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Urinary Tract Infections Caused by β-lactamase Producing Clinical Bacteria in Oncology Center, Mansoura University, Egypt

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Abstract

β-lactamases exhibited by uropathogenic bacteria represent a major issue in the treatment of urinary tract infections. The resistance of β-lactamases is aworld wide medical issue. Recently, the evaluating of bacterial β-lactamases production, and identification of their drug obstruction, should be a constant cycle. The present study was performed to deduce the antibiotic resistance and the β-lactamases gene type of isolates from Oncology Center Hospital (Mansoura University). The results came out that E. coli and K. pneumoniae were the most associated strains (42.0%, and35.0%, respectively). Antimicrobial sensitivity test displayed that K. pneumoniae was resistance to Ciprofloxacin and Levofloxacin, Cefotriaxon and Trimethoprim-sulpham by 37.8%, 39.1%, 39.3%, and 33.8%, respectively. E. coli was resistant to Ciprofloxacin and Levofloxacin, Cefotriaxon and Trimethoprim-sulpham by 41.8%, 41.8%, 45.4%, and46.7%, respectively. The detection of blaTEM gene using Polymerase Chain Reaction showed that it was introduce by 70% in K. pneumoniae and 30% in E.coli of isolates. It could be concluded that β-lactamases production among uropathogenic bacteria is present at a high rate among urinary tract Egyptian cases in Oncology Center Hospital,. Mansoura University.

Keywords: Beta-lactamase, urinary tract infections, Escherichia coli, Klebsiella pneumoniae, TEM gene.

Introduction

Infection of urinary tract is indicating to the infection any where in the urinary tract. Infections of urinary tract are run about by a wide compass of microbes, including Gram-positive and Gram-negative bacteria, as well fungi. Urinary tract disorders regularly reach ladies, children and older cases that are generally healthy. Confounded infection of tract of urine is generally associated with catheters, urinary tract irregularities, immunosuppression or resistance to antibiotics. The widely feted causative specialists for urinary tract trouble are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Streptococcus*, *Staphylococcus aureus*

and *Candida* spp., *Enterococcus* spp. (Ana *et al* 2015). In the event that infection of urinary tract is not treated prematurely and adequately, it might affect into insistent sickness and damage of kidney (Adjei and Opoku, 2004).

Patients with cancer are a special with the characteristics of immune deficiency, and multiple complications (Van *et al* 2018). The incidence rate of beta lactamase urinary tract infection in cancer patients is high continues (Sime and Biazin 2020**)**.

Bacterial beta lactamases had to be a precaution against the mortal force of cephalosporins and monobactams on cell wall combination. The beta lactamase product is the most predominant design explicable for protection from beta lactams. All beta- lactams have a relative component of action; they discourage the production of cell wall (Essack, 2001). The initiative of beta lactams on sensitive bacteria can be noticed as a two-stages process, in the main stage, the antibiotics tie to native receptors, truly distinguished as grade related penicillin-binding protein. These proteins act focal jobs in the cell cycle associated, morphogenetic blend of cell partition peptidoglycan. In actuation of penicillin-confining protein by set antibiotic has a prompt, biochemically distinguishable impact on their capacity. The later stage incorporates the physiological consequences for the sensitive cell started by this constitutive receptor ligand connection (Donald and Tipper 1985).

Generally, *Escherichia coli* and *Klebsiella pneumoniae* are kinds of Gram-negative *Enterobacteriaceae* with the high frequency causing beta lactamase urinary tract infection (Vachvanichsanong *et a l* 2020), Sanders and Sander (1992), showed up that Enterobacteriaceae β- lactamases introduced as the most usually in uropathogens, as *Klebsiella* and *E. coli*. Other enterobacteria also produce β-lactamases to a low category (Goussard and Courvlin 1999, Bush and Jacoby 2010). Multifarious spit of β- lactamases was assembled into class (A, B, C and D) (Bush *et al* 1995). β-lactamases class A which are arrested in vitro by sulbactam, tazobactam, and clavulanic acid while those having a grade with class B, class C and class D aren't slammed (Patricia 2001). A large part of beta lactamases are extrapolated from TEM gene and SHV gene mutations. β-lactamases creatures basically spread, and turned into a considerable reason for infections associated with high death rates, particularly in effective diseases like septicemia (Bjorn *et al* 2005).

