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Original research

BACTERIAL CONTAMINATION IN THE OPERATING ROOM AT HUE CENTRAL HOSPITAL, 2nd BRANCH

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ABSTRACT

Objectives: *This study aims to evaluate the microbial contamination level and identify bacterial colonization of the operating room in the hospital.*

Methods: *Three sampling procedures were used in this study, which includes swabbing, air sampler and contact agar plate. Collected samples from 8 ORs were transported and microbiologically processed using standard procedures. Bacteria were identified by morphological, biochemical tests and Vitek 2 Compact system. Microbiological criteria were based on recommendation of the Ministry of Health.*

Results: *The incidence of positive cultures was 285/600 (47.50%). The most common isolates were Gram - positive bacteria (98.25%) while low rate of Gram - negative bacteria (1.75%) was surveyed with Pseudomonas sp. (0.7%) and Enterobacter cloacae (1.05%). The water used for surgical hand washing illustrated the existence of Staphylococcus blanc (60%) and each of Pseudomonas sp. and Enterobacter cloacae (20%). Air contamination were observed with Bacillus sp. (46.90%) and Staphylococcus blanc (53.10%). Environmental surfaces were detected 7 species, of which 4 threaten strains including Staphylococcus aureus accounted for 5.55%, followed by Streptococcus β - hemolytic (0.79%), Pseudomonas sp. (0.79%) and Enterobacter cloacae (1.59%). Surgeons's hand samples were isolated with Bacillus sp. (14.55%) and Staphylococcus blanc (1.81%). The rate samples of ORs surfaces reaching level A and B accounted for 91.49% and 8.51% while none of the samples approach level C and level D. The rate of qualified samples in terms of air, water for surgical hand washing, surfaces of medical instruments and surgeons' hands was remarkably high with 87.59%, 98.44%, 95.24% and 100%, respectively.*

Conclusion: *Our study showed various bacteria isolated, of which pathogenic species occupied very low percentage. Especially, the rate of samples in the study that meet microbiological standards was remarkably high according to Ministry of Health recommendation.*

Keywords: *Operating rooms (ORs), surgical hand washing (SHW), surgical site infection (SSI), colony forming unit (CFU), brain heart infusion broth (BHI).*

I. BACKGROUND

Surgical site infection (SSI) has become a major issue that triggers to patient morbidity and death [1, 2]. A study reported that the annual cases of healthcare - accquired SSI was estimated to be 2 million in America, leading to increasing length of hospital stays with 7.4 days and incurred cost with 130 million USD [2]. Other study showed that 5% - 10% of approximetly 2 million patients undergoing surgery suffered from SSI in Vietnam [1, 2]. This gives rise to twofold increase in the length of hospital stays and treatment costs [1, 3]. The controlled environment of ORs remains potentially risky for patient due to a number of factors such as ventilating system, cleaning, sterilization, patients transportation, medical equipments, waste of injuries and water source for hand hygiene, which are able to be associated with microbial growth

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conditions [4 - 6]. This present study would reveal findings of the microbial prevalence as well as the microbial contamination level related to air, surface, water and identify bacterial colonization at 8 ORs in Hue Central Hospital 2nd Branch.

II. MATERIALS AND METHODS

The study was conducted at Hue Central Hospital and Hue Central Hospital, 2nd Branch from January 2020 to September 2021. The permission for the study was taken from Director Board of Hue Central Hospital. All samples were collected before surgical performances.

2.1. Air samping and analysis

Air sampling was implemented via an active air sampler of Germany MERCK - KGA MAS 100 brand which collects a predetermined volume of air with 1m3 in 10 minutes and capture the microorganism onto agar - based growth medium such as Blood agar, MacConkey agar, Nutrient agar. These were then transported to microbiological laboratory and incubated at 37°C in 18 - 24 hours under aerobic conditions [7]. After incubation, the quantity of colonies on Nutrient agar plates were counted as the results of total aerobic bacteria per $1m^3$ (CFU/m^3) and isolates were identified by colony characteristics, gram stain and standard biochemical test as well as Vitek 2 Compact system. Air standard in ORs is recommended by Ministry of Health given below [3, 8, 9]:

