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Prevalence of Bacterial Contamination and Antimicrobial Susceptibility Pattern among Blood and Blood Components Collected with and without diverging method at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia

By: Wondwossen Tsegaye (BSc)

Advisor: Adane Bitew (MSc, PhD)

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This is to certify that the thesis prepared by Wondwossen Tsegaye, entitled:

Prevalence of Bacterial Contamination and Antimicrobial Susceptibility Pattern among Transfusion Ready Blood and Blood Components at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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External Examiner_________________ Signature ___________Date ____________

Internal Examiner_________________ Signature ___________ Date ____________

Advisor_________________________Signature ___________ Date ____________

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Chairman of the Department or Graduate Program Coordinator

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Abbreviations

AFCSH	Armed Forces Comprehensive Specialized Hospital
AST	Antimicrobial Susceptibility Test
BAP	Blood Agar Plate
BHI	Brain-Heart Infusion
CAP	Chocolate Agar Plate
CLSI	Clinical and Laboratory Standards Institute
CNS	*Coagulase negative Staphylococci*
ENDFBBS	Ethiopia National Defense forces Blood bank service
FFP	Fresh Frozen Plasma
MAP	MacConkey Agar Plate
PC	Platelet Concentrates
PRBC	Packed Red Blood Cell
TSI	Triple sugar Iron test
TTBI	Transfusion transmittable bacterial infection
TTI	Transfusion transmittable Infection

Abstract

Background: Transfusion of bacterial contaminated blood and blood components could be a cause of morbidity and mortality. Understanding the mechanism of blood contamination is important in developing infection control strategy.

Objectives: To determine prevalence of bacterial contamination and antimicrobial susceptibility pattern among blood and blood components collected with and without diverging method.

Methods: A comparative cross-sectional study was done from January to April 2018 on blood and blood components collected with and without diverging method at the study area. A total of 376 blood sample of blood and blood components were taken. 3 ml of samples from each was inoculated on 15 ml Brain Heart Infusion followed by incubation for seven days at $37^{o}C$ then after subculture on Sheep Blood Agar, MacConkey Agar and Chocolate Agar. Finally, the antimicrobial susceptibility test was done for each isolate. Data were entered and analyzed using SPSS version 20 software. $P \leq 0.05$ was statistically significant.

Results: The overall prevalence of bacterial contamination among blood and blood components were 4.5% (N= 17/376). The prevalence of bacterial contamination of blood and blood components collected with the non-diverging and diverging method was 7.4% (n= 14/188) and 1.6 %(n= 3/188) respectively with P value of 0.05. *Staphylococcus epidermidis* were the most dominate isolates. Gram positive isolates showed more than 74% sensitive for antibiotics and also became more than 9% resistant. Most gram negative isolates became sensitive but *Pseudomonas aeruginosa* showed resistant for Gentamicin.29.4% (n= 5/17) isolated bacteria were multidrug resistant.

Conclusion: There was a difference between bacterial contamination in blood and blood components collected between diverging and non-diverging blood collection methods.

Keywords: Bacteria contamination, Diverging and non-diverging method, Blood culture

1 Introduction

1.1 Background

After the invention of blood circulation by the British physician William Harvey, the first recorded thriving blood transfusion was from dogs to other dogs. And then, human being transfused blood from lamb. In the 1800's, the blood transfusion was started among human beings then after the outcome became successful (1). The stipulation of blood products in the US at the World War II and immediate to the postwar era were significantly increased. Blood banks, transfusion services, and other blood and laboratory support services were organized and expanded (2).

Blood transfusion is a medical mediation planned to offer protected blood products in a considerable amount to clients for those who need them (3). Nevertheless, Transfusion transmittable infection (TTI) can be transferred from blood products to recipients originated from the contamination of these blood products. Common TTI causative agents such as HIV, HBV, HCV, malaria, Treponema *pallidum* (4), and the bacteria can be screened from the blood. Bacterial contamination of blood products could be the causation for morbidity and mortality when transfused them to patients (5).

In the half of 1990s, both literature and organization's haemovigilance systems indicated case report about bacterial transmission and this interest depicted by the blood community (6). Either skin commensals or gastrointestinal tract flora took the dominating bacterial isolates among them gram-positive aerobic pathogens which accounts for 75% are the principal one (7).

Blood products may be externally infected during blood collection; inadequate disinfection of donor skin, processing, an accident on blood bags and also storage conditions contributes to contaminated blood products. In addition to that, they also internally infected from asymptomatic bacteremia in the donor (8). In developed countries, the incidence of bacterial contamination and its related transfusion transmittable bacterial infection (TTBI) has been greatly condensed as a result of proper donor screening (9), uses of enhanced donor skin decontamination method, diverting the first blood volume, screening for bacterial contamination and storage-time reduction (10). As opposed to developed countries, developing countries are not put into practice the

above measures to guarantee the protection of donated blood from bacterial contamination (8).

Among African countries, most are focused on viral risks of TTIs. The threat of bacterial contamination gained through collection and processing is 2500 times higher than in developed countries until now. Furthermore, visual examination of the blood bags for hemolysis is the most applicable means of detecting for bacterial contamination in this region (11).

There is no published research is done on bacterial contamination with the diverging method in Ethiopia. However, two studies indicated the prevalence of bacterial contamination and their antimicrobial susceptibility pattern among whole blood collected by the non-diverging method (11, 12). To this end, the aim of the current study was shown the prevalence of bacterial contamination of blood products collected by two different donor blood collection methods.

1.2 Statement of the problem

Bacterial contamination is a major cause of morbidity and mortality in platelet concentrate (PCs) transfusion all over the world. In the United States, Platelet contamination with bacteria is the second most common source for transfusion-related deaths (13). In addition, according to the haemovigilance report from France showed that the risk of bacterial transfusion reaction after transfused whole blood, PRBC and platelet concentrate were 12.9 per 1,000,000 units, 10.4 per 1,000,000 units and 69.1 per 1,000,000 units respectively. In addition, the fatal out per 1,000,000 units were 12.0, 0.7 and 1.1 for platelets, RBCs and whole blood correspondingly (14).

In Africa, some published studies indicated that there was a high prevalence of bacterial contamination in blood and blood components in Ghana16.5 % (15), Kenya 8.8 % (16), Nigeria 8.8 % (17), Zimbabwe 3.1% (18) and Ethiopia15.3% (11). In most of these studies, they point out that the possible causes of contamination such as criteria of donor selection, environment, and screening, rubbing of the phlebotomy sites with unimproved disinfectant and avoiding culturing of donor blood.

The outcome of transfusing bacterial contaminated blood products to patients causes TTBI. The consequence will be developing Transfusion Associated Bacterial Sepsis (TABS) which is the most common microbiological risk of transfusion and is caused mostly by blood products (19). The sepsis which is caused by transfusing bacterially contaminated blood can result in a health burden to the patients. Moreover, clients like immunosuppressed and older individuals with poor nutritional status are the most susceptible population, but healthy individuals can have also a rapidly fatal outcome when transfused with a large load of bacteria alone or with the endotoxin (20).

In Ethiopia, most donors' blood is collected by the non-diverging method. In addition, the existence of asymptomatic blood donors having bacteremia, application of single disinfection method, environmental contamination, contamination while processing, long storage of blood products and absence of screening for bacterial contamination are the major problems for bacterial contamination. This study showed the difference in bacterial contamination of blood and blood components collected by the non-diverging and diverging method.

1.3 Significance of the study

The significance of the study was focused on:-

- ❖ Provided current information on the prevalence of bacterial contamination from blood products.
- ❖ Added information for clinicians' knowledge on bacterial contamination and safe blood.
- ❖ Added information for policymakers on improving the nation donor blood collection method.
- ❖ Becoming an update and source of evidence for further studies to carry out in the future.

2 Literature Review

2.1 Bacterial contaminants of Pathogenesis

After bacteria contaminate blood they go through three phase. The first phase is a lag phase. Few amounts of bacteria around 10-100 might exist in the blood products due to the nonspecific host defense mechanism such as complement activation in the availability of phagocytosis following opsonization they might be killed or inactivated (21). But there are some bacteria can stay alive to multiply then enter the exponential phase which is the second phase. Those bacteria with a load of around 20 that passed the nonspecific host defense mechanisms can multiply up to 10^8 -10^9 per milliliter of blood. This is a life-threatening problem of transfusion. This phase is ending up with either the on-hand nutrients are worn out or build up with toxic products of metabolism. The last phase in which bacteria may not survive but enough amount of endotoxin may have been produced to cause serious diseases condition and even death is called death phase (22).