The β-lactamases enzymes exhibited by bacteria are gradually causing urinary tract infections both in hospitalized and others cases. This is making treatment of urinary tract problems troubling and advancing more prominent application of expensive extensive range antibiotics, for instance, carbapenems. Identification of β-lactamases involving standard antimicrobial helplessness approaches and deferral in the recognition and annunciating of β-lactamases promotion by Gram-negative bacilli are related with delayed clinic stay, increase bleakness, mortality and medical care price tickets (Mehran and Rahbr 2008). The aim of our research was to estimate the antibiotic resistance of β- lactamases pathogenic bacteria in Mansoura University Oncology Center.

Material and Methods

This study imparted in the laboratories of Microbiology of the Mansoura Center of Oncology, Egypt from the first of October, 2019 to end of September, 2020. Collection of urine samples were done by the medical center nurses then transferred to the microbiology laboratory, then reprocessed for urine culture, were streaked on of MacConkey agar medium at 37 °C were also incubated for 24 h. With the appearance of bacterial growth including all bacterial isolates with a clinically significant growth were included in study. The strains were identified using the Vitek 2 system at Mansoura University Oncology Center . Samples tested for antibiotic resistance using the method of disk diffusion, for 24 h, the disks were placed on the plates at 37 °C. After incubation, the inhibition zone diameter was measured by (mm) and the testing susceptibility results were observed according to CLSI (2011).

Detecting of β-lactamase producers using polymerase chain reaction (PCR) based detection (Koneman *et al* 1997, Hefernan *et al* 2007). Extraction of DNA was performed according to guidelines of the kit of Qiagen isolation DNA kit (Germany). The targeted genes were amplified using Taq polymerase master mix (ready to use PCR reagent) using specific primers for antibiotic bla_{TEM}. DNA was amplified with the thermal cycler Robocycler (Stratagene) using the cycling parameters and the conditions of amplification (Table 1). The

DNA fragments were visualized by the agarose gel electrophoresis. DNA band of 800 bp were interpreted as positive specimen for presence of TEM gene.

Table1. PCR primer used for detecting *bla*$_{TEM}$ gene

Nucleotide sequence (5`-3`)	Target gene	Ta	Product size	Reference
F: CATTTCCGT GTCGCCCT TATTC	*bla*$_{TEM}$	58°C	800 bp	Luo *et al* *2011*
R: CGTTCATC CATAGTTG CCTGAC				

Results

The total numbers of samples include one hundred and fifty during the study period. 96 bacterial isolates were obtained from urine samples. Gram-negative bacilli were the most common. There were 42.0% *E. coli*, 35.0% and *K. Pneumoniae*, others isolates mentioned in Table 2.

Klebsiella pneumoniae was resistant to Cefpodoxime, levofloxacin Cefoprazon-sulbactam, Ceftazidime and Ciprofloxacin by 46.1%, 39.1%, 44.4%, 38.4%, and 37.8, respectively. *E. coli* was resistant to Piperacillin-tazobactam, Gentamycin, and Ceftazidime, Ciprofloxacin, and Levofloxacin by 57.1%, 58.8%, 46.1%, 41.8%, and 41.8, respectively). *K. pneumonia*e was sensitive for cefoxitin by 50%, *E. coli* was sensitive to cefoprazon-sulbactam, levofloxacin and ciprofloxacin, also with 50% (Table 3).

Table2: Bacterial isolates distribution among studied cases

Bacterial isolates	%
E. coli	42.0
K. pneumoniae	35.0
Enterococci	7
Pseudomonas	4
Proteus mirabilis	3
Citrobacter	2
Breundimona	1
Raoultella	1
Kluyvera cryocrescens	1

The β-lactamase detection by Polymerase Chain Reaction revealed the amplification of about 800 bp on agarose gel electrophoresis (Figure 1) as a positive result for the presence of *bla*$_{TEM}$ gene. The results showed that the beta lactamase *bla*$_{TEM}$ gene spread among Enterobacteriaceae, *K. pneumoniae* (70%), and *E. coli* (30%) (Table 4),