- An empty theatre bio load should not exceed $35CFU/m^3$
- During surgery bio load should not exceed $180CFU/m^3$
- None of harmful bacteria are present in operation theatres

2.2. Surfaces sampling and analysis

Sterile swabs moistened in sterile distilled water and rolled over the surfaces of medical equipments and surgeons' hands. All collected swabs were then immediatetly put into test tubes of BHI (brain heart infusion broth) which is a liquid medium used for the enrichment of microorganisims growth. These were then transported to microbiological laboratory and incubated at 37°C for 18 - 24 hours under aerobic conditions. After incubation, BHI tubes with good turbidity were chosen to inoculate on agar plates. The plates, afterwards, were continued to be put into the incubator and set the conditions as the previous step. After 18 - 24 cultivation, isolates eventually were identified by colony characteristics, gram stain, standard biochemical test and Vitek 2 Compact system [8, 9].

Contact - plate sampling is the method making the bacterial load on surfaces visible. Nutrient agar plates were used to press against the surfaces such as walls and floors for a short moment in 10s. These were then transported to microbiological laboratory and incubated at 37°C for 18 - 24 hours under aerobic conditions. After incubation, the quantity of colonies on the plates was counted as the results of total aerobic bacteria per $25cm^2$ ($CFU/25cm^2$). The surface standard in ORs is recommended by Ministry of Health given below [3, 8, 10]:

- Level A: < 1 $CFU/25cm^2$, Level B: < 5 $CFU/25cm^2$, Level C: < 25 $CFU/25cm^2$, Level D: < 50 $CFU/25cm^2$
- None of harmful bacteria are present in operation theatres.

2.3. Water sampling and analysis

In order to take water samples for surgical hand washing, faucets, firstly, were heated to pre-disinfect and flushed for about 3 - 5 minutes. Test tubes containing 10ml BHI were used to take water samples and then transported to microbiological laboratory and incubated at 37°C for 18 - 24 hours under aerobic conditions. After incubation, BHI tubes with good turbidity were chosen to inoculate on agar plates. The plates, afterwards, were continued to be put into the incubator and set the conditions as the previous step. After 18 - 24 cultivation, isolates eventually were identified by colony characteristics, gram stain, standard biochemical test and Vitek 2 Compact system [8]. The standard of water for SHW in ORs is recommended by Ministry of Health given below [11, 12]: None of bacteria are present in water for surgical hand washing.

III. RESULTS

600 samples were collected from air, water for SHW, surfaces including medical equipments, walls and floors, of which 285 (47.50%) cultures were positive (Figure 1). The most common isolates were Gram - positive bacteria with 98.25% while Gram - negative bacteria occupied only 1.75% (Figure 2).

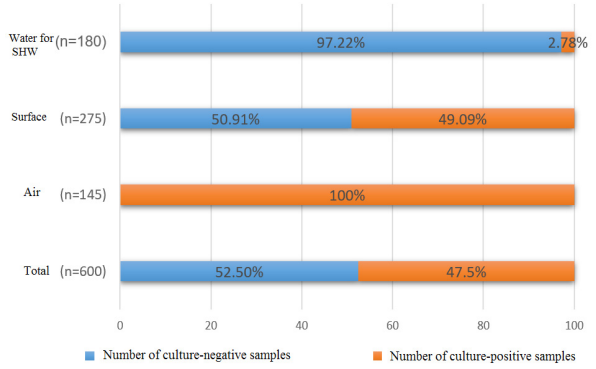

Figure 1: Percentage of positive and negative samples

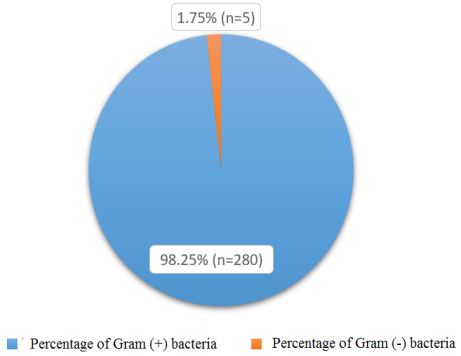

Figure 2: Percentage of Gram - positive and Gram - negative

7 bacterial species were identified in different ORs including *Bacillus sp.* accounted for 48.42% bacteria isolated, followed by *Staphylococcus blanc* (46.32%), *Staphylococcus non - coagulase* (2.46%), *Staphylococcus aureus* (0.70%), *Streptococcus β - hemolytic* (0.35%), *Pseudomonas sp.* (0.70%) and *Enterobacter cloacae* (1.05%) (Figure 3).