2.2 Bacterial contamination rates

According to centers for disease control and prevention (CDC), bacterial contamination of platelets has been risk factor for TTI in the United State long years ago. The threat has been considerably higher when comparing the threat of bacterial contamination and viral infection after transfusion. Just about 1 in 1,000-3,000 platelet units may be contaminated with bacteria. Sepsis due to transfusion and culture confirmed in at least 1 in 100,000 transfused clients and it has lead to urgent fatal consequence in 1 in 500,000 recipients. Even if the real risk of transfusion-associated sepsis is like higher, the infection as a result of contaminated blood products is under-reported (23).

Surveillance was done by *Michael R J et al.,* (2008) on patients receiving platelets from July 1991 through December 2006 in Ohio, USA. The active surveillance indicated that 52 (0.02%) bacterial contaminated out of 238,983 platelet units were detected during the surveillance period. Out of fifty-two, fifty were detected with active surveillance and the rest is a passive one. The conclusion was active surveillance detected the bacterial contamination by 32 times higher than the passive one (24).

A study was done in Mexico by *Ibánez-Cervantes G et al.,* (2017) on one hundred platelet concentrate from the national center for blood transfusion. Out of them, 9% of the

platelets were detected for bacterial contamination by BacT/ALERT 3D automated system and also nucleotide sequence identified that *Staphylococcus epidermidis* which comes from blood donor skin and soil. The implication of detecting contaminants indicated that cleaning of blood collection area and proper disinfecting of puncture site was significant (25).

A study conducted in the Netherlands by *Korte D De et al.,* (2006) using double skin disinfection and diversion the first 10-30 ml of blood to the pouch. A total of 113,093 PCs were tested for bacterial contamination. The first positivity rate before and after the application of a new method was 0.95% and 0.85 % respectively. And also the bacterial contamination rate between diverging the first flow of blood alone and in combination with double disinfection were 0.5% and 0.37% in that order. This told us diverging and double disinfecting the skin were brought a considerable reduction of bacterial contamination (26).

A review was done in Netherland by *Korte D De et al.,* (2014) with the possibility of the lowering of TTBI by promoting a group of standard activities while collecting whole blood. They indicated that by applying them, it is possible to minimize bacterial contamination by 50% to 75%. In addition, TTBI at the same time reduced. Lack of detecting a low initial load of bacteria is still a problem because the detectors lack sensitivity. Another move toward could be the application of pathogen-inactivation methods for cellular blood products to decrease the number of pathogens. Nevertheless, these methods are expensive (27).

Another review was done by *Liumbruno G M et al.,* (2009) from Italy on bacterial contamination from 2004 to 2007 on countries like UK, Netherland, Canada and USA which diverted 20 ml, 20-30 ml, 40ml and 40-50 ml amount of whole blood in that order. The percentage of bacterial contamination of blood and blood components were 47%, 49%, 90% and 47% respectively. This show as diverting a certain amount of whole blood before entering into the main blood bag decreased the bacterial contamination (28).

A Study was conducted in New Zealand by Dickson M and Dinesh D (2013) before and after the expiry date of 59,461 and 15,560 platelet concentrates which were detected with BacT/ALERT BPA aerobic culture bottle respectively. The rate of bacterial

contamination was 0.04% in both situations. In other published literature the rate was between 0.01- 0.74% and is decreased (0.01–0.18%) when diversion of the initial flow of blood is employed. The conclusion was detecting transfusion of bacterial contaminated platelet with BacT/ALERT reduce the transmission and also testing samples collected at day 2 were not identified all contaminants (29).

A study conducted by *Satake M et al.,* (2009) in Japan focused on pre and post running of the diversion method for Platelet Concentrates. The positivity rate of bacterial contamination were 36/21,786 (0.17%) and 11/21,783 (0.05%) without and with diversion method. The decreasing rate was 71%. The winded up that the outcome of diversion method on the reduction of bacterial contamination was significantly high (30).

A study carried out from Malaysia by *Jumaah N et al.,* (2014) to determine the prevalence of bacterial contamination. The bacterial contamination was12 (1.7%) out of 702 blood samples that were inoculated from 20 ml initially diverted blood during collection. Bacteria like Coagulase negative *Staphylococcus (*CNS), *Staphylococcus aureus* and gram-positive bacilli were identified. They concluded that the utilization of blood bag which has diverging pouch had major effects on the lowing of bacterial contamination during blood collection (31).

In 2015, a study conducted by *Latifi M et al.* in Iran on 92 blood and blood components. The bacterial contamination percentages of whole blood were 30%, 25% and 19% at 3 days, one week and beyond one week storage time correspondingly. In the same approach for blood component, the contamination percentage for storage time less than three days in decreasing order was plasma (21%), platelet (18%) and cryoprecipitate (14%). in contrary to the previous, the contamination percentage for storage time 3 to 7 days were platelet (28%), plasma and cryoprecipitate (7-18%). For storage time more than one week, the result was platelet (31%), plasma (9%) and cryoprecipitate (13%). Some gram-positive and negative bacteria were identified. The conclusion was donor skin disinfection should be improved and bacterial contamination was clearly identified after a week blood donation (32).

A study conducted by *Vadlavalli A K et al.,* (2016) in India to determine the bacterial contamination of Hundreds of units of whole blood. The prevalence of bacterial

contamination was 11% and isolates of gram-positive bacteria were identified. They concluded that serious intervention should be done on the blood collection system due to the existed of bacterial contamination (33).

Another study from India conducted by *Barot T et al.,* (2016) on blood and blood components indicated that out of 148 units of stored whole blood and packed red cells 5 (3.37%) samples had shown bacterial growth. The isolated contaminants were *Staphylococcus aureus*, *Klebsiella spp*. and CNS. Therefore, it is critical to improving hygiene precaution in order to minimize bacterial contamination and ensure patient safety (34).

A study conducted in Accra Ghana by *Adjei A A et al.,* (2009) on bacterial contamination of blood and blood components using culture and sensitivity. The result revealed that the total prevalence was 28 out of 303 (9.2 %). Of these, the percentage of bacterial contamination among whole blood, plasma and platelets took 13%, 3 % and 9 % respectively. Gram-positive and negative bacteria were identified. Most antibiotics were sensitive for both. They concluded that bacterial contamination in blood and blood components were common in Ghana (8).

The same study was conducted from Ghana by *Opoku-Okrah C et al.,* (2009) indicated that 14 17.5%) of blood bags from a total of 80 were positive for bacterial growth. Among the positives, 10 (71.42%) isolates such as gram positives were the commonest contaminants and also few gram-negative bacteria were identified. From those drugs, Amikacin was sensitive for the whole isolates. Their final conclusion was Ampicillin and Cotrimoxazole became resistant for all *Staphylococcus aureus* isolates and MDR strain bacteria had been generated (20).

Another similar study in Ghana by *Boye A et al.,* (2016) also indicated that 16/97 (16.5%) bacterial contamination was identified from prescreened and stored blood units. From the total of 97 blood units, gram-negative and positive bacteria were 43.8% and 56.2% for each. Bacterial contamination was common in the study are and further measure should be taken to minimize (15).

Another study from Nigeria by *Bolarinwa R A et al.,* (2011) was done on bacterial contamination of blood and blood components. The prevalence of bacterial contamination

was 8.8 % from a total of 162 blood and blood components. The isolates were gram-positive like *Bacillus spp and Listeria spp*. 29% to 100% were resistant to antibiotics. The bacterial contamination was common in Nigeria and was the serious nosocomial infections (17).

A cross-sectional study from Zimbabwe by *Makuni N et al.,* (2015) was held on the prevalence of bacterial contamination in blood and blood products. Around 196 blood samples were tested for bacterial growth using conventional culture and sensitivity. The prevalence was 6/196 (3.1%). Platelet was highly contaminated. The isolates had shown that different sensitivity feature for the antibiotics. Clients who received blood and blood components were in danger to develop sepsis (18).

A study from Uganda by *Aloysius G B M et al.,* (2013) was done on 510 samples of blood and blood components to detect bacterial contamination using culture and sensitivity method. The prevalence was 18/510 (3.5%). Different isolates were identified and among them, 94.4% were *Staphylococcus aureus.* Isolates were resistant to penicillin and cloxacillin. The bacterial contaminated blood and blood components can influence the health status of the patients particularly in malaria-endemic place (5).

Research conducted in Kenya by *Hassall O et al.,* (2009) on 435 selected whole blood units ready for pediatric transfusion. Generally, 8.8 % were detected bacterial contamination using culture. Gram-negative bacteria were 64%. Bacterial contamination was neglected and important risk of blood transfusion for children in sub-Saharan Africa (16).

A cross-sectional study was conducted in Gondar, Ethiopia by *Wondimu H et al.,* (2013) on 137 whole bloods collected for transfusion. Twenty-one (15.33%) of blood bags was contaminated with some gram-positive and negative bacteria. Some antibiotics like chloramphenicol were sensitive but MDR was seen in 66.7% of the isolates. 95.24% of the contamination source was from external. Their conclusion was bacterial contamination was a familiar setback and concentration should be given for safe transfusion practice (11).

