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Microbial Spectrum and Antibiotics Susceptibility Profile of Nosocomial Urinary Tract Infections in Mansoura Oncology Center

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A B S T R A C T

Hospital acquired infections (HAIs) are the commonest untoward incident in healthcare delivery worldwide that not only bring additional medical cost but also, extend hospital stay and affect patient's morbidity and mortality. Urinary tract infections (UTIs) account for majority of these infections. The objective is to estimate hospital acquired UTIs prevalence and antibiogram among the admitted cancer patients. Also, to evaluate the performance of chromogenic Uriselect 4 agar medium in isolation and presumptive identification of nosocomial UTIs. Among 10414 patients stayed in hospital > 48 h., 1080patients were suspected to have nosocomial UTIs. 698 (64.6 %) were catheterized and 382 (35.4%) were not. (4.49%) patients confirmed to have nosocomial UTIs. 468 (43.3%) yielded aerobic growth on CLED agar, versus 624(57.8%) on the chromogenic Uriselect4 media. On CLED, 408 isolates were identified by automated Vitek 2 system versus 348 (33.3%) were identified by manual biochemical reactions while 10% and 1.7% of the isolates were not identified by manual biochemical tests and automated Vitek2 system respectively. *E.coli* was the most frequent isolated uropathogen in cancer patients identified by manual biochemical reactions, automated Vitek 2 system and chromogenic Uriselect 4 medium(11.1%, 23.1% and 17.8% respectively).Catheterization had been the main risk factor for nosocomial infection in our patients.

Introduction

Nowadays, Hospital acquired infections (HAIs) are the major worldwide safety concern for both patients and health-care workers (Scherbaum *et al.,* 2014). HAIs monitored closely by agencies such as CDC to minimize their occurrence and improve patient safety (Monegro and Regunath, 2018). Hospital acquired infections occurs within 48 and 72 hours of patient's hospital admission (Rose *et al.,* 2019). UTIs are estimated to be 25-40% of HAIs so they represent the most common nosocomial infections (Pragash *et al.,* 2017). Catheter associated urinary tract infection (CAUTI) represent one of the highest rates of occurrence among hospitalized patients, comprising about 36% of HAIs (Aly *et al.,* 2016). Hence, urinary tract instrumentation and prolonged hospital stay are predisposing factors for developing nosocomial UTIs (Gharaghani *et al.,* 2018). The predominant causative agents are *Escherichia coli, Klebsiella spp., Proteus*

mirabilis, Enterococcus spp. and *Staphylococcus* (Xiaoyan *et al.,* 2017). Identification of causative organism is crucial to plan for treatment protocol and decrease the risk of nosocomial infection. Our objective is to estimate the prevalence and antibiogram of nosocomial UTIs in the admitted cancer patients. Also, the performance of chromogenic Uriselect 4 agar medium which was compared with cysteine lactose electrolyte deficient medium (CLED) in isolation and presumptive identification of nosocomial UTIs.

Materials and Methods

This study was conducted on 1080 specimens that obtained from patients suspected to have nosocomial UTIs (64.6 % were catheterized, 35.4% were non-catheterized) from a total of 2304 urine samples received in OCMU laboratory from 10414 patients stayed more than 48h from a total number of 49585 patients admitted to OCMU during the period from January 2019 to December 2019.

Pd (patient days): total patient's days number in hospital within this period =24414. Dd (Device days): total number of exposure to the devices for all patients during this period=22757

Symptomatic nosocomial UTIs were diagnosed based on the National Health Safety Network guidelines (NHSN, 2015). They include at least one of these signs or symptoms that affect patients without any recognized cause: fever more than 38° C, urgency, frequency, or suprapubic tenderness and positive urine culture more than10^5 cfu/ml.

Early morning midstream voided urine or catheter-catch urine samples were aseptically collected in sterilized cap. Samples were then transported to microbiological laboratory within 2 hours.

