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BACTERIAL ISOLATES AND ANTIMICROBIAL SUSCEPTIBILITY IN AL-YARMOUK TEACHING HOSPITAL IN BAGHDAD

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ABSTRACT
The hospitals environment, patients and staff provide a reservoir of microorganisms, many of which are multi-resistant to antibiotics. Nosocomial infections are an important cause of morbidity, mortality, increasing financial burden to patients and public throughout the world. Three hundred twenty two cotton swabs taken from different departments and wards of Al-Yarmouk Multi-specialty Teaching Hospital. Three groups of swabs were collected: first group was collected from materials close to patients, the second group collected from materials related to health staff, while the third group was collected from the environment surrounding the patient. Swabs processed and examined for identification of bacterial growth. Pathogenic growth constitute 70.5%, the rate of none pathogenic growth is 26.1%, while the remaining 3.4% of the swabs show no growth. The highest sensitivity is to Imipenem (94.2%), followed by Levofloxacin (92.8) and Ciprofloxacin (92.5%), while the highest resistance is to Cefotaxime (98.4%) followed by Tetracycline (94.2%). A high rate of positive swabs with multidrug resistance pathogens is a serious issue that need a lot of attention. Additional study in this field with a larger sample size covers the anaerobic bacteria as well.

KEY WORDS: Swabs, Culture, Antibiotic resistance, Nosocomial infections

INTRODUCTION

Al-Yarmouk Teaching Hospital is one of the biggest governmental multispecialty hospitals in Baghdad (the capital of Iraq); it serves most areas in Alkurkh side of Tigris River in addition to the area western south of Baghdad. Hospital acquired infections (Nosocomial infections) are often described as an infections that are acquired within the hospital environment between 2-4 days of admission into the hospital or other health care facilities (WHO, 2002). The hospitals environment, patients and staff provide a reservoir of microorganisms, many of which are multi-resistant to antibiotics (Bryce *et al*., 2007, Muhammad *et al*., 2013). Recently, a new term, "healthcare associated infections" is used for the type of infections caused by prolonged hospital stay and it accounts for a major risk factor for serious health issues (Khan *et al*., 2015). Resistant bacteria to common antibiotics that cause serious infections have become a major global healthcare problem (Alanis, 2005, Levy, 2002). Nosocomial infections are continued to be an important cause of morbidity, mortality, prolonged hospital stay and extra financial burden to patients and public throughout the world (Kumar & Singh, 2015, Aktar, *et al*., 2016, Ozer *et al*., 2015). The patterns of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another, as well as from one hospital to other and among different locations in the same hospital (Pattanayaka, 2013).The increasing number of immunocompromised patients and increased use of indwelling devices, as well as massive and widespread use of antimicrobial agents in both hospital and community settings contributes to antimicrobial resistance among bacterial pathogens causing infections. This has profound effects on both the hosts who receive these drugs and the bacteria exposed to them (Chen *et al*., 2003). It has become aberrantly clear that the major nosocomial pathogens either are naturally resistant to clinically useful antimicrobial drugs or possess the ability to acquire resistance (Atata *et al*., 2013). Microorganisms may be related to several materials in the hospital environment such as floors, walls, ceiling, doors, windows, electronic equipment and specific hospital articles in use for assistance to patients (Bouzada *et al*., 2010). Environmental surfaces can be further divided into medical equipment surfaces (*e.g*., knobs or handles on hemodialysis machines, X-ray machines, instrument carts, and dental units) and housekeeping surfaces (*e.g*., floors, walls, and tabletops). Routine environmental-surface sampling (*e.g*., surveillance cultures) in health-care setting is neither cost-effective nor warranted (Ekrami *et al*., 2011). The role of surfaces in the spread of nosocomial infection is controversial. Although contamination of the inanimate environment by pathogens has been recognized, its significance is unclear (Bolaji *et al*., 2011). The contaminated surfaces generally are not directly associated with transmission of infections to either staff or patients. The transmission is largely via hand contact with the surface (Ekrami *et al*., 2011).

Throughout the world, cross-resistance and multi-resistance patterns have been observed. Indiscriminate use of antibiotics for medical purposes has taken the brunt of the blame. In fact, all antibiotic use, whether medical,

agricultural, and necessary or not, leads to increased resistance (Bolaji *et al*., 2011).