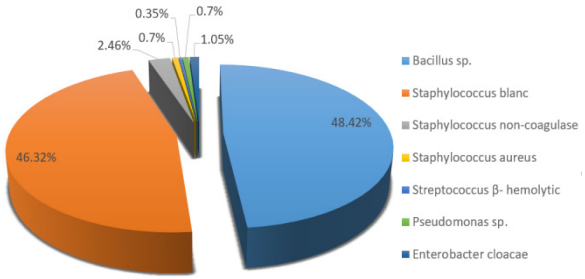

Figure 3: Types of bacteria identified from ORs

Figures 4 showed a predominance of *Bacillus sp.* and *S. blanc* which are less likely to cause diseases among 8 ORs. *Staphylococcus non - coagulase* was observed in OR1, OR3 and OR4 with 5.26%, 5.41% and 9.09% of bacteria isolates, respectively. The percentage of pathogenic bacteria was very low. *S.aureus* was only present in the OR1 with 5.26%. *Streptococcus β - hemolytic* was detected in the OR3 with 2.70%. *Pseudomonas sp.* occupied 3.03% bacteria isolated in the OR3 and 2.63% was the rate of *Enterobacter cloacae* found in OR1.

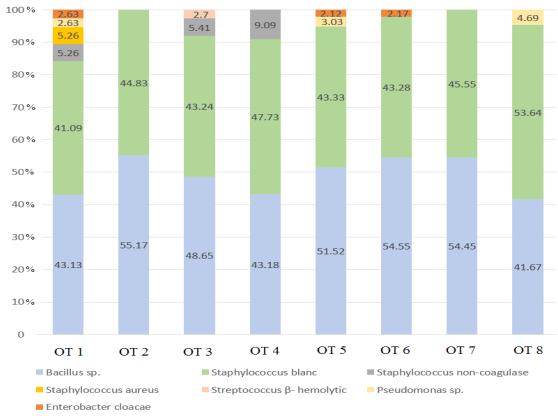

Figure 4: Types of bacteria from different ORs

Figure 5 reveals that the quantity of air samples containing over 35 CFU/m^3 was 18 (12%), of which OR1 and OR2 accounted for 6.25% positive cultures which touched the lowest rate while OR3 and OR4 accounted for 16.67% reaching the top rate compared to the others ORs. Bacteria isolated from environmental

air given in Figure 6 such as *Bacillus sp.* and *S. blanc* were not involved in the group of pathogenic species according to the Ministry of Health recommendation.

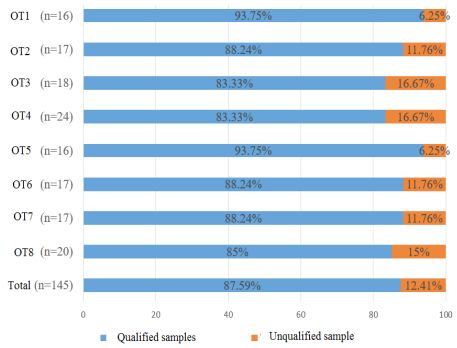

Figure 5: Microbial air contamination level

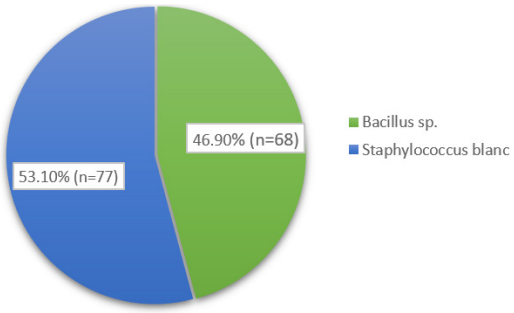

Figure 6: Types of bacteria identified from air samples

7 types of bacteria were surveyed among the medical equipments in the ORs. *Bacillus sp.* and *S. blanc* occupied 49.21% and 40.48%, followed by *Staphylococcus non - coagulase* (5.55%), *S. aureus* (1.59%), *Enterobacter cloacae* (1.59%) and each of *Streptococcus β - hemolytic* and *Pseudomonas sp.* (0.79%) (Figure 7).

