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ORIGINAL ARTICLE

Bacterial Pattern and Risk Factors of Hospital Acquired Infections in a Tertiary Care Hospital, Egypt

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Key words:
Nosocomial, Burn, ESBL, MRSA, Risk factors

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ABSTRACT

Background*: Hospital-acquired infection in hospitals especially in developing countries is a challenging health problem with a great burden on patients and national economy. The incidence and risk factors varies from place to another and from time to time which necessitates continuous updating to combat the problem.* ***Objectives:*** *A prospective cross-sectional surveillance study was done over two-year duration at Al-Ahrar hospital, Zagazig to estimate the incidence of hospital acquired infection (HAI) and highlight the risk factors associated, to determine the antibacterial susceptibility, and to generate a modified antibiotic policy against emergence of new resistant strains.* ***Methodology****: Samples were collected from different hospital wards. Isolation and identification of bacteria, antibacterial susceptibility testing was determined. Double disc synergy test and E- test ESBL were done for MIC determination.* ***Results****: The overall incidence of HAI in the hospital was 20.6%. Burn unit was the most affected (82.4%) followed by internal medicine and ICU with incidence of (57.10% and 29.40%) respectively. The most predominant organism was Pseudomonas spp. followed by E. coli and Staphylococcus aureus. Prolonged hospital stay, medical devices such as urine catheter, ventilator, CVC and some co-morbidities act as risk factors of HAI. The highest susceptibility was to imipenem, amikacin and vancomycin while all isolates were resistant to cephalosporins. All S. aureus isolates were MRSA and 18.2% were VRSA. Ten isolates (20.8%) were confirmed as ESBL by double disc synergy test and E-test ESBL.* ***Conclusion:*** *The relative high incidence of HAI and resistance among the studied isolates necessitates the implementation of strict infection control practice and antibiotic policies in our hospitals.*

INTRODUCTION

Nosocomial or Hospital acquired infections (HAIs) are defined as localized or systemic infection which is acquired in health care facility due to direct or indirect contact with its staff or environment and develops after 48 h or more of admission.[1] The incidence differs from one country to another, from hospital to another and in the same hospital from ward to ward and even in the same ward according to differences in clinical practices.[2] In developed countries HAIs are reported to affect from 5% to 15% of hospitalized patients and 50% or more of patients in intensive care units (ICUs), while in developing countries, the magnitude of the problem remains largely underestimated.[3] HAIs include different systemic and local infections including surgical site infections, central line-associated bloodstream infections, catheter-associated urinary tract infections, and ventilator-associated pneumonia.[4] This results in excess length of stay, functional disability and emotional stress of the patient, increased mortality and healthcare costs.[5] Treatment of these infections is also problematic due to the growing trends of antibiotics resistance which necessitates continuous updating of knowledge on antibiotic resistance pattern in different hospitals.[6] Hospitals, as a consequence of the high concentration of antibiotics usage, save the environment for the evolutionary battle between antibiotics and bacterial antibiotic resistance genes. Thus, in hospitals the spread and diversification of resistance patterns are drastically accelerated.[7] An increasing incidence of multidrug-resistant pathogens responsible for nosocomial infections is well noticed in different health care facilities. Methicillin-resistant *Staphylococcus aureas* (MRSA) and extended-spectrum beta-actamase (ESBL) producing bacteria are evolving in different countries worldwide with more impact on developing countries.[8,9]

ESBLs are enzymes hydrolyzing most penicillins and cephalosporins, but not cephamycins and carbapenems. These cpmpounds are inhibited by β-lactamases inhibitors: clavulanic acid, sulbatam and tazobactam. ESBL detection and characterization became mandatory for infection control practice. Two important steps are needed for ESBL detection. The first is a screening test with an indicator cephalosporin which looks for resistance or diminished susceptibility,

thus identifying isolates likely to be harboring ESBLs. The second step is a confirmation test which evaluates the synergy between an oxyimino cephalosporin and clavulanic acid, distinguishing isolates with ESBLs from those that are resistant for other reasons.[10]

The need to evaluate the incidence and risk factors of HAIs in different Health care facilities in Egypt is obvious. Al-Ahrar hospital is a relatively new Egyptian tertiary care hospital which lies at Zagazig City, and serves a large population density region. Evaluation of HAIs in this Hospital can give an impression about a large sector of Health care facilities in Egypt and can highlight the need for implementation of efficient infection control program and antibiotic policies in our hospitals.