Table3. Antibacterial resistance and sensitivity by *Escherichia coli* and *Klebsiella pneumonia*e

Antibiotics	*K. pneumoniae*			*E. coli*			*P*- value	Antibiotics	*K. pneumoniae*			*E. coli*			*P*- value
	R%	I%	S%	R%	I%	S%			R%	I%	S%	R%	I%	S%	
Amoxicillin-clavulanic	41.6	25.1	33.3	16.6	50.1	33.3	0.142	Doxacycline	57.1	2.9	40	14.2	35.8	50	0.214
Levofloxacin	39.1	60.9	0	41.8	8.2	50	<0.001*	Ertapenem	47.3	43.7	9	36.8	8.7	54.5	0.093
Azetronam	16.6	83.4	0	83.3	16.7	0	…	Cefoprazon-sulbactam	0	0	100	46.4	53.6	0	0.574
Azithromycin	0	0	0	33.3	66.7	0	…	Ceftazidime avibactam	0	100	0	100	0	0	…
Amikacin	77.3	4.2	18.5	45.4	18.9	35.7	0.254	Piperacillin-tazobactam	28.5	71.5	0	35.1	14.9	50	0.001*
Azlocillin	0	100	0	100	0	0	…	Cefoxitin	37	13	50	46.2	53.8	0	<0.001*
Ceftazidime	38.4	61.6	0	46.1	53.9	0	<0.001*	Gemifloxacin	50	0	50	33.3	66.7	0	0.261
Cefpodoxime	46.1	28.9	25	35.8	64.2	0	<0.001*	Gentamycin	35.2	36.3	28.5	58.8	27	14.2	0.015*
Ciprofloxacin	37.8	62.2	0	41.8	8.2	50	0.009*	Impeneme	48.4	21.3	30.3	36.3	18.3	45.4	0.274
Cefoprazon	44.4	55.6	0	37	13	50	0.039*	Meropem	46.1	25	28.9	38.4	24.3	47.36	0.631
Cefotriaxon	39.3	50.7	10	45.4	54.6	0	0.982	Minocycline	0	100	0	0	0	100	…
Cefotaxime	37	40.8	22.2	40.7	3.8	55.5	0.963	Norfloxacin	37.9	62.1	0	37.9	62.1	0	…
Cephaloxin	100	0	0	100	0	0	0.533	Chlortetracycline	0	100	0	100	0	0	…
Clarithromycin	33.3	66.7	0	33.3	66.7	0		Colistin	0	100	0	0	0	100	…
Ceftibuten	100	0	0	0	100	0	…	Cefixime	100	0	0	100	0	0	…
Cefazolin	100	0	0	100	0	0	…	Trimethoprim-sulpham	33.8	16.2	50	46.7	3.3	50	0.997

R(Resistance), S(Sensitive), I(Intermediate).

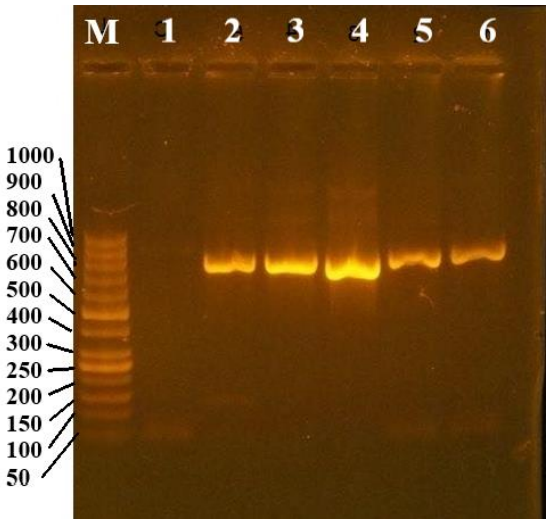

Figure1. β-lactamase detection on agarose gel electrophoresis for PCR. Lane 1, negative control for PCR product. Lane 2-6 Positive PCR product of *bla*$_{TEM}$ gene producing about 800bp band for some clinical representative samples. Lane M, 1kb DNA marker.