Figure 8 showed that 46/55 samples taken from surgeon hands gave negative results with 83.64% and there was the presence of *Bacillus sp.* (14.55%) and *S. blanc* (1.81%) which are not involved in the group of pathogenic bateria.

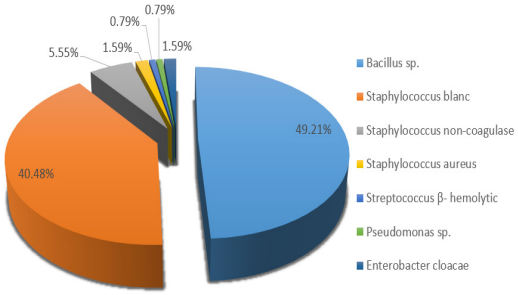

Figure 7: Medical equipments'surface contamination

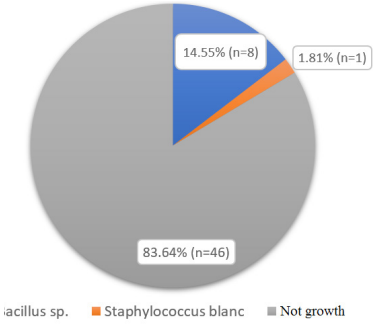

Figure 8: Surgeon hand's surface contamination

Figure 9 illustrates that none of surface cultures among 94 samples collected reach level C (< 25 CFU/25m^2) and level D (< 50 CFU/25cm^2) according to the Ministry of Health standard for classification of operation room cleanliness [3, 13].

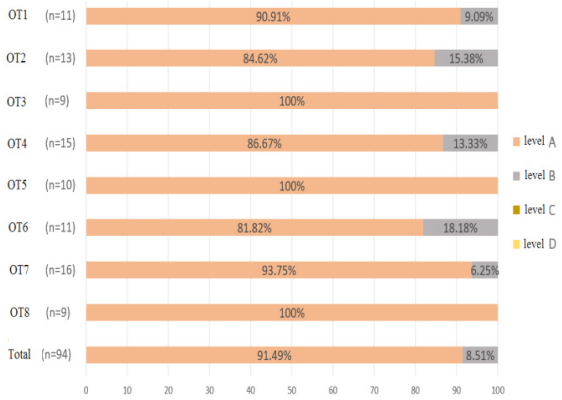

Figure 9: ORs's surface (floor & wall) contamination level

182 samples of water for SHW were collected, of which only 1.11% positive cultures result in the presence of *Enterobacter cloacae* (20%) and *Pseudomonas sp.* (20%) (Figure 10 & 11).

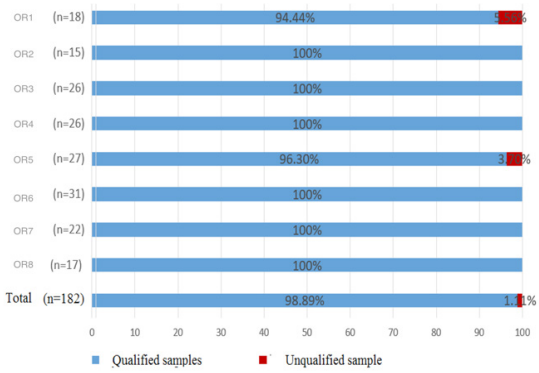

Figure 10: Water for SHW contamination level

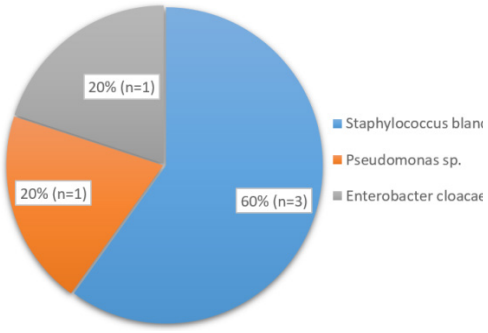

Figure 11: Types of bacteria identified from water for SHW

IV. DISCUSSION

A number of studies showed various results in the rate of positive cultures. Kausar et al (2020) reported that the positive rates occupied 72.82% [14]. Additionally, 48.3% and 24.7% were also the proportion of the positive ones according to Baban et al (2019) and Laham et al (2011), respectively [15, 16].