The objectives of this work were to detect of the incidence of hospital acquired infections at Al-Ahrar hospital, antibacterial susceptibility of isolated organisms against different sets of antibacterial groups, determination of specific resistance pattern if any, and lastly to generate a modified antibiotic policy to guard against emergence of new resistant strains.

METHODOLOGY

Subjects:

Current study included 214 inpatients (103 males and 111 females) with mean age of (40.3±21) admitted to Al-Ahrar Hospital without showing any signs of infection for at least 48 hours after time of admission. The study included 92 patients from Surgery unit, 51 patients from Intensive care unit (ICU), 35 patients from Gynecology & Obstetric unit, 17 patients from Burn unit, 9 patients from Heart and chest unit, 7 patients from Internal medicine unit and 3 patients from Urology unit.

Bacteriological methods:

All patients were subjected to careful history taking. Samples were collected under complete aseptic condition using sterile syringes, swabs, catheters and containers. Endotracheal aspirate were obtained from ICU patients with suspected ventilator-associated pneumonia using sterile suction catheters. Urine was collected from patients with suspected urinary catheter associated infections according to Cheesbrough [11]. Culture and identification using routine microscopic and biochemical methods were done to all relevant isolates. Gram negative bacilli were fully identified using API20E. Isolates were subjected to antibiotic susceptibility testing using Kirby-Bauer disc diffusion method following CLSI standards.[12] Cefoxitin 30 µg disc was used for MRSA detection. Double disk synergy test was used for isolates presumed to be ESBL producers.[13] Disks containing (cefotaxime 30 µg and ceftazidime 30 µg) were applied next to a disk with clavunalic acid (amoxicillin-clavulanic acid) with a 2 cm center to center distance. Positive result was indicated by extension of the edge of the inhibition zone of ceftazidime and cefotaxime on the side near to amoxicillin-clavulanic acid disc. MIC determination for ESBL was performed using E-test strips (Liofilchem Diagnostics, Italy).

Risk factors:

For determination of risk factors associated with HAI, Univariate analysis of predictors for hospital acquired infection was done. The following factors were observed; Age, sex, ICU stay, central venous catheterization, urinary catheterization, endotracheal intubation, mechanical ventilation, underlying disease or comorbidity such as liver disease, diabetes mellitus, chronic heart disease and chronic renal failure.

RESULTS

Among the 214 samples obtained from different units in the hospital 44 (20.6%) of samples were confirmed to have hospital acquired bacterial infection. The burn unit was the most affected unit with an incidence of 82.40%. This was followed by internal medicine, urology and ICU with incidence of (57.10%, 33.30% and 29.40%) respectively. Surgical site infections were detected with an incidence of (22.20%, 14.30%, and 3.30%) from Cardiothoracic, Gynecology & Obstetrics and Surgery units respectively. Table 1 demonstrates the risk factors which were found to be associated with increased incidence of HAI.

Table 1: Univariate analysis of predictors for hospital acquired infection.

Variables	All (N=214)	Infected (N=44)		Non-infected (N=170)		p-value
		No.	(%)	No.	(%)	
Female	117	27	(23.1%)	90	(76.9%)	0.317‡
Male	97	17	(17.5%)	80	(82.5%)	
Age (years) (Mean ± SD)		40.3	±21	40.8	±18.1	0.874*
<7 days LOS	196	28	(14.3%)	168	(85.7%)	<0.001‡
≥7 days LOS	18	16	(88.9%)	1	(10.1%)	
ICU samples	51	15	(29.4%)	36	(70.6%)	0.078‡
Non ICU samples	163	29	(17.8%)	134	(82.2%)	
Heart diseases	12	9	(75%)	3	(25%)	<0.001‡
Non Heart disease	202	35	(17.3%)	167	(82.7%)	
Liver diseases	5	3	(60%)	2	(40%)	0.060‡
Non Liver disease	209	41	(19.6%)	168	(80.4%)	
Renal diseases	8	7	(87.5%)	1	(12.5%)	<0.001‡
Non Renal disease	206	37	(17.9%)	169	(81.1%)	
Diabetic	17	9	(52.9%)	8	(47.1%)	0.002‡
Non Diabetic	197	35	(17.8%)	162	(82.2%)	
CVC	7	6	(85.7%)	1	(14.3%)	<0.001‡
Non CVC	207	38	(18.4%)	169	(71.6%)	
Ventilator	13	9	(69.2%)	4	(30.8%)	<0.001‡
Non Ventilator	201	35	(17.4%)	166	(82.6%)	
Urinary catheter	73	30	(41.1%)	43	(58.9%)	<0.001‡
Non Urinary catheter	141	10	(9.9%)	127	(90.1%)	

Continuous variables were expressed as mean ± SD; categorical variables were expressed as number (percentage); *Independent samples Student's t-test; ‡ Chi-square test; $p<0.05$ is significant.