Table4. Distribution of *bla*$_{TEM}$ gene among some *K. pneumoniae* and *E. coli* clinical isolates

		K. pneumoniae	**E. coli**	**P-value**
bla$_{TEM}$ gene	Positive	70%	30%	0.06

Discussion

The most extensively recognized bacteria responsible for infection of urinary tract in the current research are *E. coli* and *K. pneumoniae*. Qurshi (2005) reported that *K. pneumoniae* and *E. coli* were introduced as principle for infection of urinary tract among youths in Pakistan. Likewise, the predominance of *Klebsiella* species in our study was 35% while the ratios revealed in Ethiopia was 19- 21% (Tensaie 2001) and Cameroon was 18.51% (Pieboj *et al*., 2004).

In this study, *K. pneumoniae* was resistant to Cefpodoxime, Cefotriaxon, levofloxacin, Ceftazidime and Ciprofloxacin by 46.1%, 39.3%, 39.1%, 38.4%, and 37.8 % respectively. Also *E. coli* was resistant to Piperacillin-tazobactam, Gentamycin, Ceftazidime, Ciprofloxacin, and Levofloxacin by 35.1%, 58.8%, 46.1%, 41.8%, and 41.8, respectively. El- Sweify *et al* (2015) reported a predominance of 44.33% of carbapenem resistance for *K. pneumoniae*. Others registered a lower frequency at 13.92% in the National Center of Cancer Institute of Egypt (Hossam and Amany 2009) and14.2% in Al- Azhar University (Khalid *et al* 2010). According to Mekonnen *et al*. (2023), presence of bacterial infection in urinary tract was 24.1%, and most of the bacterial isolates 68.75% were gram-negative bacteria, the *E. coli* 28.75% and *K. pneumoniae* 12.50%.

Comparably typical studies appeared changing rate from 20% to 40% in Greece (Bratu *et al* 2005; Giakoupi *et al* 2009), and approached consequent of 83% was showed in America (Marquez *et al* 2013). The pervasiveness of carbapenem resistance *K. pneumoniae* was 33.3% (Dalia and Doaa 2017). Our results showed that TEM gene is spread among Enterobacteriaceae by 20% in clinical isolates. Worldwide spread of β-lactamases exhibiting strains gives an extraordinary importance to the exploration of these strains in original area and emergency gatherings for reassessment of the immediate treatment prescriptions. In Egypt, multitudinous examinations have delved the pervasiveness of beta lactamases among Enterobacteriaceae dissociated from medical clinics and local domain gained urinary tract infections (Fam *et al* 2011; Abdel- Moaty *et al* 2016; Hasuna *et al* 2020). Additionally, our commonness of β-lactamases showing isolates is veritably advanced than that revealed in a several other Egyptian surveys; namely, 17% by Fam *et al* (2011) and 38.8% by Shash *et al* (2019). The pervasiveness of β- lactamases differences as indicated by species, geological zones, kinds in infection control programs, various exemplifications of empiric antibiotic rules and, unexpectedly, after some time.

El-Nagdy (2016) found that 139 out of 200 *E. coli* isolates from urinary tract infected patients were multidrug resistant *E. coli* and he also recorded that 129 out of 200 of *E. coli* are positive for extended spectrum beta lactamase.

According to Wang *et al*., (2023), *E. coli* presented the absolute percent 82.50% for production the beta lactamase enzyme, so it served as the most contributed to the overall trend in the prevalence of beta lactamase enzyme urinary tract infection in that study, and also *K. pneumoniae* with 16.82%, presented the subsidiary effect on the all prevalence of beta lactamase enzyme urinary tract infection.

Besides, specific pressure ran about by the misemployment of antibiotics in certain nationalities prompts the rising of β- lactamases production. Since the beginning of the new

thousand periods, *E. coli* has turned into the most regularly restricted β- lactamase exhibiting bacteria worldwide with CTX-M being the most frequently disentangled types (Hong *et al* 2018) as 43.9% of *E. coli* and 56.1% of *K. pneumoniae* produced β- lactamase. The genes from SHV and TEM types among the β-lactamase producing isolates were 14.4% and 20.6%, respectively. Proportions of positive β-lactamase isolates from out- cases to hospitalized patients were 24% to 33% (Fatemeh *et al* 2012).