Allurine specimens were cultured on both CLED agar and chromogenic Uriselect 4 media simultaneously then incubated at 37°C. Significant growth on both plates was identified.

Presumptive bacterial growth identification was done on chromogenic Uriselect 4 medium according to their colour and colony morphology as shown by manufacture supplement catalogue (as shown in Photos A to K). Colonies on CLED agar were identified by gram stain, manual biochemical reactions such as; coagulase, catalase, citirate utilization, ureas, TSI and oxidase and confirmed by by automated Vitek 2 System. Tests for antimicrobial susceptibility were done on Mueller-Hinton agar according to Kirby-Bauer disk diffusion method. Nitrofurantoin, Cefepime, Ampicillin-Sulbactam, Ceftazidime, Cefoxitin, Ceftriaxone, Ciprofloxacin, Levofloxacin, Ofloxacin, Amoxicillin-Clavulanic, Norfloxacin and Cefotaxime were antimicrobial agents used.

Results and Discussion

Patients included in this study were 444males and 636 females, with range of age from 31 to 66 years.

Calculated equations were performed based on (Angshuman *et al.,* 2015).

Among 1080 urine samples 468(43.3%) patients confirmed to have nosocomial UTI, among them 306/468 (65.3%) were CAUTI and 162/468 (34.7%) were not CAUTI. Incidence density (CAUTI rate) was 13.44/1,000 catheter days. DAI rate was 28.33%, but HAIs rate was 4.49% (Table 1).

Most nosocomial UTIs are associated with urinary catheters (Babamahmoodi *et al.,* 2015). Catheterized patients show greater risk for developing urinary tract infection (2.6

times) more than non-catheterized patients (Melaku *et al.,* 2012).

Another study reported that the incidence rate for device associated infections (DAI) was 19.55% (113/578). This is much lower than our findings. Nevertheless, the device utilization was very high in case of urinary catheter (0.838) in the same study which agrees with our results. DAI rate/100 cases was 6.23 while DAI/1000 cases was 4.25 (Jana *et al.,* 2015).

Sawsan *et al.,* 2020 reported the incidence rate of CAUTI was 29.5%. The difference from the result could be due to the fact that this study included only cancer cases who were more liable to nosocomial UTI due to immunodeficiency. Moreover, patient days were much longer in the current study compared to others in the existing literature.

In this study, CLED agar culture revealed bacterial growth in 468 samples (43.3%), gram negative bacilli were detected in 348 cases (74.4%), whereas gram positive organisms were encountered in 120 cases (25.6%).This findings were similar to the study conducted by Akter *et al.,* 2014 which resulted in (42.67%) bacterial growths and (57.33%) showed no growth. Bitew *et al.,* (2017*)* revealed that 256 of bacterial isolates were recovered, 68.4% of the isolates were Gram-negative and 31.6% isolates were Gram-positive bacteria. Furthermore, another study reported that gram negative bacteria accounted for 73.2% of cases with UTI, while the remaining cases were due to gram positive organisms (Yerega Belete *et al.,* 2019).

(Table 2) represent the growth on chromogenic Uriselect 4 medium that yielded 792 isolates. The most frequent isolated organism among the gram-negative bacilli was *E-coli* 192(17.8%), followed by *Citrobacter* 120 (11.1%). Regarding gram positive organisms Enterococci 216 (20%) were the most frequent.

In this study, (Table 3) shows the identification of organism growth was 43.3% and 57.8% in CLED agar and chromogenic Uriselect4 medium respectively. In addition, CLED agar yielded single organism in all specimens, while Uriselect4 detected mixed organisms in 19.2% of specimens. On comparing CLED agar with chromogenic Uriselect4 media, there was a significant difference between the two media in organism identification ($p < 0.001$), this means that chromogenic Uriselect 4 media was superior to CLED agar in isolation and identification of UTI organisms.