Another cross-sectional study from Debre Markos, Ethiopia by *Esmael A et al.,* (2014) was conducted on bacterial contamination of stored blood ready for transfusion. 12.5 %

of the whole blood was contaminated with bacteria out of 120 preserved whole blood were showed bacterial growth. Blood group O Rh-positive became the highest to be contaminated. Gram-positive and negative bacteria were identified. Tetracycline and Cotrimoxazole were resistant drugs for gram-positive and negative respectively. Their conclusion was further surveillance and/or study should be done (12).

There was no published research that was done in comparing bacterial contamination of blood products collected with the diverging and non-diverging method in Ethiopia. But some researches were done independently either diverging or non-diverging blood donors collection method outside Ethiopia. The current study was determined prevalence of bacterial contamination and antimicrobial susceptibility pattern among blood and blood components collected with and without diverging method at AFCSH.

2.3 Common bacterial contaminants

Gram-positive skin commensals such as *Staphylococcus epidermidis* and *Bacillus cereus* are the organisms most often recovered from donated blood. Such contamination is thought to occur principally during phlebotomy, as a result of incomplete disinfection and/or skin core removal by the collection needle. These organisms typically do not grow at 1 to 6°C but survive and multiply readily at the platelet storage temperature of 20 to 24°C (35).

In the case of gram-negative bacterial contamination, asymptomatic donors with transient bacteremia are presumed to be responsible for most cases of contamination. For example, in the case of *Yersinia enterocolitica* contamination of red cells, implicated donors typically are found to have elevated antibody titers to *Yersinia enterocolitica*, implying a recent infection (36).

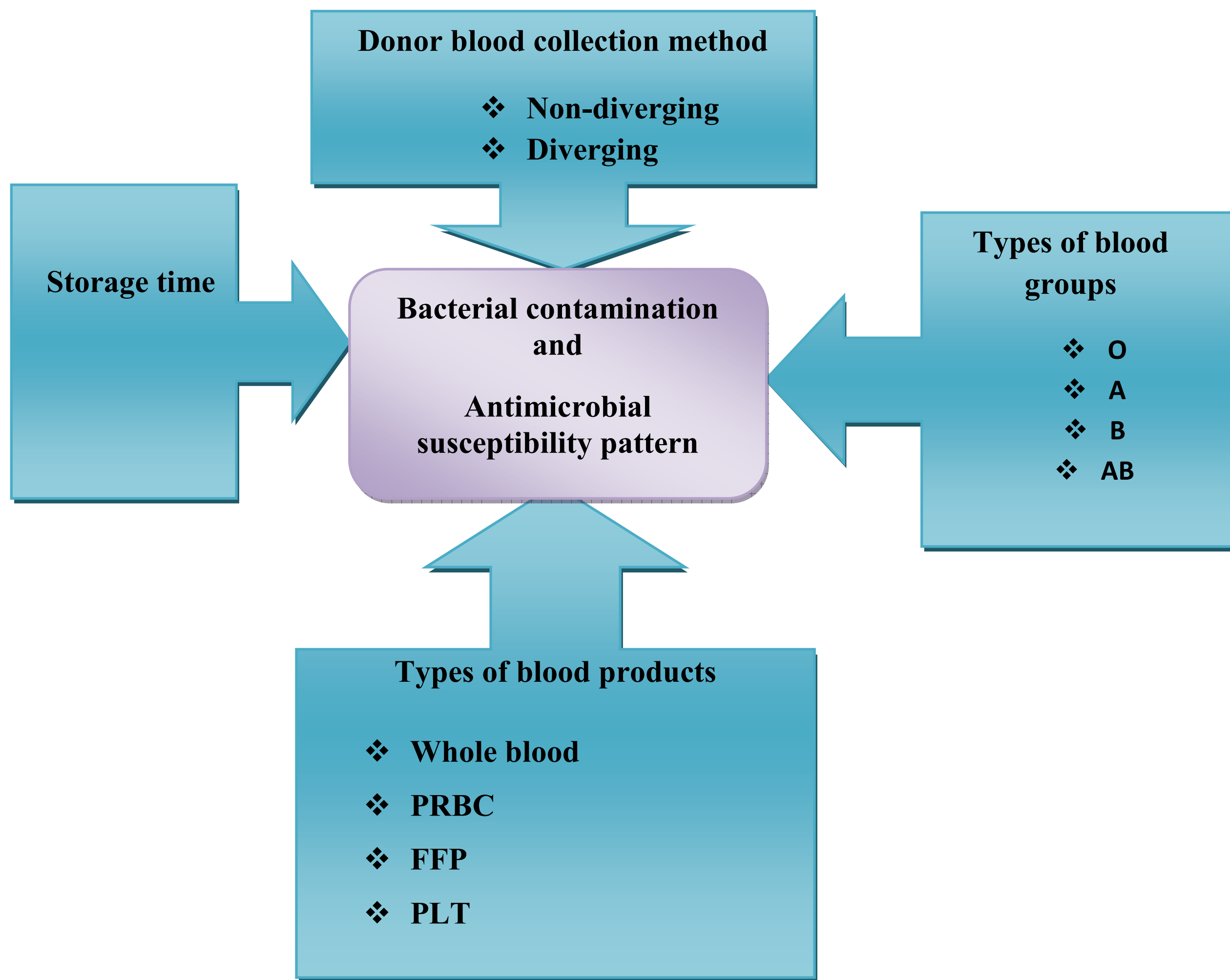

Figure 1: Conceptual framework for this study Addis Ababa, Ethiopia December 2017/18.

3 Objectives

3.1 General objectives

To determine prevalence of bacterial contamination and antimicrobial susceptibility pattern among blood and blood components collected with and without diverging method.

3.2 Specific Objective

- To determine the prevalence of bacterial contamination of blood and blood components.
- To compare bacterial contamination of blood and blood components collected by diverging and non-diverging method.
- To determine the antimicrobial susceptibility pattern of isolated bacteria.

4 Hypothesis

There was no difference between bacterial contamination in blood and blood components collected between diverging and non-diverging blood donor collection methods.

5 Materials and Methods

5.1 Study area

The study was conducted in Addis Ababa which is the capital city of Ethiopia. AFCSH is located in Lideta sub-city. It is organized under HMD, Ministry of Defense. It provides medical service to members of the Ethiopian defense forces, their family and community. AFCSH has 15 wards with 600 beds. There are 378 healthcare professionals with different levels and field of training. Based on the 2016/17 annual report the hospital provides services for 100,005 outpatients and 4,229 inpatients as well as 962 deliveries and 295,549 laboratory investigations. On average, about 8- 12 units of blood and blood components are transfused daily. In 2016/17 G. C, the maximum annual required blood and blood components were 4818 units of blood and blood components (37). The hospital has the access to receive blood and blood components that were collected by diverging method from Ethiopia national defense forces blood bank service (ENDFBBS) and non-diverging one from Ethiopia national blood bank service (ENBBS).

5.2 Study Design and Study period

A comparative cross-sectional study was conducted from January 2017 to April 2018.

5.3 Sample Sources

All blood and blood components collected from ENBBS and ENDFBBS for transfusion purpose during the study period were the sample sources.

5.3.1 Study Sample

All blood and blood components delivered from ENBBS and ENDFBBS for transfusion purpose during the study period were study sample.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

- Transfusion ready blood and blood components.

5.4.2 Exclusion criteria

- Blood and blood components that showed any leakage on the bag.

5.5 Study variables

5.5.1 Dependent variables

- ❖ Bacterial contamination in blood and blood components.
- ❖ Antimicrobial susceptibility pattern.

5.5.2 Independent variables

- ❖ Types of donor blood collection method.
  - o Diverging
  - o Non-diverging
- ❖ Storage time of blood and blood components.
- ❖ Storage temperature of blood and blood components.
- ❖ Types of blood and blood components.
- ❖ Types of blood group.

5.6 Sample size determination

The sample size was calculated based on two population proportion formula. The value of P_1 was taken as 15.33 %(0.1533) (11) from the previous study on whole blood and P_2 as 50%. Considered 95% confidence interval and commonly used values for C_p, power; $C_{0.05,\ 95\%,}$ were 13. The sample size is calculated using the following standard formula (38).

The sample size

$$n = \frac{[p_1(1 - p_1) + p_2\,(1 - p_2)]}{(p_1 - p_2)^2} \times c_{p,\text{power}}$$

n= sample size

P_1 = proportion of occurrence of the event to be studied 15.33 % (0.1533).

P_2 = proportion of occurrence of the event to be studied 50 % (0.5).