Table 2: Demonstrates the distribution of bacteria responsible for the HAIs cases in this study, with the site of infection. A total of 48 pathogens were isolated from 44 patients.

Type of bacteria			Burn	Sur	ICU	Int. Med	Gyn	C. Thox
	n=48	(%)	n=15 (%)	n=3 (%)	n=16 (%)	n=4 (%)	n=6 (%)	n=4 (%)
*** Gram negative bacilli**	29	60.4						
1. Pseudomonas	14	29.22	12(80)	--	1(6.25)	--	1(16.67)	--
2. *E. coli*	11	22.9	1(6.67)	--	5(31.25)	3 (75)	2(33.33)	--
3. Klebsiella	3	6.2	--	1(33.3)	--	--	--	2(50)
4. Proteus	1	2.1	1(6.67)	--	--	--	--	--
*** Gram +ve bacteria**	19	39.6						
1. *Staphylococcus aureus*	11	22.9	--	2(66.7)	8(50)	--	1(16.67)	--
2. Enterococci	4	8.3	--	--	2(12.5)	1(25)	--	1(25)
3. Coagulase Neg. Staph. (CONS)	4	8.3	1(6.67)	--	--	--	2(33.33)	1(25)

Table 3: Antibiotic susceptibility of HAI cases by disc diffusion method

Antibiotic	Antimicrobial Susceptibility Patterns Of HAI Cases		
	Susceptible	Intermediate	Resistant
	%	%	%
Imipenem	88.6	0.0	11.4
Amikacin	62.1	34.5	3.4
Azithromycin	37.9	10.3	51.7
Augmentin	15.8	0.0	84.2
Ciprofloxacine	20.5	4.5	75.0
Vancomycin	52.6	0.0	47.4
Clindamycin	26.3	0.0	73.7
Tetracycline	6.9	3.4	89.7
Gentamycin	3.4	6.9	89.7
Sulphamethoxazole	6.7	0.0	93.3
Erythromycin	5.3	0.0	94.7
Oxacillin	0.0	0.0	100.0
Cephradine	0.0	0.0	100.0
Cefuroxime	0.0	0.0	100.0
Ceftazidime	0.0	0.0	100.0
Ofloxacin	0.0	0.0	100.0
Cefotaxime	0.0	0.0	100.0
Ampicillin	0.0	0.0	100.0
Imipenem	88.6	0.0	11.4

NB: Vancomycin was used with gram positive organisms only.

Antibiotic sensitivity of the isolated strains (Table 3) showed that highest susceptibility to imipenem, amikacin and vancomycin (88.6%, 62.1% and 52.6%) respectively. All isolates were resistant to cephalosporines (Cephradine, Cefuroxime, Ceftazidime, Cefotaxime) oxacillin and ampicillin.

Almost all gram negative isolates were sensitive to imipenem (100% sensitivity). This was followed by amikacin for which 15 out of 29 isolates were sensitive (51.7%) (6 *E. coli*, 8 Pseudomonas and 1 *Klebsiella*). Seven gram negative isolates was sensitive to azithromycin (1 E. coli, and 6 Pseudomonas). Four isolates were sensitive to ciprofloxacin (All Pseudomonas) and 2 isolates were sensitive to gentamycin (1 Pseudomonas and 1 Klebsiella).

Regarding *Staphylococcus aureus* isolates, all of the strains were found to be Methicillin resistant (MRSA) and 2 of them were found to be intermediate to vancomycin (VISA) although several studies show that the disc diffusion breakpoint is unreliable for detecting VISA and VRSA strains. Fortunately the 2 VISA isolates were found to be sensitive to Imipenem. The Enterococci isolates were all sensitive to vancomycin. Among the 4 isolated CONS one of the isolates was resistant to all tried antibiotics. The remaining isolates were sensitive to at least to imipenem and/or vancomycin.

The extreme resistance of Gram negative isolates to cephalosporins was a motive to test the gram negative isolates for ESBL production using double disc synergy test for (Fig. 1). ESBLs can be defined as β-lactamases capable of conferring bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid. Ten isolates (5 *E. coli*, 4 Pseudomonas and one Proteus) out of 29 were found to be ESBL indicated by distortion of the zone in the distance between (amoxicillin/clavulanate) disc and cefotaxime or ceftazidime discs. Using Epsiloemeter (E test) strips *(Liofilchem Diagnostics, Italy)* confirmed 3 only of the ten isolates as being ESBL strains.