Bajpi *et al* (2017), concluded the pervasiveness of β- lactamase TEM, SHV, and CTX- M genes among the individualities from Enterobactriaceae as 78.2% *E. coli* and *Klebsiella* isolates were recognized out of the 80% individualities. Additionally, the frequency of TEM gene was 55.1% in *E. coli* and 58% for *Klebsiella*. Jena *et al* (2017) have delved the predominance of SHV TEM and CTX genes of β-lactamase producing *E. coli* isolated from urinary tract infection. The predominant TEM gene was 93.43% followed by CTX was 82.21 %, and SHV was 4.3%. In another research, Bali *et al* (2010) showed that among the beta lactamase producing *E. coli* (71.93 %) was TEM gene.

Conclusion

Our research had offered an overview of the common urinary bacteria introduced in Oncology Hospital Mansoura and appeared a specific diverseness. The spectrum of antibiotic conditioning showed that some antibiotics apply the effectiveness on urinary tract infections. Mansoura Hospital of Oncology as well as in other developing nationalities, many propensities were at point of distinguished as conditioning which advancing the resistance development of bacterial strain to antibiotics as a self- medication, TEM gene is far reaching among clinical isolates. Wide spread of beta lactamase producing strains gives an extraordinary importance to disquisition of these strain in local area and medical clinics for assessment of the instant therapy prescriptions.

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الملخص العربي

عنوان البحث: التهابات المسالك البولية التي تسببها البكتيريا السريرية المنتجة للبيتا لاكتاماز في مركز الأورام ، جامعة المنصورة ، مصر

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تعد التهابات المسالك البولية أحد الأسباب الرئيسية لوصف الأدوية واستهلاك المضادات الحيوية من أجل استخدام أفضل علاج بالمضادات الحيوية لمرضاهم ، يجب أن تتوفر للأطباء بيانات موثقة وحديثة حول علم الأوبئة ومقاومة المضادات الحيوية للبكتيريا المسببة للأمراض البولية ، لذلك فإن المراقبة المنتظمة في كل دولة مطلوبة. هدفت هذه الدراسة إلى التحقق من التنوع البكتيري الممرض ومعدلات مقاومة مضادات الميكروبات للبكتيريا المسببة للأمراض البولية بمستشفى المنصورة للأورام (مصر - مدينة المنصورة) في الفترة من ١ أكتوبر ٢٠١٩ إلى ٣٠ سبتمبر ٢٠٢٠ وكذلك المساهمة في الرصد والمراقبة والتكيف للعلاج بالمضادات الحيوية. تم إجراء التحليل لـعينات البول فى مستشفى مركز الأورام بالمنصورة وفقًا للبروتوكول الروتيني لتحليل البول. تمت تحليل النتائج للحصول على معدل انتشار التهاب المسالك البولية ومعدل المقاومة البكتيرية للمضادات الحيوية واتجاه تطورها بمرور الوقت ومعدل مقاومة الأدوية المتعددة. تم الكشف عن وجود البكتريا في حوالي ١٠٠ عينة حيث كانت إشريشيا كولاي و كلبسيلا بنيومنيا أكثر سلالات تم التعرف عليها بنسبة ٤٢,٠٪ و ٣٥,٠٪ على التوالي. أظهرت اختبارات المقاومة للمضادات الحيوية أن بكتيريا كلبسيلا بنيومنيا كانت أكثر مقاومة لـ سيفترياكسون بنسبة ٣٩,٣٪ ، بينما كانت مقاومة إشريشيا كولاي لمضادات سيبروفلوكساسين و ليفوفلوكساسين بنسبة ٤١,٨٪. يبدو أن تنوع بعض البكتيريا المسببة للأمراض البولية التي تم الحصول عليها مقاومة لمضادات حيوية مختلفة كان يتبع اتجاهًا مثيرًا. في انتظار مواجهة طريق علاجي مسدود مع ظهور بكتيريا متعددة المقاومة، فإن تحديد الأسباب الخاصة بالمنطقة أمر بالغ الأهمية لتكييف العلاج بالمضادات الحيوية.
كما وجد ان الجين الوحيد الموجوده لانزيم البيتا لاكتاميز فى إشريشيا كولاي و كلبسيلا بنيومنيا هو جين ال .TEM gene.