$C_{p,\ \text{power}} = C_{0.05,\ 95\%}$ is defined, 13

$$n = \frac{0.153(1-0.153) + 0.5(1-0.5)}{(0.153-0.5)^2} \times 13$$

n= 41

Since the calculated sample size was 41. 10 % of the non-include rate were considered so that the sample size became 45. As a result, low sample size was calculated. To increases the sample size, we took 47 units from whole blood, 47 units from Packed red blood cells (PRBC), 47 units from Fresh Frozen Plasma (FFP) and 47 units from platelet concentrate (PC) from the non-diverging method. And another same amount of blood and blood components collected from the diverging method. A total of 376 units of blood and blood components were included.

5.7 Sampling method

Convenient sampling method was applied for culturing blood and blood components.

5.8 Data collection procedure

5.8.1 Sample characteristics

Information like unit number, types of blood and blood components, types of blood group, collection and expiry date and storage temperature were obtained from each unit of labeled blood and blood components bag then recorded on the log sheet.

5.8.2 Laboratory analysis

5.8.2.1 Sampling collection

Prior to the actual study, pretest was done with 16 blood and blood components. Each unit of whole blood and PRBC was mixed before sampling with handshaking and striper and then we detached 20-25 cm segment after sealed the tubing at 5-10 cm away from the end of each blood bag's tubing. Each detached segment was labeled with coded labeling paper. In the biological safety cabinet, each unit of FFP, platelet, and detached segment was decontaminated first using packed swab saturated with 70% isopropyl alcohol and waited for one minute and then with 2% tincture iodine then waited for three minutes.

After decontaminated the sterilized brain-heart infusion broth (Oxoid, Basingstoke, UK) blood culture bottle stopper in the same way like above, 3 ml of blood samples were drawn from each segment using sterile disposable syringe and then dispensed into 15 ml of BHI broth aseptically. The specimens were delivered to the AFCSH Microbiology laboratory incubation room then isolated, identified and tested for susceptibility (20).

5.8.2.2 Bacterial isolation and identification

The inoculated BHI was incubated at 37°C aerobically then observed daily for any possible signs of bacterial growth such as pellicle formation, hemolysis, gas formation clotting and turbidity for 7 days. After overnight incubation, the BHI bottles were mixed then one ml blood sample was taken from the bottle with a sterile disposable syringe and subculture two drops of sample on sheep Blood agar plate (BAP), MacConkey agar plate (MAP) and Chocolate agar plate (CAP) blindly. Inoculated BHI bottles; that show bacterial growth at 2 to 7 days, were also subcultured on sheep BAP, MAP, and CAP and also resubcultured until we got the pure colony. The inoculated MAP was incubated aerobically but BAP and CAP were incubated with 5-10% CO_2 atmosphere. Whenever bacterial growth was observed on the incubated media, identification of bacterial species was done based on bacterial cultural characteristics, gram stain and biochemical tests. The biochemical tests were done depending on their gram reaction result. For gram-positive bacteria, we used catalase, coagulase, CAMP, bile esculin, novobiocin, TSI tests and so on. We also used for gram-negative bacteria TSI, citrate, indole, motility, urease, and oxidase. The result of biochemical referred to the chart of gram-negative and positive bacteria to identify the species level of the bacteria. In addition, differential media like Mannitol salt agar was used to differentiate staphylococci species (39).

5.8.2.3 Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed according to Kirby-Bauer disk diffusion method on Mueller hinton agar plates (MHA). Antibiotics were selected based on the recommendation of clinical and laboratory standards institute guideline (CLSI). The known volume and concentration of recommended antibiotics for gram-positive bacteria such as Amikacin (30 µg) Cefoxitin (30 µg), Clindamycin (2 µg), Erythromycin (15 µg), Penicillin (10 units),), Gentamicin (10 µg), Trimethoprim-sulphamethoxazole

(1.25/23.75 µg), Tobramycin (10 µg) Ceftriaxone (30 µg), Ampicillin (10 µg) , Chloramphenicol (30 µg) and Cefotaxime(30 µg) and also antibiotics for gram-negative bacteria like Amikacin(30 µg), Amoxicillin+ clavuninic acid (10/20 µg) , Cefepime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg) , Meropenem (10 µg), Piperacillin-tazobactam (10/100 µg), Tobramycin (10 µg) and Trimethoprim-sulphamethoxazole (1.25/23.75 µg) were used. The zone of inhibition of those drugs was measured after 18-24 hr inculcation to the adjacent millimeter of the caliper. Interpretation of identified bacteria as susceptible, intermediate, or resistant according to the recent CLSI specified interpretive criteria (40).

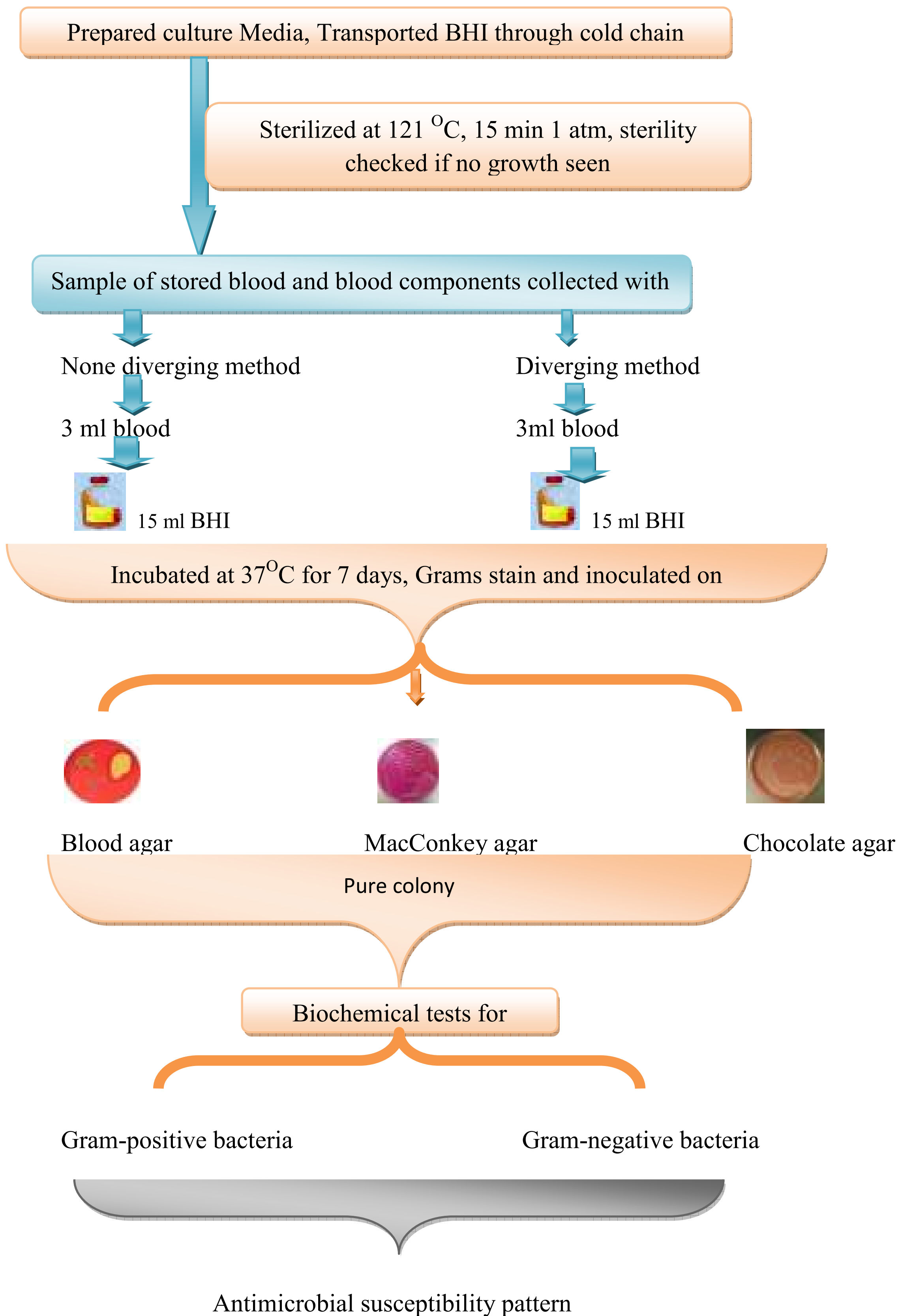

Figure 2: Path of workflow of the research

5.8.3 Principles

5.8.3.1 Blood Culture

Blood specimens are submitted through BHI bottles for culturing from blood sample source. After overnight incubation at 35-37 ^{0}C then blindly subcultured. Samples are continued to examine up to seven days for visible signs of bacterial growth such as turbidity, heamolysis, clots etc. The resulting broth cultures are then sub cultured onto sheep blood, chocolate and MacConkey agar, and incubated for 18 – 24 hrs at 37°C and bacterial growths in the form of discrete colonies will be examined (41).

