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Full Length Research Paper

Occurrence of Multidrug Resistant Bacteria (Mdrb) among Operating Theatres in Various Hospitals of Al-Basrah Province

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A total of forty four samples were taken from operating rooms of various hospitals in Basrah province during January to April 2014, the samples were collected from beds, walls, instruments, lights, dresses and floor of operating rooms. Thirty three bacterial isolates were diagnosed, Pseudomonas aeruginosa was the predominant bacterial type in 18 samples in percent (54%), followed by other bacterial types as follows: Bacillus subtilis (9.09%), Actinomyces sp., Staph.aureus and Streptomyces sp. (6.06%), while other bacterial types were isolated in percent 3.03% were Actinobacter sp. Serratia marcescens, E.coli, Klebsiella, Staph.saprophyticus and Candida albicans (p <0.05). An antibiotic susceptibility test was carried out in the present study for bacterial types against seven commercial antibiotics by disc-agar diffusion method. Multi drug resistant strains and Resistance/Sensitive ratio were determined for all bacterial isolates.

Keywords: Mdrb, operating theatre, hospital.

INTRODUCTION

Surgical site infection (SSI) is the second most common health care associated infection next to hospital acquired urinary tract infection [1]. The prevalence of SSI varies from country to country depending on level of adherence to infection prevention practice measures in a given health care setting [2].The infection, which is an important clinical indicator for quality of patient care and infection control [3], is primarily determined by the overall contamination level of hospital environment like indoor air together with the surgeon's technique during the operation, patient's degree of susceptibility, insertion of foreign material or implants, appropriateness of surgical preparation, adequacy and timing of antimicrobial prophylaxis [4]. Surgical operations and interventional procedures were performed in areas with various levels of microbiological control of the ventilation. Identification of type, number and variety of different bacteria that are present in different hospital rooms, especially the operation room is of great importance.

They found that 10% of infections that infect the patients was acquired during the period of staying at the hospital and this may increase mortality and morbidity as well as total cost. [5] The contamination of operation rooms is one of the important sources that was a threat to the lives of patients, especially those for cardiac surgery, transplantation surgery, prostatic surgery, bladder cancer. Inanimate objects which become contaminated with pathogenic bacteria and then spread the infection to others are often referred to as fomites. Most outbreaks of infection associated with inanimate objects are caused by items that should be sterile, but have been contaminated.[1] The hypothesis that environmental microorganism cause human diseases arises from two facts, firstly, our interaction with the inanimate environment is

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constant and close, secondly environmental objects are usually contaminated often with important human pathogens. There are no systematic studies of the relative importance of various environmental factors in ensuring a safe operating room environment; however, it is known that contaminated fluid or equipment in the operating room may result in contamination of surfaces and lead to outbreaks of wound infection with Pseudomonas aeruginosa or Serratia marcescens.

Surgical patients often have other health problems that are unrelated to their surgical complaint, e.g., asthma or diabetes mellitus, and may predispose them to infection.[6] Surgery is a trauma to the body and carries a risk of infection e.g. wound infections. In addition, there are post surgical complications e.g., postoperative ischemia, that contribute a further risk. The shorter the preoperative period, the lower the risk of acquiring resistant hospital organisms.[6] Hospital environment is a reservoir of wide varieties of microorganisms. Several strains of pathogenic bacteria have been frequently reported colonizing medical equipments (like Stethoscopes). These pathogens include superbugs like Vancomycin Resistant Enterococcus spp., Methicillin Resistant and Sensitive Staphylococcus species and Multidrug resistant, P. aeruginosa, E. coli, Klebsiella spp. And Streptococcus spp. [7-9]. Medical equipments used in the non-critical care setting are less likely to have standard disinfection and cleaning protocols than equipments in the critical care setting. Thus, medical care equipments are more likely to carry considerable number of pathogenic microorganisms [7].

The contamination of stethoscope particularly the diaphragm is reported mainly due to lack of regular disinfection (before and after examining each patient). A study from India reported that, 45% of general practitioners disinfect their stethoscope once a year or never and 35% disinfect their stethoscope monthly [6]. ORs' and SWs' environment (which places patients at a greater risk than the outside environment) could be polluted with bacterial pathogens released into it from various sources (10). Environmental surface reservoirs like floors, patients and carrier health personnel, construction activities and delayed maintenance can act as a source for microbiological pollution through shedding and environmental disturbance during different activities [11,12]. Pathogens commonly present on the floor include Staphylococcus aureus dispersed by patients and staff, and (in much smaller numbers) Gram-negative rods, such as Pseudomonas aeruginosa [13]. Spores of Clostridium tetani and gas-gangrene bacilli are also present on floors, probably deposited in larger numbers from shoes and trolley wheels than by deposition from the air [14]. Some of the bacteria lie loosely in the dust, while others are ingrained into the surface and between cracks [15]. The removal of this reservoir is one of the normal aims in the control of hospital infection [16]. Dispersal of bacteria into the air has been greatly reduced through the replacement of brooms by vacuum cleaners in wards [17].