Fig. 1: Positive double disc synergy test by using CTX, CTZ with AMC discs.

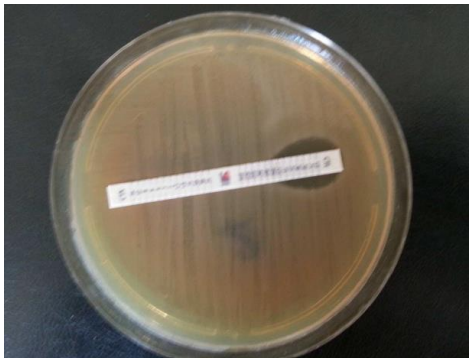

Fig. 2: E-test with (CTX/CTL ratio>8)

DISCUSSION

Nosocomial infections pose substantial risk to patients receiving care in hospitals. This problem is aggravated by inadequate infection control in developing countries due to poor hygiene, resources and structural constraints, deficient surveillance data and lack of awareness regarding nosocomial infections[14].

There is no documented or statistical evaluation of incidence of hospital-acquired infections (HAIs) in general hospitals belong to Egyptian Ministry of Health. Al-Ahrar hospital is a tertiary hospital serving a large number of populations. The study aimed to draw a chemotherapeutic portrait for HAI at Al-Ahrar hospital as an example of Egyptian tertiary care hospital. The study revealed that the incidence of HAIs at Al-Ahrar hospital was 20.6%. This incidence is considered high when compared to hospitals with similar demographic features[15,16]. However it was similar to rates reported in Benin[17] and in Primary–Care Hospitals in Nigeria[18]. This relatively high incidence can be explained by inclusion of burn unit. Most of burn patients were later infected because of skin breach and the severe immuno-compromised status in addition to prolonged hospitalization and invasive therapeutic and diagnostic procedures [19]. Burn unit showed the highest incidence of HAI (82.4%) in current study. Similar rates were previously reported by Oncul et al. [2009] who reported 166 nosocomial infections among 169 patients in burn unit in Turkey[20].

Internal Medicine Unit was the second in incidence (57.1%), as most patients in this unit were previously discharged from ICU and had prolonged hospital stay. This was followed by those from Urosurgery Unit (33.3%) and ICUs (29.4%). General Surgery unit showed the least incidence of HAI (3.3%) which can be explained by early antimicrobial prophylaxis in addition to rapid discharge of operated cases.

Similar to what was previously reported that gram negative bacilli were the most common cause of HAI in developing countries [21], gram negative bacilli were responsible for 60.4% of isolates in the current study. This matched with many older reports from different countries [22,23] However, a study in Assiut University Hospitals revealed that gram positive organisms predominated over the gram negative ones in the reported HAI along the period of their study [24]. It is noteworthy to be mentioned that this study was held on ICU patients only which could be a reason for the different rates from the current study.

The most frequently isolated organism in this study was pseudomonas (29.2%), followed by *E. coli* (22.9%), methicillin-resistant *Staphylococcus aureus* (MRSA) (22.9%), then enterococci (8.3%) and coagulase negative staphylococci (CoNS) (8.3%). Most of pseudomonas strains (85.7%) recovered in our hospital were collected from burn patients. Bacteriology of burns was a core of many studies which concluded that pseudomonas was the most predominant nosocomial infection in these patients [25,26]. The heavy spread of the organism as a contaminant on floors, beds sinks and even in disinfectants in hospital environments especially burn hospitals, make pseudomonas frequently isolated as an opportunistic pathogen from hospitalized burn patients[27].

Many studies reported similar patterns of bacterial HAI from different hospitals. Dogru et al. [28] in a Turkish medical surgical intensive care unit reported that pseudomonas spp and MRSA were the most frequently detected organisms in device associated nosocomial infections. In a Canadian multi-center study, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, were the most common isolates. MRSA represented 22.3% of all *S. aureus* isolates and vancomycin-resistant enterococci (VRE) made up 6.7% of all enterococcal isolates. (ESBL) producing *E. coli* and *K. pneumoniae* occurred in 3.0 % of isolates[29].

Among the risk factors evaluated in our study, invasive devices, length of hospital stay and chronic debilitating diseases revealed significant association with acquiring HAI. The usage of invasive devices is known to increase the opportunity to catch infections in hospitals. Urinary catheters, ventilator and central venous catheter (CVC) showed statistically significant difference ($P<0.05$) in the current study. This agreed with other Egyptian reports by Rasslan et al.[30] and Abdelfattah et al.[31]. They attributed the high rates of device associated HAI to unnecessary increase length of stay, low compliance in hand hygiene and serving many patients with same nurse.