5.8.3.2 Disc diffusion antimicrobial susceptibility testing method

Paper antimicrobial discs with a known volume and concentration of antibiotics are placed on sensitivity testing media consistently inoculated with the isolates. The antimicrobial diffuses from the disc into the medium and the growth of the organisms is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc (42).

For the detail information about the principles, test procedures and interpretation of all test methods are indicated in Annex IV.

5.9 Quality Assurance

5.9.1 Pre-analytical phase

- ❖ Units of blood and blood component information's such as unit number, blood group, collection date, expiration date and types of blood and components were recorded from labeled blood bags.
- ❖ The storage time was calculated from the difference between date of sample drawn and donor blood collected date.
- ❖ In addition, Samples such as whole blood, PRBC, FFP and PC were collected with sterile syringe and needle with aseptic techniques.
- ❖ The specimens were transported to the examination microbiology laboratory within 30 minutes.
- ❖ Well organized data collection formats were used.

- We followed the manual, SOP of the Microbiology laboratory and local and international guideline and manufacturer instructions.

5.9.2 Analytical phase

- Materials and equipments were adequately controlled. The following main tasks were done in this phase
  - Washing, Cleaning and autoclaving of glass wares.
  - Recording of temperature for refrigerator, deep freezer and incubator.
  - Sterility checked for
    - Autoclave
      - ATCC 7953 *Geobacillus stermophilus* spores self contained.
    - Prepared BHI
      - By incubating and sub culturing on chocolate media.
    - Prepared sheep blood agar, chocolate agar and MacConkey agar and other biochemical media
      - By incubating in the incubator
  - Aseptic techniques and safe handling of infectious material
  - Preparation and Quality control of culture media.
  - Staining techniques
  - Quality control of stains.
    - *Staphylococcus aureus* control strain ATCC 25923.
    - *Staphylococcus epidermidis* control strain ATCC 12228
    - *Escherichia coli* control strain ATCC 25922.
    - *Pseudomonas aeruginosa* control strain ATCC 27853.
    - *Enterococcus faecalis* control strain ATCC 29212
    - *Streptococcus pyogenes* control strain ATCC 19615
    - *Proteus mirabilis* control strain ATCC 35659
    - *Klebssiella pneumonia* ATCC 13883
  - Inoculation of broth and agar culture media and plating out techniques.
  - Techniques used to identify pathogens.
  - Reading and interpretation of cultures.

- Performing antimicrobial test.
- Autoclaving, disposing of cultured media.

5.9.3 Post-analytical phase

- Recording, verifying and reporting of the results in the result log sheet were done.
- Interpreting test reports correctly.
- Collected data were checked and entered first into excel sheet then SPSS version 20 with two individuals.
- Isolates were stored.

5.10 Data Analysis and Interpretation

Data entry and analysis were done using SPSS statistical software version 20. Descriptive statistics like percentage were calculated. Binary and multiple Logistic regression analysis also were used to see the relationship between the dependent and independent variable and assess their significant level. In all cases, p-value, less than or equal to 0.05 were considered as statistically significant. Finally, the results were presented in words, percentages, graphs and tables. Variables that became statistically significant association ($p \leq 0.05$) at binary logistic analysis was entered and analyzed by multiple logistic regression analysis.

5.11 Operational Definition

Haemovigilance: the set of surveillance procedures covering the entire blood transfusion chain, from the donation and processing of blood and its components, through to their provision and transfusion to patients, and including their follow-up (43).

Blood components: Specific parts which can get from donated whole blood such as PRBC, FFP, Platelet and Cryoprecipitate used to treat a patient who required them (44).

Diverging method: It is practice of blood collection method applied by changing the first milliliters flow of venipuncture blood direction to reduce fragments and contamination from the skin and air (45).

Diverging pouch/Bactivam: Reduction of bacterial contamination provided with sampling bag of 40mL (46).

Non-diverging method: is a donor blood collection method which used to collect blood without changing the first flow of venipuncture blood direction.

Bacterial Contamination: the occurrence of bacteria on inanimate objected from different sources (47).

Multidrug resistant (MDR): bacteria resistant to two or greater number of antibiotic from different classes (48).

5.12 Ethical Considerations

This research project was approved and given ethical clearance by ethics and research committee of the department of medical laboratory sciences, School of Allied Health Sciences and College of Health Sciences of Addis Ababa University. Permission was obtained from the AFCSH administrator. Samples were coded and the confidentiality of donors' blood was maintained throughout the study. The principal investigator informed to the laboratory director of AFCSH about those blood and blood components showed bacterial growth to take any action.

5.13 Dissemination of results

The study report was submitted to Department of Medical Laboratory Sciences, School of Allied Health Sciences and College of Health Sciences of Addis Ababa University. The result will be presented to the study sites. The study abstract will be submitted to the local association like Ethiopia Medical Laboratory Association to present the result during continuous medical education events organized through this association. Extra paper will be submitted to the international or national peer-reviewed journal for publication.

6 Results

6.1 Demographic characteristics

A total of three hundred seventy six blood samples were collected from transfusion ready blood and blood components which were collected by non-diverging and diverging blood donor collection methods and shipped to AFCSH. From each method, one hundred Eighty eight blood and blood components were conducted during the study period.

Among different types of blood group, O blood group with Rh-positive was the highest to be examined (N= 137, 36.4%. The next blood types were B+ (N=82, 21.8%) and A+ (N=78, 20.7%) were the second and third correspondingly.

In addition, the minimum and maximum storage temperatures for whole blood were 3.5 and 5°C, PRBC 3.5 and 4.8^{o}C, FFP -40 and -38°C and also platelet 23 and 25°C.

Among the different types of blood and blood components, majority of the storage time of whole blood , PRBC, FFP and platelet were at up to 1 week 26(27.7%), 1-2 week 51(54.3%), more than six weeks 30(31.9%) and 1-5 days 94(100%) respectively. Detail information is displayed in table 1.

Table 1: Sample characteristics of blood and blood components in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Types of blood and blood components	Donor blood collection method		Total
	Non-diverging	Diverging	
	N (%)	N (%)	N (%)
Whole blood	47(25)	47 (25)	94 (25)
PRBC	47 (25)	47 (25)	94 (25)
FFP	47 (25)	47 (25)	94(25)
Platelet	47(25)	47 (25)	94(25)
Total	188 (100)	188 (100)	376 (100)

	Types of blood groups								
	A+	**A-**	**B+**	**B-**	**AB+**	**AB-**	**O+**	**O-**	**Total**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Whole blood	20(21.3)	2(2.1)	23(24.5)	1(1.1)	12(12.8)	1(1.1)	32(34)	3(3.2)	94(100)
PRBC	18(19.1)	2(2.1)	19(20.2)	4(4.3)	11(11.7)	2(2.1)	36(38.3)	2(2.1)	94(100)
FFP	21(22.3)	1(1.1)	17(18.1)	2(2.1)	18(19.1)	1(1.1)	39(31.9)	4(4.3)	94(100)
Platelet	19(20.2)	1(1.1)	23(24.5)	2(2.1)	4(4.3)	1(1.1)	39(41.5)	5(5.3)	94(100)
Total	78(20.7)	6(1.6)	82(21.8)	9(2.4)	45(12)	5(1.3)	137(36.4)	14(3.7	376(100)
	Storage temperature in ^{O}C								
	-38	**-39**	**-40**	**23**	**24**	**25**	**3.5-5**		**Total**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**		**N (%)**
Whole blood	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	94(100)	94(100)	0(0)
PRBC	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	94(100)	94(100)	0(0)
FFP	44(46.8)	21(22.3)	29(30.9)	0(0)	0(0)	0(0)	0(0)	94(100)	44(46.8)
Platelet	0(0)	0(0)	0(0)	23(24.5)	53(56.3)	18(19.1)	0(0)	94(100)	0(0)
Total	44(11.7)	21(5.6)	29(7.7)	23(6.3)	53(14.1)	18(4.8)	188(49.8)	376(100)	44(11.7)
	Storage time								
	1-5 days N (%)	**Up to 1 week N (%)**	**1-2 weeks N (%)**	**2-3 weeks N (%)**	**3-4 weeks N (%)**	**4-5 weeks N (%)**	**5-6 weeks N (%)**	**> 6 weeks N (%)**	**Total N (%)**
Whole blood	0(0)	26(27.7)	18(19.1)	25(26.6)	21(22.3)	4(4.3)	0(0)	0(0)	94(100)
PRBC	0(0)	11(11.7)	51(54.3)	17(18.1)	12(12.8)	3(3.2)	0(0)	0(0)	94(100)
FFP	0(0)	5(5.3)	13(13.8)	2(2.1)	1(1.1)	25(26.6)	18(19.1)	30(31.9)	94(100)
Platelet	94(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	94(100)
Total	94(100)	42(11.2)	82(21.8)	44(11.7)	34(9)	32(8.5)	18(4.8)	30(8)	376(100)

Key:

N= Number

FFP= Fresh Frozen Plasma

PRBC = Packed Red Blood Cell

^{o}C = degree centigrade

6.2 Prevalence of overall bacterial contamination

The overall prevalence of bacterial contamination among blood and blood components were 4.5% (N= 17/376). Among these blood and blood components, the highest bacterial contamination growth (N= 6, 35.3%) were observed from whole blood and PRBC followed by platelet (N=3, 17.6%) and FFP (N=2, 11.8%). There was no statistically significant association between the bacterial contamination and types of blood and blood components (COR (CI): 2.07 (05-8.53), P value for COR = 0.31).