The present study aimed to evaluate the bacterial quality of operating rooms in various hospitals, Isolate different bacterial types, determine the antibiotic susceptibility of main bacterial types and determine multi drug resistant bacteria isolated from operating rooms.

MATERIAL AND METHODS

Sample Collection

A total of 44 swabs were collected from the operating theatres of 5 main hospitals:

- Al-Sadder teaching hospital
- Port hospital
- Al-Basrah hospital for gynecology and obstetrics
- Al-Mudayna hospital

These samples were through January to April 2014 using Sterile swab saturated with brain heart infusion (Oxoid, Germany). The swabs were used for aerobic cultures. Anaerobic cultures were not done.
All specimens were transported to the laboratory and cultured within 2 hours of collection.
All media were sterilized by autoclave 121°C for 15 minutes and glassware were sterilized by oven 180°C for 1-1.5 hours.

Isolation and Characterization of Bacteria

The swab specimens were inoculated on various ordinary media; blood agar base, nutrient agar, MacConkey agar (Oxoid, Germany). The plates were incubated at 37 °Cfor 18-24 hours under aerobic conditions, after that the culture plates were examined according to the appearance, color and morphology of the colonies and then various biochemical tests were used for characterization of bacteria.

Antibiotic Susceptibility of Isolates

An antibiotic sensitivity test was carried out on all isolates using paper disk diffusion technique to determine growth inhibition zone (GIZ) and measured by mm. A total of seven antibiotics were tested. The bacteria were streaked on a nutrient agar and the antibiotics were placed on the agar using sterile forceps. Each disk was placed far from each other (by 2.5 cm) to avoid interruption between the zones of inhibition (the zone around the antibiotic disk that has no growth). The plates with the antibiotic disks were then incubated at 37°C for 24 hrs to observe the zones of growth inhibition produced by antibiotics. The antibiotic disks were as follows: Tetracycline, Ampicillin, ciprofloxacin, cefotaxime, Erythromycin, Vancomycin, Penicillin.
All antibiotic discs were supplied from (Bioanalyse Co. Turkey).

Statistical Analysis

SPSS (statistical program for social sciences) Ver.17 and X^2(chi-square) test were used to find statistical significant differences between the items.

$P < 0.05$ was significant.
$P \geq 0.05$ was not significant.

RESULTS

Table 1 shows A total of 44 inanimate samples were collected from operation theatres of 5 main hospitals in Basrah.
The samples: bed 8, wall 3, instruments 18, light 7, dress 5, floor 3.

Table 1: Numbers of samples collected from inanimate sites of operating rooms of various Basrah hospitals

Sample type		Numbers of samples	%	+ve culture
Inanimate samples	**Bed**	8	18.18%	11
	Wall	3	6.81%	1
	Instrument	18	40.9%	11
	Light	7	15.9%	3
	Dresses	5	11.36%	5
	floor	3	6.81%	2
Total		44	100%	33

p < 0.05

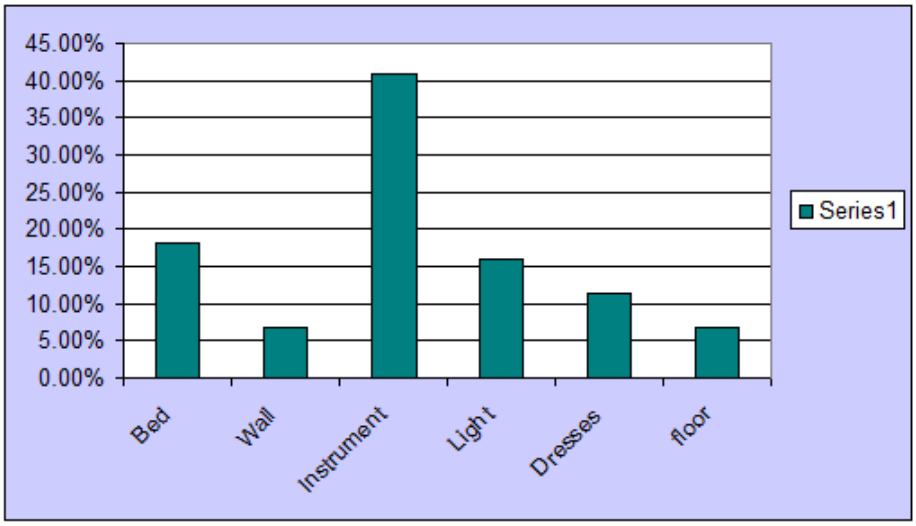


Table 2: Bacterial types isolated from different inanimate sources of operating theatres