There was good agreement between the two techniques. Uriselect4 media had sensitivity and specificity of 100 and 74.5% respectively, with a diagnostic accuracy of 85.6%. Perry *et al.,* (2003) showed that chromogenic Uriselect4 medium lead to the recovery of 98.3% of strains, while it was 90.9% for CLED agar. This study agree with our findings that chromogenic Uriselect4 media were more effective in identification of uropathogens.

(Table 4), shows that 10% and 1.7% of the isolated organisms were not identified by manual biochemical tests and automated Vitek2 system respectively. The result indicated that E. coli was the most commonly isolated gram-negative bacteria (11.1%) by manual biochemical test and (23.1%) by vitek. This is due to the fact that, the commonest gastrointestinal tract and bowel flora is E. coli which ascends to urinary tract (Yerega Belete *et al.,* 2019).

Table.1 Device-associated infection rate, device utilization ratio, DAI/1000 patient days or 1000 device days

Infection	DAIs (n=306)	Device days(Dd)	Patients days(Pd)	Device utilization ratio (Dd/Pd)	DAI rate/100 suspected patients (n/1080)%	DAI/1000 device days(incidence density)	HAIs rate/100 patients (*n*/10414)%
CAUTIs	306	22757	24414	0.932	28.33	13.44	4.4

Table.2 Phenotypic identification of the isolated organisms on chromogenic Uriselect 4 medium

Isolated organisms on chromogenic Uriselect 4 media			N	%
Gram negative Bacilli	E-coli		192	17.8%
	citrobacter		120	11.1%
	Klebsiella		24	2.2%
	Enterobacter		72	6.7%
	Serratia		12	1.1%
	Proteus		48	4.4%
Gram positive Cocci	Enterococci	spp	96	8.9%
		E-faccium	96	8.9%
		E-fecalis	24	2.2%
	Staphylococci		96	8.9%
Yeast	Candida		12	1.1%

Table.3 Urine culture on CLED agar versus chromogenic Uriselect 4 medium

		Samples N=1080				
		CLED		Uriselect4		*P*
		N	%	N	%	
Culture	No growth	612	56.7%	456	42.2%	<0.001[C]
	Growth	468	43.3%	624	57.8%	
	Single	468	100%	504	80.8%	
	Mixed	0	0%	120	19.2%	

Table.4 Frequency of isolates by Manual biochemical identification and automated Vitek2 system

	Manual biochemical tests		Vitek2	
E-coli	120	11.1%	108	23.1%
Citrobacter	84	7.8%	96	20.5%
Klebsiella	24	2.2%	24	5.1%
Enterobacter	12	1.1%	36	7.7%
Staph	48	4.4%	60	12.8%
Enterococci	60	5.6%	72	15.4%
Candida	12	1.1%	12	2.6%
Unidentified	108	10.0%	8	1.7%

Table.5 Antimicrobial susceptibility pattern of the all isolated organisms (456) in this study

	456 Isolates			
	Sensitive		Resistant	
	N	**%**	**N**	**%**
Levofloxacin	368	80.7%	88	19.3%
Nitrofurantion	367	80.5%	89	19.5%
Norfloxacin	346	75.9%	110	24.1%
Ofloxacin	344	75.4%	112	24.6%
Cefoxitin	332	72.8%	124	27.2%
Ceftazidime	326	71.5%	130	28.5%
Cefepim	307	67.3%	149	32.7%
Ciprofloxacin	304	66.7%	152	33.3%
Cefotaxime	296	64.9%	160	35.1%
Ceftriaxone	247	54.2%	209	45.8%
Amoxicillin.Clavulinic	146	32.02%	310	67.98%
Ampicillin.Sulbactam	83	18.2%	373	81.8%

