empiric therapy of suspected UTIs. Constant monitoring of susceptibility pattern of specific pathogens in different time intervals is recommended. This will help to check emergence of resistance strains and thus help patient management and trigger decision makers to update the empirical therapeutic strategy.

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