Blood group O with Rh-positive (n= 6, 35.3%) showed the most contaminated one than others. The highest number bacterial contamination was observed on whole blood (N= 6/17, 35.3 %) and packed PRBC (N= 6/17, 35.3 %). The association among blood group and storage temperature to the bacteria contamination were not applicable due to the existence of no bacterial growth on blood group O and B with Rh-negative.Detail information is displayed in table number 2 and fig 3.

Table 2: Prevalence of overall bacterial contamination and association factors among collection method, blood and blood components in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Variables		Bacterial G N (%)	Bacterial NG N (%)	Total N (%)	COR (CI)	P value for COR	AOR (CI)	P value for AOR
Donor blood collection method	Non-diverging	14 (7.4)	174 (92.6)	188 (50)	4.96 (1.4-17.6)	0.013	7.75 (1-60.1)	0.05
	Diverging	3 (1.6)	185 (98.4)	188 (50)	1		1	
Blood & blood components	WB	6 (35.3)	88 (24.5)	94 (25)	2.07 (0.5-8.53)	0.31		
	PRBC	6 (35.3)	88 (24.5)	94 (25)	2.07 (0.5-8.53)	0.31		
	Platelet	3 (17.6)	91 (25.3)	94 (25)	0.66 (0.1-4.04)	0.65		
	FFP	2 (11.8)	92 (25.6)	94 (25)	1		1	
Total		**17 (100)**	**359 (100)**	**376 (100)**				

Key:

N= Number

G= Growth

NG= No Growth

COR= Crude odd ratio

CI= Confidence interval

AOR= Adjusted odd ratio

WB: Whole blood

PRBC = packed red blood cell

FFP: Fresh Frozen plasma

p value= ≤ 0.05

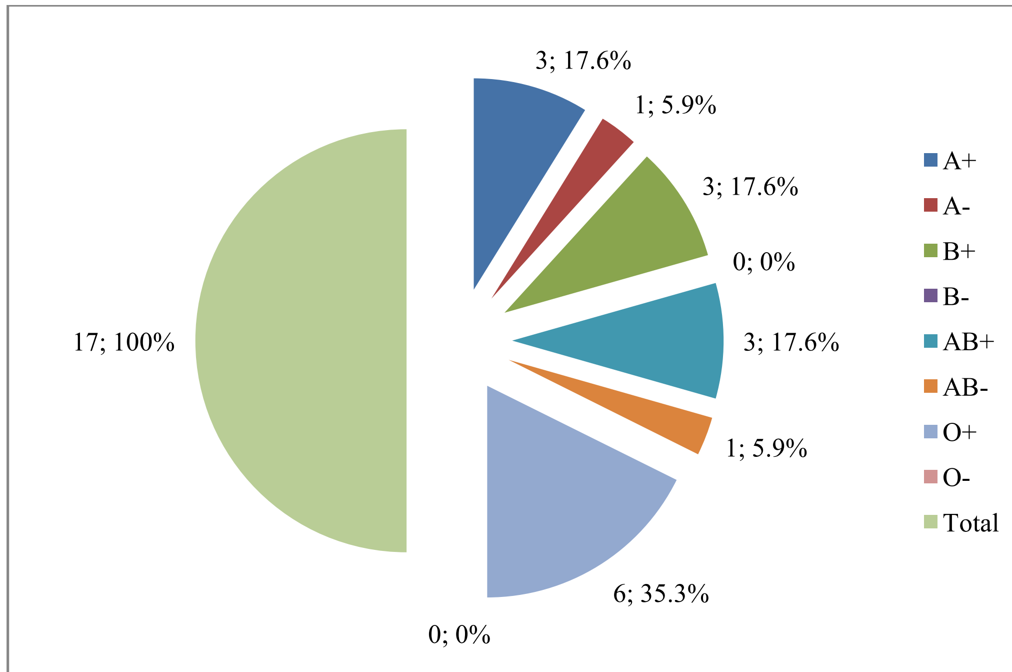

Figure 3: Frequency of the overall bacterial growth among blood and blood components' blood group in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

From the overall prevalence of bacterial contamination (N= 17/376, 4.5%), gram-positive and negative were isolated. Of these, gram-positive bacteria (N= 14/17, 82.4%) took the largest number and gram-negative bacteria accounted (N= 3/17, 17.6%). Among the isolated gram-positive bacteria *Staphylococcus epidermidis* (N= 6, 35.3%) became the dominant isolate followed by *Staphylococcus aureus* (N= 4, 23.5%). Detail information is displayed in figure 4.

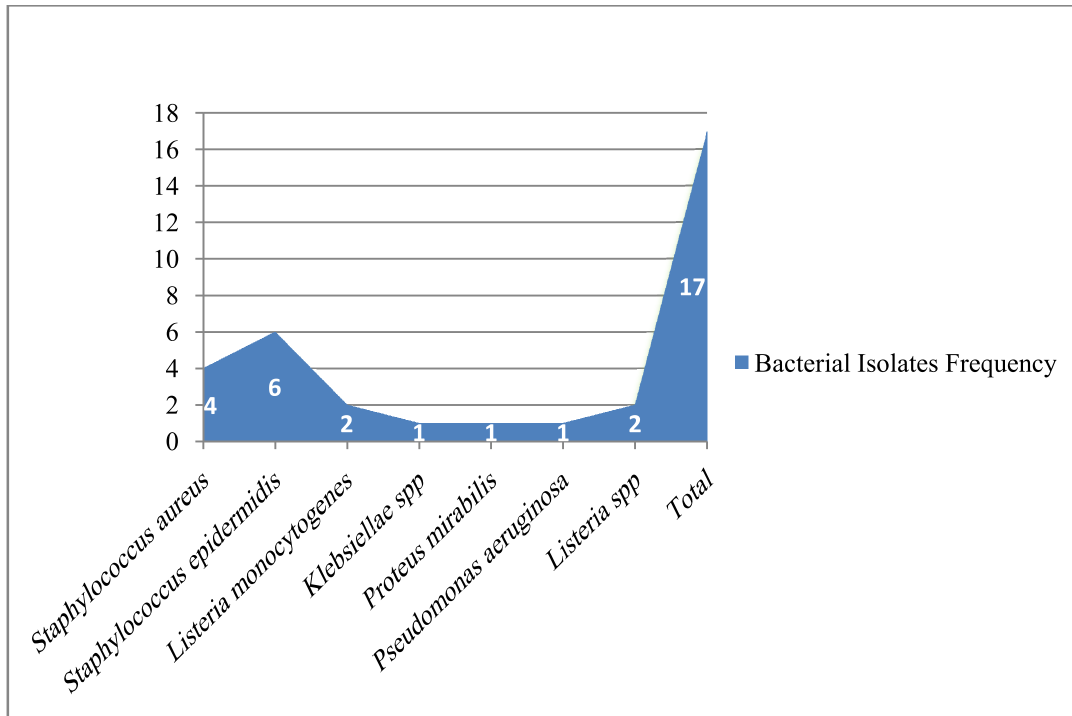

Figure 4: Frequency distribution of the isolated bacteria from blood and blood components in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

6.3 Comparison of bacterial contamination between diverging and non-diverging method

The prevalence of bacterial contamination from those blood and blood components which were collected with non-diverging 7.4% (n= 14/188) was higher than diverging method 1.6% (n= 3/188). There was a statistical significant association between blood donor collection method and bacterial contamination (AOR (CI): 7.8 (1-60.1), P value: 0.05).

The most isolated bacteria from blood and blood components collected with non-diverging method were *Staphylococcus epidermidis* (n= 4, 28.57%) and *Staphylococcus aureus* (n= 4, 28.57%). Whereas identified isolates from blood and blood components collected with diverging method were only *Staphylococcus epidermidis* (n= 2, 66.7%). Detail information is displayed in figure 5.

