

ISOLATION, IDENTIFICATION AND ANTIBIOTIC SENSITIVITY TEST OF THE MICROORGANISM ISOLATED FROM OPERATION ROOM AND CARDIOPULMONARY BYPASS CIRCUIT SYSTEM DURING OPEN HEART SURGERY IN SANA'A CITY, YEMEN

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ABSTRACT

Aim: The aim of the current study was to isolate, identify and determine antibiotic sensitivity test of the microorganism isolated from operation room and cardiopulmonary bypass circuit system during open heart surgery in Sana'a City, Yemen.

Material and methods: two hundred and ten patients were enrolled in this study. A total of 540 samples were collected in the current study which including 210 crystalloid prime solution samples, 210 samples from Blood from line of oxygenator, 30 swab samples from anesthesia machine, 30 swab samples from surgical instrument, 30 swab samples from health care worker hands in operation room after washing and 30 air samples from air in the operation room. The Identification of Isolated bacteria, yeasts and fungi and antibiotic sensitivity test by disc diffusion method were carried according to The Clinical & Laboratory Standards Institute (CLSI).

Results: Among the 420 samples were collected from crystalloid solution and from cardiopulmonary bypass (CPB) machine during operation samples, 12 and 6 samples respectively, were showed positive growth of microorganisms. Out of 30 samples which were collected from anesthesia machine tube and from the air of operation room, 11 and 27 respectively, were positive culture. 2 positive samples which was collected from instruments after open heart surgery. In addition, 4 samples from the hands of open-heart surgery workers were positive. The most isolated were Staphylococcus epidermidis, S. aureus S. saprophyticus, Lactobacillus spp. Pseudomonas aeruginosa and Bacillus subtilis. Aspergillus carbonarius, Candida guillirmondii and Geotrichum candidum. The most of the bacterial pathogens were sensitive to Linezolid, Vancomycin and Cephalothin.

Conclusion: The air of operation room, blood and crystalloid solution in the cardiopulmonary bypass machine, anesthesia machine tubes, open heart surgery instruments represent the sites of contamination with bacteria and fungi in the operation room. The predominant bacterial pathogens were isolated from the positive samples were the Gram-positive bacteria such as Staphylococcus epidermidis and Staphylococcus aureus. The Linezolid and vancomycin antibiotics showed the highest antimicrobial activity against isolated pathogenic bacteria.

Keywords: Cardiopulmonary Bypass Circuit; Open Heart Surgery; Sana'a City; Aspergillus Carbonarius; Staphylococcus Epidermidis.

I. INTRODUCTION

Cardiac surgery, or cardiovascular surgery, is a surgery on the heart or great vessels performed by cardiac surgeons. It is often used to treat complications of ischemic heart disease (with coronary artery bypass grafting); to correct congenital heart disease or to treat valvular heart disease from various causes, including endocarditis, rheumatic heart disease, and atherosclerosis. It also includes heart transplantation (Jernberg et al 2010, Yuh et al 2007). The heart-lung machine or also known as cardiopulmonary bypass (CPB) has been a part of routine practice in cardiothoracic surgery for a long time. Despite the high contemporary quality standards for cardiopulmonary bypass, perioperative nosocomial infections remain a problem of considerable importance in cardiothoracic surgery patients, with incidences varying between 5.0% and 21.7% and are associated with significant increases in morbidity and mortality (Evans et al 2018). The occurrence of positive CPB cultures in these patients is especially critical, as a relationship with postoperative infections cannot completely be excluded. Its exact incidence and involvement in postoperative infections however, remain to be elucidated (Biffi et al 2017). The cultures taken during CPB can be subdivided into one of the two

following group preoperative cultures of the CPB priming fluid, or cultures of whole blood taken during CPB (Pierce et al 2018). Several previous research studies revealed that incidences of bacterial infection near to 17% of positive blood cultures from cardiopulmonary bypass CPB. The coagulase-negative staphylococci (CoNS) represents the majority of isolated microorganisms, even though *Bacillus* spp., the *Bacteroides fragilis* group, and Gram-negative coliform bacteria were found occasionally. Furthermore, splanchnic hypoperfusion with subsequent relative mucosal ischemia of the intestines may lead to bacterial translocation during cardiopulmonary bypass CPB (Evora et al 2016). Likewise, it can be contaminated during preparation of circuit without using sterile gloves or from air born contamination during preparation or from the perfusionist itself. A possible route of transmission of *Staphylococcus aureus* leads to bacterial mediastinitis in a heart transplant recipient (Hosokawa et al 2020).