Bacterial types	Total number of isolates %	Number of isolates %						Statistical analysis
		Bed	**Dress**	**Wall**	**Light**	**Floor**	**instrument**	
Bacillus Subtilis	**3(9.09%)**	**2**	**1**	**0**	**0**	**0**	**0**	P ≥ 0.05
Actinomyces sp.	**2(6.06%)**	**2**	**0**	**0**	**0**	**0**	**0**	P ≥ 0.05
Staphylococcus aureus	**2(6.06%)**	**1**	**0**	**0**	**0**	**0**	**1**	P ≥ 0.05
Pseudomonus aeruginosa	**18(54.54%)**	**3**	**2**	**1**	**3**	**2**	**7**	P< 0.05
Streptomyces sp.	**2(6.06%)**	**1**	**1**	**0**	**0**	**0**	**0**	P ≥ 0.05
Actinetobacter sp.	**1(3.03%)**	**1**	**0**	**0**	**0**	**0**	**0**	P ≥ 0.05
Serratia marcescens	**1(3.03%)**	**0**	**0**	**0**	**0**	**0**	**1**	P ≥ 0.05
E.coli	**1(3.03%)**	**0**	**0**	**0**	**0**	**0**	**1**	P ≥ 0.05
Klebsiella	**1(3.03%)**	**0**	**1**	**0**	**0**	**0**	**0**	P ≥ 0.05
Staphylococcus saprophyticus	**1(3.03%)**	**0**	**0**	**0**	**0**	**0**	**1**	P ≥ 0.05
Candida albicans (Yeast)	**1(3.03%)**	**1**	**0**	**0**	**0**	**0**	**0**	P ≥ 0.05
Total	**33**	**33.33%**	**15.15%**	**3.03%**	**9.09%**	**6.06%**	**33.33%**	

p < o.o5

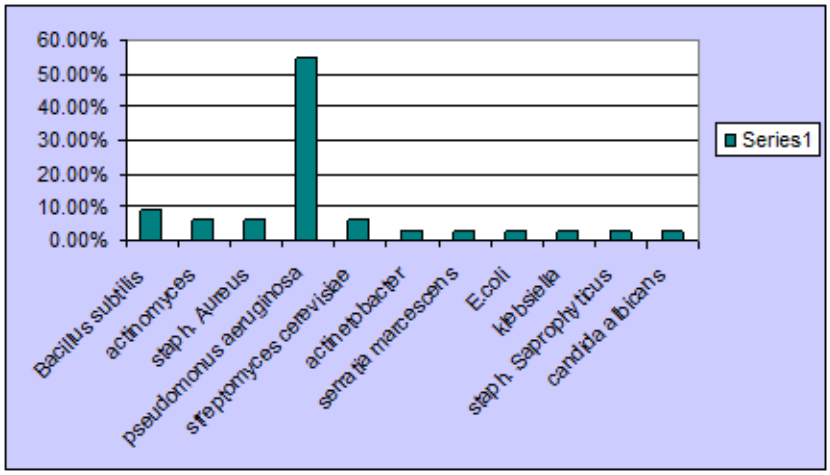


Table 3: Standard antibiotic ssuscetibility test according to diameters of inhibition zone supplied by Bioanalyse Co. Turkey

Antimicrobial agent	**Symbol**	**Conc.mcg**	**Zone diameter(mm)**	
			Resistant	**sensitive**
Ciprofloxacin	(cip)	10mcg.	20 or less	29 or more
Amoxicillin+clavulanic acid	(AMC)	20mcg.	19 or less	20 or more
Gentamycin	(CN)	10mcg.	10 or less	15 or more
Vancomycin	(VA)	30mcg.	9 or less	12 or more
Lincomycin	(L)	2mcg.	9 or less	15 or more
Chephalexine	(CL)	30mcg.	14 or less	18 or more
Penicillin	(p)	10mcg.	11 or less	22 or more
Erythromycin	(E)	15mcg.	13 or less	18 or more
Ampicillin	(AM)	10mcg.	(11-21) or less	(14-30) or more
Tetracycline	(T)	30mcg.		19 or more
Streptomycin	(s)	10mcg.	14 or less	15 or more
Trimethoprim + sulphamethoxazole	(STX)	1.25mcg./23.75mcg.	11 or less	16 or more