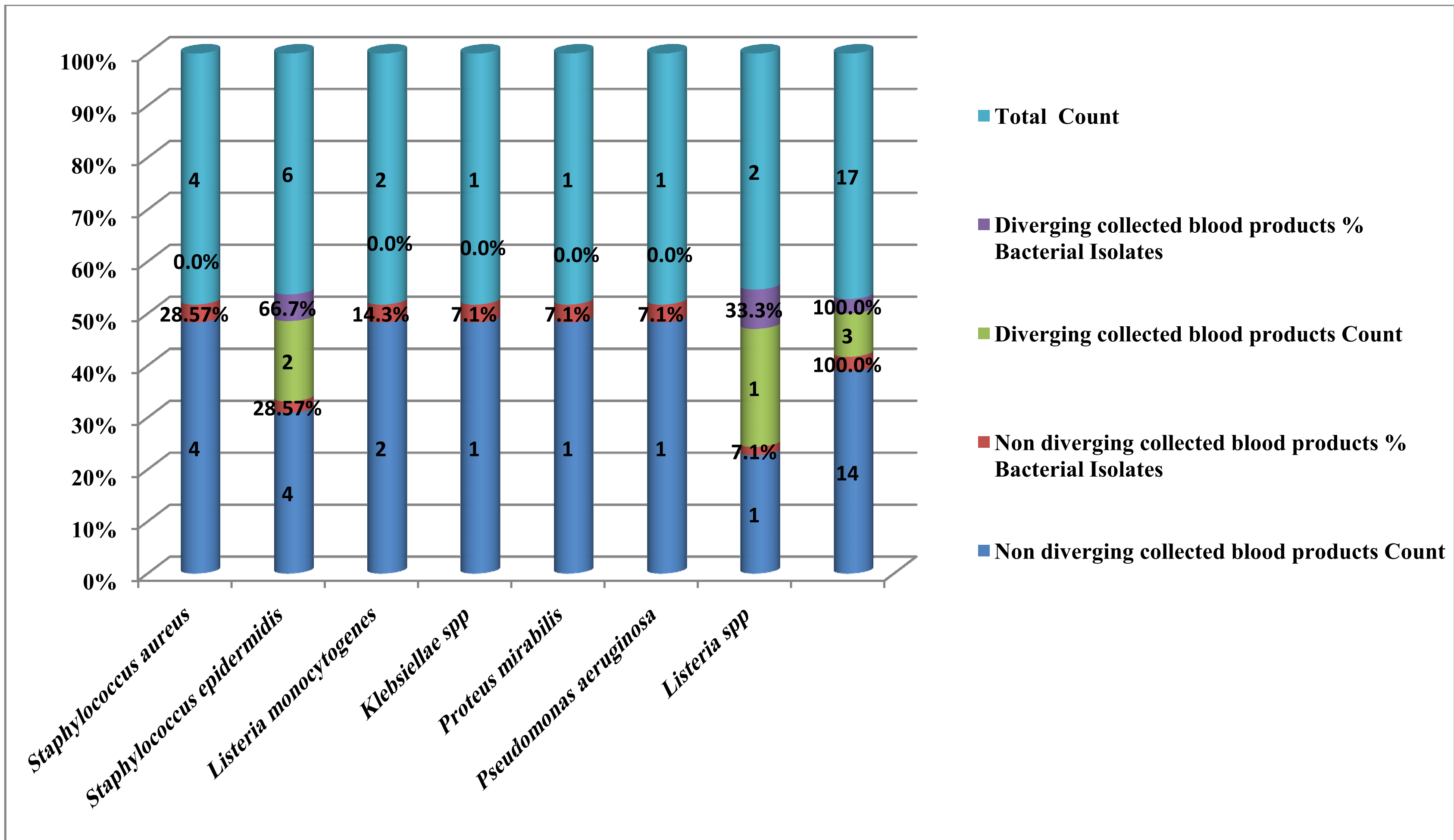

Figure 5: Comparison of the frequency distribution of isolated bacteria from blood and blood components between non-diverging and diverging donor blood collection method in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

6.4 Antimicrobial Susceptibility Patterns of Isolated Bacteria

In this study, around eighteen different antibiotics spectrums were tested against seventeen isolated bacteria from stored blood and blood components. The rate of sensitivity among gram-positive and negative bacteria ranges from 0% - 100 %. More than 74%, 6% and 9% of the gram-positive isolates were sensitive, intermediate and resistant respectively. Similarly, the sensitivity pattern among the gram-negative bacteria were 85.2 % sensitive, 11.1% intermediate and 3.7% resistant (Table 3, 4 and 5).

The most sensitive antibiotics among identified gram-positive isolates from those blood and blood components collected with non-diverging method were Ceftriaxone (100%), Ampicilin (100%), Tobramycin (100%) and Cefotaxime (100%).

Gentamicin (9%), Trimethoprim-sulphamethoxazole (9%) and Erythromycin (9%) were showed as the least intermediate one. The decreasing order of antibiotics resistant pattern were Trimethoprim-sulphamethoxazole (27%), Erythromycin (27%), Cefoxitin (25%) and Penicillin (18%) (Table 3).

Parallely, gram-negative isolates were sensitive for the antibiotics such as Amoxicillin+ clavulanic acid (100%), Piperacillin-tazobactam (100%), Cefepime (100%), Ceftriaxone (100%), Tobramycin (100%), Amikacin (100%), Ciprofloxacin (100%) and Trimethoprim-sulphamethoxazole (100%). Gentamicin and Meropenem became intermediate with 33% and 67% respectively. The only antibiotic that showed resistant to *Pseudomonas aeruginosa* 33.3% was Gentamicin (Table 4).

Staphylococci epidermidis and *Listeria spp* were isolated from blood and blood components collected with diverging blood collection method. Most antibiotics were sensitive for these organisms except Ceftriaxone (100%), Chloramphenicol (100%) were intermediate. In addition, Trimethoprim-sulphamethoxazole (67%) was the most resistant antibiotic (Table 5).

Finally, among the isolated seventeen organisms, 5(29.4%) were shown to be Multidrug resistant (MDR≥2 drugs) for one group of spectrum of antibiotics. *Staphylococci epidermidis* 3(17.6%) took the highest MDR bacteria followed by *Listeria monocytogenes* 1 (5.88%) *and Listeria spp* 1 (5.88%) (Table 6).

Table 3: Antimicrobial susceptibility pattern of gram-positive bacteria isolated from blood culture among stored blood and blood components collected by non-diverging method in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Bacterial Isolates	N	P	GEN N (%)	CTR N (%)	CLD N (%)	AMK N (%)	CXT N (%)	AMP N (%)	COT N (%)	PEN N (%)	ERY N (%)	TOB N (%)	CHL N (%)	CXM N (%)	Total N (%)
S aureus	4	S	3 (75)	ND	3 (75)	3 (75)	4 (100)	ND	3 (75)	3 (75)	3 (75)	4 (100)	ND	ND	26 (81)
		I	1 (25)	ND	1 (25)	1 (25)	0 (0)	ND	1 (25)	0 (0)	0 (0)	0 (0)	ND	ND	4 (13)
		R	0 (0)	ND	0 (0)	0 (0)	0 (0)	ND	0 (0)	1 (25)	1 (25)	0 (0)	ND	ND	2 (6)
S. epidermidis	4	S	4 (100)	ND	4 (100)	4 (100)	2 (50)	ND	2 (50)	3 (75)	2 (50)	4 (100)	ND	ND	25 (78)
		I	0 (0)	ND	0 (0)	0 (0)	0 (0)	ND	0 (0)	0 (0)	1 (25)	0 (0)	ND	ND	1 (3)
		R	0 (0)	ND	0 (0)	0 (0)	2 (50)	ND	2 (50)	1 (25)	1 (25)	0 (0)	ND	ND	6 (19)
L.monocytogenes	2	S	2 (100)	2 (100)	ND	ND	ND	2 (100)	1 (50)	2 (100)	1 (50)	2 (100)	1 (50)	2 (100)	15 (83)
		I	0 (0)	0 (0)	ND	ND	ND	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	1 (6)
		R	0 (0)	0 (0)	ND	ND	ND	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	2 (11)
Listeria spp	1	S	1 (100)	1 (100)	ND	ND	ND	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	9 (100)
		I	0 (0)	0 (0)	ND	ND	ND	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		R	0 (0)	0 (0)	ND	ND	ND	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sub Total	1	S	10 (91)	3 (100)	7 (88)	7 (88)	6 (75)	3 (100)	7(64)	9 (82)	7(64)	11 (100)	2 (67)	3 (100)	75 (85)
	1	I	1 (9)	0 (0)	1 (22)	1 (22)	0 (0)	0 (0)	1 (9)	0 (0)	1 (9)	0 (0)	1 (33)	0 (0)	6 (6)
		R	0 (0)	0 (0)	0 (0)	0 (0)	2(25)	0 (0)	3 (27)	2 (18)	3 (27)	0 (0)	0 (0)	0 (0)	10 (9)
Total			**11(100)**	**3 (100)**	**8 (100)**	**8 (100)**	**8 (100)**	**3 (100)**	**11 (100)**	**11 (100)**	**11 (100)**	**11 (100)**	**3 (100)**	**3 (100)**	**91 (100)**