The aim of the current study was to isolate, identify and determine antibiotic sensitivity test of the microorganism isolated from operation room and cardiopulmonary bypass circuit system during open heart surgery in Sana'a City, Yemen.

II. MATERIAL AND METHODS

The current study was conduct as the cross-section study were two hundred and ten patients were enrolled in this study. A total of 540 samples were collected in the present study which including 210 crystalloid prime solution samples, 210 samples from Blood from line of oxygenator, 30 swab samples were collected from each of the following places anesthesia machine, surgical instrument, health care worker hands in operation room after washing and from air in the operation room. All the samples were transported immediately to the microbiology laboratory in Al-thawrah General Hospital in Sana'a city for culturing into three media, Blood Agar, MacConkey Agar and Sabouraud dextrose agar (SDA) medium. The Blood Agar and MacConkey agar media of all samples were incubated aerobically at 37°C for 24–48 hours for isolation of bacteria (Teklehymanot et al 2017). In addition, the SDA medium was incubated at 28°C aerobically for 7 days for isolation of fungi (Zhou et al 2020). The Identification of Isolated bacteria, yeasts and fungi and antibiotic sensitivity test by disc diffusion method were carried according to The Clinical & Laboratory Standards Institute (CLSI,2012). Statically Analysis of the data was performed by SSPS version 22. Statistical significance was set at Asymp. Sig. or p-value ≤ 0.05 Considered significant.

III. RESULTS

A total of 420 cultures were analyzed, half being priming fluid and half CPB blood cultures. 12 (5.71%) and 6 (2.86%) samples were respectively showed positive growth of microorganisms. Out of 30 samples which were collected from anesthesia machine tube and from the air of operation room, 11 (36.67%) and 27 (90.00%) respectively, were positive culture. 2 positive samples (13.33%) which was collected from instruments after open heart surgery. 4 samples (13.33%) from the hands of open-heart surgery workers were positive as shown in Table1 and Figure1.

The most isolated bacteria and fungus isolated were *Staphylococcus epidermidis*, *S. aureus*, *S. saprophyticus*, *Lactobacillus* spp. *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Aspergillus carbonarius*, *Candida guilliermondii* and *Geotrichum candidum*. In addition, most of the bacterial pathogens were sensitive to Linezolid, Vancomycin and Cephalothin. Whereas the *Pseudomonas aeruginosa* was resistant to Cefadroxil and Sulphonamide as shown in table 2.

Table 1: sources of samples, type of samples and number and percentage of the positive samples (n= 540)

Source of sample	Type of samples	No. of samples	Positive cultures	Positive %	X2	P- Value
Cardiopulmonary bypass machine	Crystalloid solution	210	20	9.52	164.743	< 0.05
	Blood	210	6	2.86	186.686	< 0.05
Anesthesia machine tubes	Swabs	30	14	46.67	2.333	> 0.05

Air of operation room	Air	30	27	90	19.200	< 0.05
Open heart surgery instruments	Swabs pre-Operation	15	0	0	K – S(Z) 2.00	< 0.05
	Swabs post Operation	15	4	26.67	8.067	
Hands of open-heart surgery workers	Hand swabs	30	4	13.33	16.133	< 0.05

Table 2: Bacteria and fungi which was isolated from all samples

Position of samples		CPSS	CPB	AM	HCWH	SI	AOR	Total	%
Name of M.O.	No. of (+) Samples /T	20/210	6/210	14/30	4/30	4/30	27/30	75/540	100
Bacteria								61	81.33
S. epidermidis		6	2	4	2	3	15	32	42.67
S. aureus		4	2	2	2	N	6	16	21.33
S. saprophyticus		2	N	5	N	1	N	8	10.67
Lactobacillus spp		2	N	N	N	N	N	2	2.67
Bacillus subtilus		N	N	N	N	N	1	1	1.33
Pseudomonas aeruginosa		2	N	N	N	N	N	2	2.67
Molds								14	18.67
Aspergillus carbonarius		4	N	N	N	N	N	4	5.33
Candida guillirmondii		N	N	3	N	N	1	4	5.33
Geotrichum candidum		N	N	N	N	N	4	4	5.33
Fusarium oxysporum		N	2	N	N	N	N	2	2.67
Total		20	6	14	4	4	27	75	100