Regarding the length of hospital stay, the percentage of HAI showed high statistically significance with increased hospital stay over 7 days (P-value<0.001). Similar conclusions were reached by Abdelfattah et al.[31] and Razine et al.[15]

Patients with chronic diseases are virtually immune-compromised and more prone to develop infections. This is surely aggravated by hospital

admission. In the current study, patients with chronic renal and heart diseases and diabetics were significantly more at risk to develop nosocomial infection, whereas liver disease patients were not. The same risk factors were reported in many other [24,32,33]. The non-significant association with hepatic disease is mostly attributed to the low number of chronic hepatic cases involved in the current study.

One of the main alarming threats in hospital associated infections is the antibiotic resistance patterns mostly encountered in hospitals, which make the battle more difficult to be controlled, leading to an increase in morbidity and mortality.[34]

In our study, the antibiotic susceptibility pattern revealed a limited panel of antibiosis that could be effectively used in such infections. Only, imipenem and amikacin were effective against most of isolates. Vancomycin revealed to be an effective choice against gram positive infections.

Interestingly, all isolated staphylococci were methicillin resistant either MRSA or MRCoNS. This is similar to previous report in a pilot study in Egyptian hospitals which reported that 93% of isolates were MRSA[35] Two out of the 11 *Staphylococcus aureus* isolates were vancomycin resistant (VRSA) as so. However, all isolated enterococci recovered in the present study showed complete susceptibility to vancomycin (100%).

The prevalence of vancomycin resistance varies significantly among different geographical distribution and according to many factors[36]. In Egypt, the vancomycin resistance is still in its infant stages. The incidence of its resistance, as observed in the current study, is not comparable with that of any other available antimicrobial drugs. This may be attributed to its limited use in the general community being only taken by infusion with no oral or direct intravenous or intramuscular injection available. Most of its intake is restricted to hospitals in resistant cases and only upon a sensitivity report. Also, the horizontal gene transfer particularly by conjugation, in gram positive bacteria against which glycopeptides work, is thought to be much less than that occurs in gram negative ones[37].

The least reported susceptibility was against Trimethoprim-sulfamethoxazole (6.7%), erythromycin (5.3%) and gentamycin (3.4%). The interesting point was that 100% of isolates were resistant to cephalosporines. The decreased efficacy of cephalosporins in Egyptian hospitals[24,38] and even in environmental samples[39] was previously observed in different Egyptian reports. This reflects the risk of non-rationalized use of antibiotics especially the cephalosporins in our hospitals and in community.

The distribution of antibiotic sensitivity among different isolates in different wards of the hospital was provided to hospital management and infection control team in the hospital to be used as a keystone for creating antibiotic policy.

The current study revealed that ESBL producing organisms represented 20.8% among gram negative isolates, proved by double disc synergy test (DDST). Positive E-test with CTX/CTL ratio>8 was only confirmed in 3 isolates while no reading was detected in 7 isolates. This might be due to presence of inhibitor resistant TEM (IRT) enzymes which cannot be detected by ESBL E-test strips as reported by the manufacturer. This is supported by an older report from the same locality revealed that *bla*VIM and *bla*TEM were the most prevalent resistance genes detected in the imipenem-resistant gram negative bacilli isolated[38]. The reported prevalence in this study was less than that reported by See *et al.* [35] who investigated HAI from 46 ICUs in Egyptian hospitals and revealed that 71% of isolates were ESBL, however all the isolates tested in this report were *Klebsiella pneumonia* and tested by combined disc diffusion method. An additional reason for the lower percentage of ESBL is false negative results. It was reported that SHV-6 ESBL and Amp C type β-lactamase producers are poorly detected by double disc diffusion or E tests methodologies [40]. The genetic basis for reasons of resistance to 3rd generation cephalosporin in the isolates should be considered for further work.

In conclusion, incidence of HAI in Egyptian hospitals is still higher than international benchmark rates. Although the Ministry of Health has taken many steps to implement infection control practices in its affiliated hospitals, however it seems that more steps and actions are needed to commit to international standards. The burn units and ICU were the main units at risk due to the immune-compromised nature of the patients inside. The association of HAI to multidrug resistance was clearly observed in the study. MRSA and 3rd generation cephalosporin resistance were the most prominent. This high incidence of resistance necessitates the implementation of strict antibiotic policy towards their intake either in hospitals or in the community.

Conflicts of interest:

The authors declare no conflicts of interest.

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