Control of antibiotic resistance requires aggressive implementation of several strategies: ongoing surveillance of resistance; using hygiene controls and antibiotic controls to limit spread of strains of resistant bacteria; and enlisting administrative support (Weinstein, 2001). The aim of the present study is to identify the rate of predominantly isolated bacterial microorganisms and their drug resistance patterns for the environment of a multispecialty Al-Yarmouk teaching hospital, and to put a base line data, which assist the control programs.

MATERIALS & METHODS

A cotton swabs taken from different departments and wards of Al-Yarmouk Multi-specialist Teaching Hospital, they were 322 swabs. Each ward contains many rooms with average of six patients in each room. Simple randomization method used to select the room to be included in the study, the second room from each ward was selected, and patient number 2 was chosen to be the studied sample. Culturing 14-32 swabs from the selected room to explore the bacterial inhabitants. Three main groups of swabs were taken the first group was related to patients including patients' skin, dress, and cloths. The second group collected from materials related to health staff including white coats, dressing truly, cannula, and health instruments. While the third group was from the environment surrounding the patient including patient's bed, side desk, door handles, ground, floor, and walls.

On Sunday, of each week sterile cotton swabs moistened with sterile normal saline was used to do swabbing on weekly interval. Samples collection started in October 2015 to February 2016. Three hundred twenty two swabs were subjected to examination to identify the bacterial inhabitant in the hospital. The swabs were labeled. These swabs immediately transported to the bacteriology unit, microbiology department of the Teaching Laboratories in AL- Yarmouk teaching hospital for processing. In the laboratory, swabs inoculated in Thioglycolate broth/ Tryptase soya broth and incubated overnight 24 hrs at 35 ±2°C to encourage growth. Observing the turbidity in soya broth for sub-culturing on Blood, MacConkey and Sabouroed dextrose agar for 24-48 hrs at 35±2°C for colony isolation and morphological identification.

Pure isolated colonies were Gram differentiated and then biochemically identified using Coagulase test, Mannitol salt agar, Urease tests, Indol, KIg, Simmon citrate, Catalax test, Oxidase test, Api 20 Staph, Api 20 Strept, Api Candida and Api 20 E.

Disk agar diffusion according to Kirby Bauer standardized antimicrobial susceptibility single disk method was carried using Muller Hintoen agar (Pierce-Hendry, and Dennis, 2010). Antibiotic used were: Trimethoprim-sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Cefixime (Cef), Azithromycin (AZ), Methicillin (Meth), Vancomycin (Van), Amikacin (Ami), Ciprofloxacin (Cip), Erythromycin (E), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev), Oxacillin (Oxa). (Bioanalys /Ankara-Turkey).

Statistical analysis

Collected data were entered into computer utilizing IBM SPSS software V20 program for grouping and statistical analysis. Tables were constructed frequencies and percentages were calculated and presented.

RESULTS

The contribution of different department and wards of the hospital in swabs collected were represented in table-1. This procedure covered almost all department of the hospital. The largest number of swabs (32) were collected from the administration building at a rate of 9.9% followed by emergency department (27)8.4%, while the least contribution was from the female side of the orthopedic department (14)4.3%.

TABLE 1: Distribution of swabs according to different departments and wards

Department/ward	n	%
Administration building	32	9.9
Emergency department	27	8.4
Medical ward/female side	23	7.1
RCU	22	6.8
Rheumatology and Neurology ward	20	6.2
Orthopedic ward/male side	20	6.2
Medicine ward/ male side	19	5.9
Surgery ward/female side	19	5.9
Dialysis unit	17	5.3
Communicable diseases ward	17	5.3
RCU Recovery Unit	16	5.o
Gynecology ICU	16	5.0
Surgery ward/male side	16	5.0
Obstetrics ward	15	4.7
Uro-surgery ward	15	4.7
Gynecology ward	14	4.3
Orthopedic ward/female side	14	4.3
Total	322	100

Table-2 showed the frequency and percentage of swabs taken from the three main areas surrounding the patient: from the rooms 120 swabs (37.3%) were collected, patients' related swabs were 69(21.4%), and health related swabs were 133(41.3%). Out of 322 swabs, drown from different places of the hospital 227 recovered pathogenic growth at a rate of 70.5%. The rate of none pathogenic growth was 26.1% while the remaining 11 swabs showed no growth 3.4% (table-3).