S. = Staphylococcus; CPSS, crystalloid prime solution samples; CPB, line of oxygenator; SI, surgical instrument; HCWH healthcare workers' hands in operation room after washing. AOR, air in the operation room. (+) ve, positive; T, Total

Table 3: Antibiotic susceptibility testing of isolated bacteria which was isolated from all samples

Antibiotic	S. epidermidis N = 31		S. aureus N = 17		S. saprophyticus N = 8		Lactobacillus spp. N = 2		Bacillus subtilus N = 1		Pseudomonas aeruginosa N = 2	
	N	%	N	%	N	%	N	%	N	%	N	%
LZD	19	61.3	13	76.5	-	-	-	-	1	100	NT	NT

OX	4	12.9	4	23.5	-	-	1	50	-	-	NT	NT
AMP	-	-	1	5.9	-	-	1	50	-	-	-	-
AML	2	6.5	5	29.4	2	25	1	50	-	-	-	-
CX	11	35.5	4	23.5	2	25	1	50	-	-	NT	NT
P	-	-	-	-	-	-	1	50	-	-	NT	NT
CE	-	-	3	17.7	-	-	1	50	-	-	NT	NT
VA	19	61.3	9	52.9	2	25	1	50	-	-	NT	NT
L	8	25.8	3	17.7	-	-	-	-	-	-	NT	NT
E	7	22.6	3	17.7	-	-	-	-	-	-	NT	NT
GMN	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2	100
KAN	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2	100
CFR	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-
S	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	-	-
CEF	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2	100

Oxacillin (OX), Ampicillin (AMP), Amoxicillin (AML), Cloxacillin (CX), Penicillin-G (P), Cephadrine (CE), Vancomycin (VA), Lincomycin (L), Linzolid (LZD), Erythromycin (E), Gentamycin (GMN), Kanamycin (KAN), Cefadroxil (CFR), Sulphonamide (S), Cephalothin (CEF), Not Tested (NT), Negative (No Zone) (-)