It is obvious that *Bacillus sp.* and *Staphylococcus blanc* were the most common isolated from the ORs. The rate of *Bacillus sp.* isolated in a number of studies was fewer than those in this study. To illustrate, *Bacillus sp.* seized 26.17% reported by Kausar et al (2020) [14] and 26.67% according to Bekkari H et al (2016) [17]. *Staphylococcus non-coagulase* is considered as an exogenous organism that can be normal skin flora of medical personnel and patient. One of the most dangerous bacteria isolated in the study was S. aureus that its isolates rate was much fewer than those in other studies such as Kausar et al (2020) [14] with 15.1% and Baban et al (2019) with 52% [16]. Besides, despite the existence of a number of dangerous strains like *Streptococcus β - hemolytic, Pseudomonas sp.*, their proportion was still extremetly low with 0.35%, 0.70% and 1.50%, respectively.

A study of Raksha et al (2019) found various species such as *Staphylococcus non - coagulase, E.coli, Streptococcus.sp, S. aureus* and *Micrococci* [18]. A report from Bekkari et al (2016) showed the presence of *Pseudomonas vesicularis* and *Streptococcus.sp* isolated in the environment of ORs [17]. Three species detected in the study of S. Ensayef (2009) were *Staphylococcus epidermidis*, *S.aureus* and *Pseudomonas aeruginosa* [19]. Nahed A. Al Laham et al (2011) gave information about 6 types of bacteria in the studied ORs including *Enterobacter, E.coli, Klebsiella, Acinetobacter, Pseudomonas and Streptococcus* [15].

The environmental hygiene and air treatment in the studied ORs was very good with none of pathogenic strains isolated and 87.59% air sample meets the microbiological standard.

S.aureus, Streptococcus β - hemolytic, Pseudomonas sp., Enterobacter cloacae were 4 species that fail to the meet the standard of microbiological surfaces in the ORs. *S.aureus* was detected in an anaesthetic face mask in OR1. In spite of low rate of *S.aureus* (1.59%) isolated, it must be concerned due to the risk of infection relating to skin, blood… as well as strong antibiotic resistance [20]. *Streptococcus β - hemolytic* (0.79%) was appeared on the surface of a medical equipment trolley in OR3. *Pseudomonas sp.* (0.79%) was found in a control button of electric scaple and *Enterobacter cloacae* (1.59%) was present on a surgical lighting in OR6.

It is noticeable that 91.49% cultures approached level A while the others were belong to level B with 8.51%. Particularly, 100% samples collected from OR3, OR5 and OR8 reached level A. It is amitted that environmental sanitation in the ORs of Hue Central Hospital - 2nd branch was very good. In our results, relatively clean sites were floors and walls of all ORs.

The result reflected that the surgical hand washing practice of surgeons in the studied ORs was very good with the high rate of negative cultures. As for

the positive ones, the bacterial isolates like *Bacillus sp.* and *S. blanc* are less likely to cause diseases and easily eliminated by routine hand hygiene.

Although the rate of positive samples of water was extremely low, it must be concerned that the two species *Enterobacter cloacae* and *Pseudomonas sp.* are found widely in the environment and fairy common pathogens involved in infections aquired in a hospital setting.

V. CONCLUSION

Microbial quality of air, surface and water in ORs might be considered as a mirror reflecting hygienic conditions of health - care facilities. Our study showed various bacteria isolated, of which pathogenic species occupied very low percentage. Especially, the rate of cultures in the research that meet the microbiological standards was remarkably high according to Ministry of Health recommendation.

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