TABLE 2: Distribution of swabs according to different sites of patients' environment

Site of swab(N=322)		n	%
Patient's related n=120 (37.3%)	bed	41	12.7
	side desk	37	11.5
	cannula	22	6.8
	patients' cloth	20	6.2
Health related n=69 (21.4%)	white coat	30	9.3
	instrument	26	8.1
	dressing trolley	13	4.0
Room n=133 (41.3%)	walls	42	13
	grounds	40	12.4
	waste container	18	5.6
	Air Conditioning system	17	5.3
	door handle	16	5.0
Total		322	100

TABLE 3: Swabs examination outcome

Type of isolate	Number of isolate	n	%
Pathogenic growth	one isolate	170 (74.9)	
	two isolate	57 (25.1)	
	total	227(100)	70.5
None pathogenic		84	26.1
No growth		11	3.4
Total		322	100

TABLE 4: sensitivity/resistance to different antibiotics

Antibiotic	Sensitivity			
	Sensitive	Moderately sensitive	Resistant	Total
	n(%)	n(%)	n(%)	n(%)
Ciprofloxacin	196(92.5)	7(3.3)	9(4.2)	212(100)
Amikacin	191(90.1)	2(0.9)	19(9)	212(100)
Levofloxacin	194(92.8)	5(2.4)	10(4.8)	209(100)
Imipenem	195(94.2)	5(2.4)	7(3.4)	207(100)
Netlimicine	175(87.1)	2(1)	24(11.9)	201(100)
Doxycycline	19(10.2)	2(1.1)	166(88.7)	187(100)
Tetracycline	10(5.3)	1(0.5)	176(94.2)	187(100)
Cefotaxime	3(1.6)	0(0)	184(98.4)	187(100)
Trimethoprim-sulfamethoxazole	16(8.7)	1(0.5)	167(90.8)	184(100)
Cefexime	25(14.4)	5(2.9)	144(82.7)	174(100)
Azithromycin	12(63.2)	3(15.7)	4(21.1)	19(100)
Clindamycin	7(58.3)	-	5(41.7)	12(100)
Vancomycin	9(81.8)	-	2(18.2)	11(100)
Oxacillin	5(45.5)	-	6(54.5)	11(100)
Rifampicin	5(45.5)	-	6(54.5)	11(100)
Erythromycin	5(50)	-	5(50)	10(100)
Methicillin	2(20)	-	8(80)	10(100)

The sensitivity/resistance pattern of the isolated bacteria represented in (table-4). The highest sensitivity is to Imipenem (94.2%), followed by Levofloxacin (92.8) and Ciprofloxacin (92.5%). While the highest resistance is to Cefotaxime (98.4%) followed by Tetracycline (94.2%).

Table-5 represents the response of the isolated bacteria to different types of antibiotics in the culture media. *Acinetobacter baumanni* have a rate of 100% resistance to Cefotaxime, Ceftriaxone, and Tetracycline, for Doxycycline the rate was 92.5%, Trimethoprim-sulfamethoxazole was 78.6%. However, it was only 13.3% resistant to Imipenem rate of sensitivity 86.7%. *Citrobacter freundii* show 100% sensitivity to Ciprofloxacin & levofloxacin at the same time it show 100% resistance to Tetracycline, Doxycycline, Ceftriaxone, and Cefotaxime. *Staphylococcus haemolyticus* show 100% resistance to Ciprofloxacine, Levofloxacine, Doxycycline, Telimicine, and Trimethoprim-sulfamethoxazole. Another finding was that *Pseudomonas aeruginosa* was 100% resistant to doxycycline but sensitive to all other antibiotic used in this work.