Table 4: Antibiotic susceptibility pattern of *Staph.aureus*

Drug type	**No. of isolates**	**Resistant(%)**	**Sensitive (%)**
TE	2	0	100%
Cip	2	1(33.3%)	1(33.3%)
P	2	1(33.3%)	1(33.3%)
AM	2	1(33.3%)	1(33.3%)
E	2	1(33.3%)	1(33.3%)
V	2	0	100%

Table 5: Antibiotic susceptibility pattern of *Bacillus Subtilis*

Drug type	No. of isolates	Resistant(%)	Sensitive(%)
TE	1	0	100%
P	1	0	100%
CFM	1	0	100%
E	1	0	100%
V	1	0	100%

Table 6: Antibiotic susceptibility pattern of *Staphylococcus saprophyticus*

Drug type	No. of isolates	Resistant (%)	Sensitive (%)
TE	1	0	100%
Cip	1	0	100%
P	1	0	100%
v	1	0	100%

Table 7: Antibiotic susceptibility pattern of *Streptomyces cervisiae*

Drug type	No. of isolates	Resistant(%)	Sensitive(%)
TE	1	0	100%
p	1	0	100%
CFM	1	0	100%
V	1	0	100%

Table 8: Antibiotic susceptibility pattern of *E.coli*

Drug type	No. of isolates	Resistant(%)	Sensitive(%)
TE	1	0	100%
P	1	0	100%
CFM	1	0	100%
E	1	0	100%
V	1	0	100%

Table 9: Antibiotic susceptibility pattern of *Pseudomonus aeruginosa*

Drug type	No. of isolates	Resistant(%)	Sensitive(%)
TE	10	10 (100%)	0
Cip	14	14(100%)	0
P	16	15(93.75%)	1(6.25%)
AM	15	15(100%)	0
CFM	9	2(22.22%)	7(77.77%)
E	9	8(88.88%)	1(11.11)

$P \geq 0.05$ (no significant differences between effects of various antibiotics)

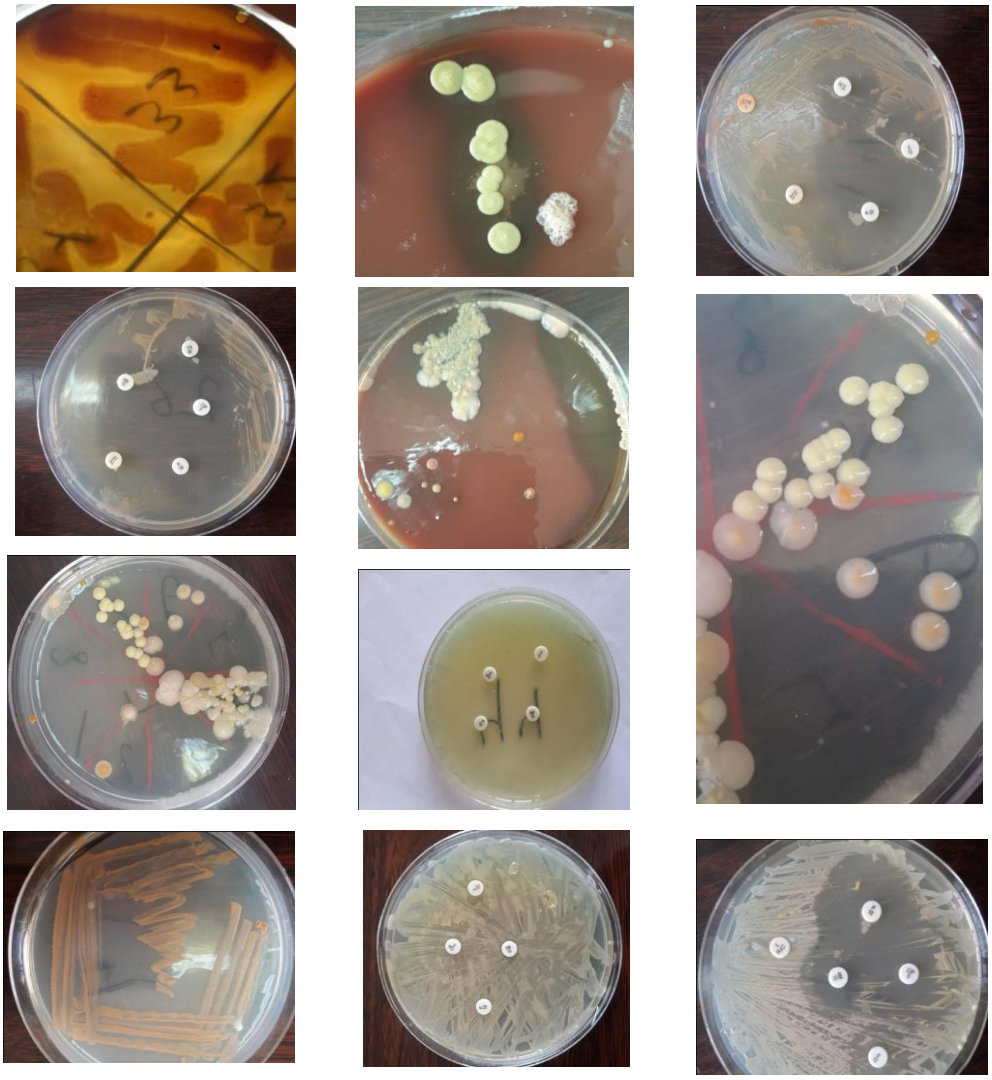
Table 10: Antibiotic susceptibility of the isolated microorganisms