In the present study, the identified microorganisms by manual biochemical tests were E. coli 120 (11.1%), followed by *Citrobacter* 84 (7.8%), *Klebsiella* 24(2.2%), and *Enterobacter* 12 (1.1%). On the other hand, *Enterococci* were the commonest gram-positive organism 60 (5.6%), followed by Staphylococci 48 (4.4%), and Candida 12 (1.1%). In other study, culture-positive samples yielded a total of 199 bacterial isolates. *E.coli* was the leading bacteria isolated from samples 59.30% followed by *Staph. Saprophyticus19*.09%, *Enterococcus* spp.11.56%, *Klebseilla* spp.5.53%, *Pseudomonas* spp. 2.01%, Proteus spp. 1.51% and *Enterobacter* spp.1.00% (Akter *et al.*, 2014). In addition, other studies reported that *E. coli* 34.1% and *Pseudomonas* spp. 22.0% were the most isolated uropathogen. As regarding Gram-positive bacteria, S. saprophyticus 54.6% and S. aureus 27.3% were the commonest isolated bacteria (Yerega Belete *et al.*, 2019).

By using automated Vitek2 system in this study, 408 isolates were identified *E.coli*, *Citrobacter*, and *Klebsiella* species were identified in 23.1%, 20.5%, and 5.1% respectively. *Enterococci*, *Staphylococcus*, *Enterobacter*, and Candida were detected in 15.4%, 12.8%, 7.7% and 2.6% respectively, while 1.7% weren't identified by Vitek2 system.

Bitew *et al.*, (2017) showed that *Staphylococci* were the commonest gram-positive organism identified by automated Vitek2 system 21.4%, followed by *Enterococci* 5.9%, Streptococci 3.5% and kocuria 0.8%. Regarding gram-negative species, *E. coli* 52.7% and *K.pneumoniae* 7%. (Table 5), shows assessment of antimicrobial susceptibility pattern of the isolated organisms on Muller-Hinton agar by disc diffusion method. With exception of 12 Candida species isolates, the remaining 456 isolates revealed 80.7% sensitivity to Levofloxacin, 80.5% to Nitrofurantoin, 75.9% to Norfloxacin followed by 75.4% to Ofloxacin while Amoxicillin-Clavulinic and Ampicillin-Sulbactam were the most resistant.

Abdollahi *et al.*, (2016) introduced carbapenem and third generation

cephalosporins as the most effective antibiotics against pathogens recovered from Iranian cancer patients.

On evaluating the microbial susceptibility pattern of each organism showed a high rate of resistance (>80%) to Amoxicillin-Clavulinic and Ampicillin-Sulbactam for most isolates. While E.coli was more sensitive to Cefoxitin 90%, Nitrofurantoin and Levofloxacin 80%, Citrobacter showed sensitivity to Ceftazidime 85.7 and Staphylococci showed sensitivity to Nitrofurantoin 93%.

Other studies reported that Gram-negative bacterial sensitivity was high for ciprofloxacin, Norfloxacin and Ceftriaxone 70%, 63.4%, 60% respectively. And sensitivity for Gram-positive isolates were high for Ciprofloxacin 77.8%, Penicillin 72.8%and Erythromycin72.8%.Ampicillin, Penicillin and Cotrimoxazole were resistant with all isolated S. aureus. (Yerega Belete *et al.,* 2019).

In conclusions: the rate of catheter associated urinary tract infections (CAUTI) as an important device associated healthcare acquired infections was 65.3%.The most frequent gram negative uropathogen isolated in cancer patients was *E.coli* which was identified by manual biochemical reactions, automated Vitek 2 system and chromogenic Uriselect 4 media: 11.1%, 23.1% and 17.8% respectively.

On using automated Vitek 2 system, 8 (1.7%) isolates weren't identified. Using disk diffusion method; Levofloxacin and Nitrofurantoin were the most sensitive antibiotics for CAUTI 80.7% and 80.5% respectively, while Ampicillin/Sulbactam and Amoxicillin/Clavulanic were the most resistant antibiotics 81.8% and 67.98% respectively. Chromogenic Uriselect 4 media is cost effective and superior to CLED agar in growth of 156 urine samples (14.4%) and isolation of 120 mixed growths.

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