TABLE 5: The resistance rate of isolated bacteria to different antibiotics

	Antibiotics									
	AK	NET	CIP	LEV	IPM	DO	TE	SXT	CFM	CTX
Bacterial isolate	R %	R %	R %	R %	R %	R %	R %	R %	R %	R %
Acinetobacter baumannii	14.3	50	21.4	16.7	13.3	92.9	100	78.6	100	100
Citrobacter freundii	25	42.9	-	-	12.5	100	100	87.5	100	100
Citrobacter kos	-	-	-	-	-	-	100	-	-	100
Citrobacter youngae	100	100	100	100	-	100	100	100	100	100
Enterobacter amnigenus	50	50	-	-	-	100	100	100	100	100
Enterobacter cloacae	9.1	18.2	-	-	-	100	100	95.5	95.0	100
Enterobacter sakazaki	-	14.3	-	-	-	100	100	100	85.7	100
Escherichia coli	20	16.7	8.3	8.3	-	100	100	100	66.7	100
Escherichia vulneris	-	-	-	-	-	57.1	85.7	100	71.4	100
Flavemonas oryzihabitans	-	11.1	11.1	11.1	-	100	100	100	100	100
Flavobacterium oryzihabitans	-	-	-	-	-	100	100	-	100	100
Gram negative bacilli	8.7	8.7	-	-	-	90.5	100	95.2	95.5	100
Gram negative coccobacilli	25	-	-	25	-	100	100	25	75	75
Gram negative short bacilli	-	-	-	-	-	100	100	-	100	100
Klebsiella ornithinolytica	-	100	-	-	-	-	100	-	100	100
Klebsiella oxytoca	-	50	-	-	-	100	100	100	100	100
Klebsiella pneumoniae	-	-	-	-	-	77.8	77.8	100	70	100
Klebsiella pneumoniae ozaenae	-	-	-	-	-	66.7	100	100	-	100
Klebsiella terrigena	-	-	-	-	-	-	-	100	-	100
Kocuuria varians rosea	-	-	-	-	-	-	100	-	-	-
Leclercia adecarboxylata	-	-	-	-	-	100	100	100	75.0	100
Moellerella wisconsensis	-	-	-	-	-	100	100	100	100	100
Pantoea spp	4.8	4.7	2.3	2.4	4.7	85.7	90.5	90.7	83.7	95.3
Pasteurella pneumotropica/ haemolytica	-	-	-	-	-	-	-	100	100	100
Proteus mirabillis	-	-	-	-	-	100	100	100	-	100
Pseudomonas aeruginosa	-	-	-	-	-	100	-	-	-	-
Pseudomonas fluorescent/ putida	-	-	-	-	-	-	-	-	-	-
Pseudomonas luteola	-	-	-	-	-	-	-	-	-	-
Pseudomonas oryzihabitans	50	50.0	-	-	50	-	-	-	-	-
Pseudomonus fluorescent	-	-	-	-	-	-	-	-	-	-
Pseudomonus luteola	-	-	-	-	-	-	-	-	-	-
Pseudomonus oryzihapitans	-	-	-	-	-	-	-	-	-	-
Serratia fonticol	-	-	-	-	-	100	100	100	100	100
Serratia odorifera	-	-	-	-	-	66.7	66.7	100	100	100
Serratia plymuthica	-	-	-	-	-	100	100	100	100	100
Serratia rubidaea	-	-	-	-	-	80	80	80	40	100
Serritia rubidaes	-	-	-	-	-	100	100	100	100	100
Shigella spp	-	-	-	-	-	-	-	100	100	100
Staphylococcus aerus	-	-	-	-	-	100	100	100	-	-
Staphylococcus epidermidis	20	33.3	25	25	-	66.7	100	66.7	-	-
Staphylococcus haemolyticus	-	-	100	100	-	100	100	100	-	-
Staphylococcus hominis	-	-	-	-	-	-	100	100	-	-
Staphylococcus saprophyticus	33.3	33.3	-	33.3	-	100	100	100	-	-
Stenotrophomonas maltiphilia	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia	-	-	-	-	-	-	-	100	-	-

AK: Amikacin, CFM: Cefixime, LEV: Levofloxacin, SXT: Trimethoprim Sulphamethoxazol, TE: Tetracycline, IPM: Imipenem, CIP: Ciprofloxacin, NET: Netilimicine, CTX: Cefotaxime, DO: Doxycycline, R: Resistance percentage.