Drug type	Number of isolates	Sesitive	Resistant
TE	17	8(47%)	9(53%)
Cip	18	2(11.11%)	16(88.88%)
P	24	15(62.5%)	9(37.5%)
AM	17	2(11.76%)	15(88.23%)
CFM	12	10(83.33%)	2(16.66%)
E	13	5(38.46%)	8(61.53%)
V	16	8(50%)	8(50%)

Table 11: Relationship between causative agents and antibiotics

Bacteria	No. of isolates	Resistance to					
		1 antibiotic	2 antibiotics	3 antibiotics	More then 3 antibiotics	Total resistance %	Tatal sensitive%
Staph. Aureus	2	0	0	0	1	50%	50%
Bacillus subtilis	3	0	0	0	0	0	100%
Stap. Saprophyticus	1	0	0	0	0	0	100%
Streptomyces sp.	2	0	0	0	0	0	100%
E.coli	1	0	0	0	0	0	100%
Pseudomonas aeruginosa	18	0	0	0	11	61.11%	38.88%

P ≥ 0.05 (no significant differences between effects of various antibiotics)



Figures: illustrates the various bacterial types isolated in the present study with antibiotics susceptibility test.

Table 2 shows the total of 33 isolates of bacterial types isolated from various inanimate sources of operation theatres, including the following numbers of isolates and percentages: bacillus subtilis 3(9.09)%, actinomyces 2(6.06%), staph.aureus 2(6.06%),pseudomonas aeruginosa 18(54.54%), streptomyces cerecisiae 2(6.06%), actinobacter 1(3.003%), Serratia marcescens 1(3.003%), E.coli 1(3.003%), Klebsiella 1(3.003%), staph. Saprophyticus 1(3.003%), Candida albicans 1(3.003%).

Table 4,5,6,7,8,9,10 and 11 show the prevalence of multidrugs resistance of bacteria, antibiotics; we found that resistance against more than three antibiotics had the biggest percentage (44.44%) with resistance of four antibiotics. There was no significant differences between the effects of various antibiotics.

The antibiotic ciprofloxacin had the biggest percentage of resistance (88.88%) and the antibiotic cefotaxime was the most effective one.

DISCUSSION

This study confirmed that various inanimate objects in the operating room theatre associated directly or indirectly with surgical procedures were variously contaminated with known bacterial and fungal pathogens. Although the direct involvement of these fomites in disease transmission was not investigated in this study, the isolation of Bacillus Subtilis, Actinomyces sp., Staphylococcus aureus, Pseudomonus aeruginosa. Streptomyces sp., Actinetobacter sp., Serratia marcescens, E.coli, Klebsiella, Staphylococcus saprophyticus, Candida albicans (Yeast) presents a serious concern for possible transmission of infection.

The antibiotic ciprofloxacin had the highest percentage of resistance (88.88%).

The selection of the antibiotic is determined by the diagnosis of the causative pathogens, then doing the antibiotic susceptibility test. The antibiotic susceptibility test will provide us information about the resistant and sensitive strains and according to this we can begin the management by using the appropriate antibiotic. The wide spread of antibiotics, using them over the counter and not completing the course of treatment is creating a huge problem of developing resistant strains.

CONCLUSION

- The operating theatres were highly contaminated with various types of bacteria.
- Pseudomonas aeruginosa was the predominant bacterial type isolated from operating rooms, followed by other gram +ve and gram –ve bacteria.
- Multidrug resistant bacteria (MDRB) were isolated in the present study in high ratio.
- The presence of MDRB in operating theatre may be as predisposing factor for infection and must be eradicate and the theatre must be sterilized in anytime to get healthy conditions.

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