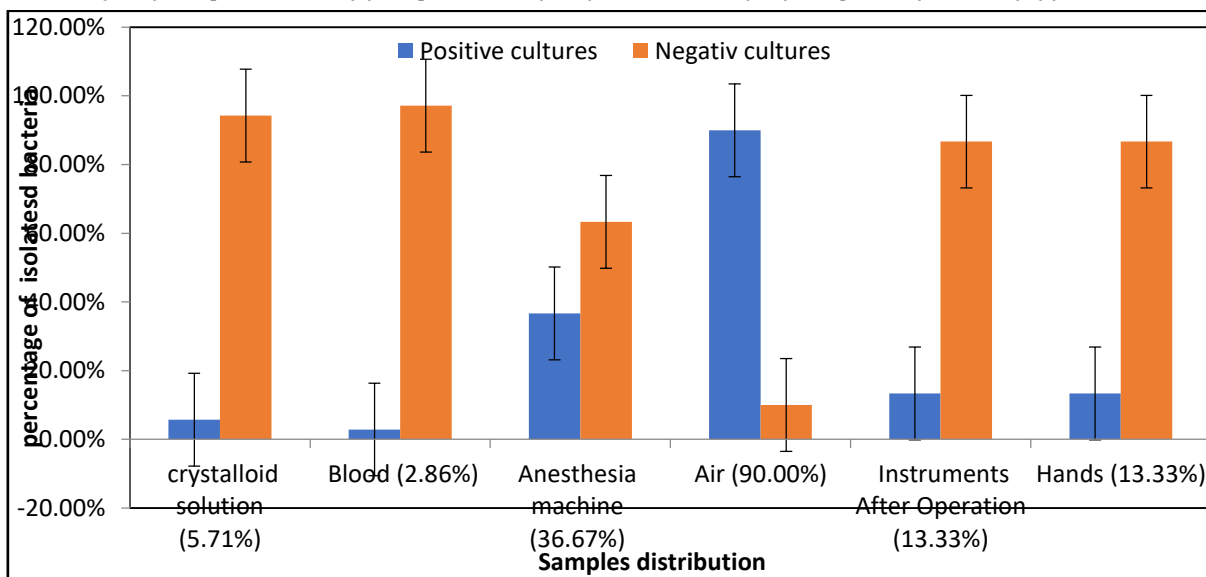


Figure 1: Distribution of the samples collected from open heart surgery operations according to culture results.

IV. DISCUSSION

The unique available study investigating the isolation, identification and antibiotic sensitivity test of the microorganism isolated from operation room and cardiopulmonary bypass circuit system during open heart surgery in Sana'a city, Yemen. The incidence of positive cardiopulmonary bypass priming fluid cultures contrasts with the available literature. Priming cardiopulmonary bypass fluid cultures yielded positive bacterial culture in 5.7% of the samples collected from crystalloid solution, these results were different from another previous research conducted by Hamers et al (2011) which found (1.0%) blood cultures tested positive. In the current study, the incidence of positive CPB blood samples showed 2.89% of the samples collected from the blood of cardiopulmonary bypass machine during operation were positive culture. The similar observation was also reported by Schwieger et al (1989), they reported that 2.4% of blood cultures collected from

cardiopulmonary bypass machine were positive for bacteria. Hamers et al (2011) also detected that 5.6% of cultures was positive for bacterial culture drawn from perfusion blood

The incidence of positive culture CBP blood samples in this study was lower than the results observed by Gudbjartsson et al (2016), they reported that about 17.7% of the perfusion blood cultures of patients yielded organisms. Also, this study results were lower than the results observed by Cheng (1990) who showed that the positive culture rate of blood remnant from CPB machine was 18.4%. He also reported that the increase of blood contamination rate during the operation may be related to the following factors, the inevitably increased number of bacteria in the air of the operating room during CPB operation, or prolonged CPB time (greater than 80 min); and wet and warm weather. In the current study, about 36.7% of the samples were positive culture which was isolated from anesthesia machine tubes. The results from this study were less than the results obtained by Spertini et al (2011). They found that 43% of the samples collected from the internal of breathing-circuit-systems were positive bacterial culture. On the other hand, the results of the current study were more than the results obtained by Pelligand et al (2007), where they found that 25.6% of the samples collected from anesthesia machine showing positive culture yielded of microorganisms.

The contaminated air of the operation room of the cardiac surgery was another important source of infection in the operation room. In this study contamination by airborne bacteria was reported in 90% of the samples collected from the air of open-heart surgery operation room. The results were consistent with findings from previous studies as reported by (Pelligand et al 2007) and Gebremariam and Declaro (2014). Stauning et al (2018), observed that 84% of the air samples collected from operation rooms showed positive culture of microorganisms. In addition, the results from the current study showed that 13.3% of the samples collected from the hands of health care workers in the open-heart surgery operation room showed positive culture. This finding is similar to the result reported by (Loftus et al 2008), they found that the hands of anesthesia providers in operation rooms, patient intravenous tubing and the immediate patient environment were contaminated immediately before or during patient care with wide range of bacterial pathogens, leading to transmission of bacteria.

V. CONCLUSION

The contamination of open-heart patients may occur intraoperatively or during the early postoperative period. Cardiopulmonary bypass machine such as blood and crystalloid solution, anesthesia machine tubes, open-heart surgery instruments are all possible sites of contamination with bacteria and fungi. The airborne bacteria and fungi colonizing in the open-heart operating rooms, operation personnel, and equipment, and the patients themselves are all potential sources of microbial contamination. Therefore, no evidence was found to support routine culturing of CPB samples in patients undergoing cardiothoracic surgery. Only airborne bacteria and other sources of contamination must be minimized. The most pathogenic bacteria isolates were *Staphylococcus epidermidis* and *Staphylococcus aureus* which were sensitive to the linezolid and vancomycin antibiotics.

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