DISCUSSION

he sample drawn was relatively small it was less than what we planned to collect, this was because of the limited material used in the process of swabbing and sensitivity testing. Another cause is the high-risk areas such as Surgical theaters, Burn unit, Hemodialysis unit, Obstetric room…etc. were excluded because an ongoing infection control program covered them. Rate of pathogenic isolation was 70.5%, which is rather high. This is higher than the result of a study conducted in 2011 in Iran by Ekrami AR *et al.* where the rate of positive swabs was 57.4% (Ekrami *et al.*, 2011). The high rate of positive swabs in this study could be attributed to many factors such as the building of the hospital is an old one with deficient maintenance, distorted infrastructures, interrupted rules that organize people who visit their patients, and inefficient staff working in cleaning and disinfection of different department and sections of the hospital. Olowokere *et al*. isolate pathogens from inanimate surfaces in their study in 2013 **(**Olowokere *et al.*, 2013). That is why, clinicians and researchers should be aware of the risk of cross-transmission of pathogens from inanimate surfaces in order to adopt appropriate infection control measures (Russotto *et al.*, 2015).

Bacteria were isolated from all the surrounding of the patients: the environment; the patients himself; and health related personnel and materials. These isolates could be considered as a source of infection to patients. This is

supported by the study of Ekrami A *et al*. who stated that these bacteria on inanimate surfaces are a potential source of infection from the hands of the health care workers to their patients **(**Ekrami *et al*., 2011). However, according to another study conducted by Kramer *et al*., 2006, surfaces are not directly connected to transmission in most hospital infections. It is suggested that microorganisms associated to hospital infections are able to survive during large periods, thus being a continuous source of contamination in cases where population control is not efficiently conducted (Kramer *et al*., 2006). The problem of bacterial resistance to antibiotic, which is the only tool to toggle the pathogenic bacteria so far, is alarming and increasing (Raza *et al*., 2013). This resistance also described as worrisome (Bouzada *et al*., 2010). Levy *et al*. had reported multidrug resistance frequencies in a hospitalized population with intense exposure to antibiotics (Levy *et al*., 1988). Because of this resistance infection will take longer to eradicate, costing much, and higher risk of transmission of infections (Atata *et al.,* 2013).The emerging resistance trend that draw attention is the high rate of multidrug resistant *A. baumannii* (Chen *et al*., 2003) (Atata *et al*., 2013). This makes its treatment of difficult (McConnell *et al*., 2011). In addition, it raise a problem for nosocomial infection control and prevention (Ghadiri *et al*., 2012), due to its ability to acclimatize to selective changes in the environment (Howard *et al*., 2012). In this study *A. baumannii* still sensitive with a relatively low rate of resistance 36%, this probably because the *A. baumannii* was drawn from inanimate surfaces *i.e.* in vitro which is differ from the rate of resistance of the same bacteria in a samples drown from patients in different wards in the hospital during the same period. However, many studies show high rate of resistance of *A. baumannii* (McConnell *et al.*, 2011, Ghadiri *et al*., 2012, Howard *et al*., 2012, Tien *et al*., 2007). In this study *P. aeruginosa* was found to be sensitive to most antibiotic used, it was reported in different parts of the word as causes for numerous nosocomial infections with natural resistance to many drug groups and its ability to acquire resistance against all relevant treatments (Strateva & Yardanova, 2009, Rostamzadeh *et al*., 2016). The result of this in vitro study was different from Rostamzadeh *et al* showed highest resistance (99.5%) of *P. aeruginosa* against Trimethoprime Sulfamethoxasole and Imipenem (33%) (Rostamzadeh *et al*., 2016). This study limited by its concentration on aerobic bacteria while the anaerobic pathogen was not covered by this study. Beside the limitation of materials resources.

CONCLUSION

A high rate of positive swabs with multidrug resistance pathogens is a serious issue that needs a lot of attention. Scientific base for antibiotic use and prescription is essential in reducing the resistance. Strict measure of prevention and infection control inside hospital is essentials to minimize hospital-based nosocomial infections.

RECOMMENDATIONS

Additional study in this field with a larger sample size covers the anaerobic bacteria as well. Further study conducted on the wounds and tissue fluids of the admitted patients to draw a more complementary map of the pathogenic isolates in the hospital.

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