Key: N= Number P= Pattern ND= Not done GEN = Gentamicin CTR = Ceftriaxone CLD = Clindamycin AMK= Amikacin CXT = Cefoxitin AMP = Ampicillin COT = Trimethoprim-sulphamethoxazole PEN= Penicillin ERY= Erythromycin TOB= Tobramycin CHL= Chloramphenicol CXM= Cefotaxime S= Sensitive I= Intermediate R= Resistant

Table 4: Antimicrobial susceptibility pattern of gram-negative bacteria isolated from blood culture among stored blood and blood components collected by non-diverging method in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Bacterial Isolates	N	P	GEN	CPR	CTR	AMK	MER	CFP	PIP-TAZ	COT	AUG	TOB	Total
			N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Klebsiella spp	1	S	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	9 (90)
		I	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10)
		R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Proteus mirabilis	1	S	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	8 (80)
		I	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)
		R	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomonas aeruginosa	1	S	0 (0)	1 (100)	ND	1 (100)	1 (100)	1 (100)	1 (100)	ND	ND	1 (100)	6 (85.7)
		I	0 (0)	0 (0)	ND	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	0 (0)	0 (0)
		R	1 (100)	0 (0)	ND	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	0 (0)	1 (14.3)
Sub Total	3	S	3 (33.3)	3 (100)	2 (100)	3 (100)	1 (33)	3 (100)	3 (100)	2 (100)	2 (100)	3 (100)	23 (85.2)
		I	3 (33.3)	0 (0)	0 (0)	0 (0)	2 (67)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (11.1)
		R	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.7)
Total			**3 (100)**	**3 (100)**	**2 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**2 (100)**	**2 (100)**	**3 (100)**	**27 (100)**

Key: N = Number P= Pattern ND= Not done GEN = Gentamicin CPR= Ciprofloxacin CTR = Ceftriaxone AMK= Amikacin MER = Meropenem CFP = Cefepime PIP- TAZ= Piperacillin-tazobactam COT = Trimethoprim-sulphamethoxazole AUG = Amoxicillin+ clavulanic acid TOB = Tobramycin S= Sensitive I= Intermediate R= Resistant

Table 5: Antimicrobial susceptibility pattern of gram-positive bacteria isolated from blood culture among stored blood and blood components collected by diverging method in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Bacterial Isolates	N	P	GEN N (%)	CTR N (%)	CLD N (%)	AMK N (%)	CXT N (%)	AMP N (%)	COT N (%)	PEN N (%)	ERY N (%)	TOB N (%)	CHL N (%)	CXM N (%)	Total N (%)
S. epidermidis	2	S	2 (100)	ND	1 (50)	2 (100)	2 (100)	ND	1 (50)	2 (100)	1 (50)	2 (100)	ND	ND	13 (81)
		I	0 (0)	ND	0 (0)	0 (0)	0 (0)	ND	0 (0)	0 (0)	0 (0)	0 (0)	ND	ND	0 (0)
		R	0 (0)	ND	1 (50)	0 (0)	0 (0)	ND	1 (50)	0 (0)	1 (50)	0 (0)	ND	ND	3 (19)
Listeria spp	1	S	1 (100)	0 (0)	ND	ND	ND	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	6 (67)
		I	0 (0)	1 (100)	ND	ND	ND	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	2 (22)
		R	0 (0)	0 (0)	ND	ND	ND	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11)
Sub Total	3	S	3 (100)	0 (0)	1 (50)	2 (100)	2 (100)	1 (100)	1(33)	3 (100)	2(67)	3 (100)	0 (0)	1 (100)	19 (74)
		I	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	2 (11)
		R	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	2 (67)	0 (0)	1 (33)	0 (0)	0 (0)	0 (0)	4 (15)
Total			**3 (100)**	**1 (100)**	**2 (100)**	**2 (100)**	**2 (100)**	**1 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**3 (100)**	**1 (100)**	**1 (100)**	**25 (100)**

Key: N= Number P= Pattern ND= Not done GEN = Gentamicin CTR = Ceftriaxone CLD = Clindamycin AMK= Amikacin CXT = Cefoxitin AMP = Ampicillin COT = Trimethoprim-sulphamethoxazole PEN= Penicillin ERY= Erythromycin TOB= Tobramycin CHL= Chloramphenicol CXM= Cefotaxime S= Sensitive I= Intermediate R= Resistant

Table 6: Multidrug resistant level of isolated bacteria stored blood and blood components collected by non-diverging and diverging method in AFCSH Addis Ababa city, Ethiopia, January to April 2018.

Bacterial Isolates	Number of isolates	Isolates from NDM		Isolates from DM		MDR≥2
		R0 N (%)	R1 N (%)	R0 N (%)	R1 N (%)	N (%)
S. aureus	4	4(23.5%)	0(0)	0(0)	0(0)	0(0)
S. epidermidis	6	3(17.65%)	1(5.88%)	0(0)	2(11.76%)	3(17.65%)
L. monocytogenes	2	1(5.88%)	1(5.88%)	0(0)	0(0)	1(5.88%)
Listeria spp	2	1(5.88%)	0(0)	0(0)	1(5.88%)	1(5.88%)
Klebsiellae spp	1	1(5.88%)	0(0)	0(0)	0(0)	0(0)
Proteus mirabilis	1	1(5.88%)	0(0)	0(0)	0(0)	0(0)
Pseudomonas aeruginosa	1	1(5.88%)	0(0)	0(0)	0(0)	0(0)
Total	17	12(70.6%)	2(11.76%)	0(0)	3(17.64%)	5(29.4%)

Key:

N= Number

MDR= Multidrug-resistant

R0 = isolates which were sensitive for all/intermediate for at least one antibiotics/resistant for one antibiotics

R1= Resistant for one group of antibiotics

NDM = Non-diverging method

DM = Diverging method

7 Discussion

7.1 Prevalence of bacterial contamination

The awareness of bacterial contamination among blood and blood components which usually uses for blood transfusion as well as their sources of contamination is imperative for setting up the preventive measure at blood bank centers and blood transfusion centers. Moreover, identification of contaminated blood and blood components, characterization of bacterial isolates and also assessing bacterial antimicrobial susceptibility pattern could have a positive impact on clinical practice and public health importance. It is also significant to offer action and improve the blood collection practice, education and policy.

In our study, the overall prevalence of bacterial contamination among blood and blood components was 4.5% (N= 17/376). Among these blood and blood components, the highest bacterial contamination growth were observed from whole blood and PRBC (N= 6, 35.3%) followed by platelet (N=3, 17.6%) and FFP (N=2, 11.8%). But, the prevalence bacterial contamination from those blood and blood components which were collected with non-diverging and diverging method were 7.4% (n= 14/188) and 1.6 % (n= 3/188) respectively.

In the current study, blood group type O+ 35.3% (N= 6/17,) showed the most contaminated one subsequently to blood group A+ 17.6% (N= 3/17) and B + 17.6% (N= 3/17). Studies from Debre Markos, Ethiopia carried out by *Esmael A et al* (12) also agreed with this study.

The prevalence of bacterial contamination among those blood and blood components which were collected with non-diverging donor blood collection method was 7.4% (n= 14/188) and almost close to previous studies reported from Africa countries like Ghana by *Adjei A A et al* 9 % (n=28/303) (8) and Nigeria by *Bolarinwa R A et al* 8.8%, (n=14/162) (17).

The bacterial contamination of blood and blood components collected with diverging done in this study is more or less similar to another finding like in Malaysia carried out by *Jumaah N et al* was 1.7% (n= 12 /702) (31).

Among the overall four types of blood and blood components; such as whole Blood, PRBC, FFP and platelet, the highest bacterial contamination was observed in both whole blood and PRBC followed by platelet and FFP. Another study was done in Ghana by *Adjei A A. et al* also supported that whole blood became the first to be contamination by bacteria followed by FFP and platelet in equal proportion except that PRBC is not included in their study (8).

The isolated bacteria in this study were both gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes, and Listeria species*) and gram-negative bacteria (*Klebsiellae species, Proteus mirabilis and Pseudomonas aeruginosa*). Our finding was agreed with different studies like in Nigeria by *Bolarinwa R A et al* (17) and India conducted by *Barot T et al* (34).

However, our finding was lower than studies done in Debre Markos , Ethiopia 12.5 % (n= 15/120) (12) and Gondar 15.3% (n= 21/137) (11) and also in Ghana 16.5 % (n= 16/97) (15), 17.5% (n= 14/80) (20) , this difference may be due to the difference in sample size, sample taken from only whole blood, the present study on the other hand higher in prevalence of bacterial contamination than other studies done in Zimbabwe 3.1% (n= 6/196) (18) , Uganda 3.5% (n=18 /510) (5) . The reason for increased prevalence may be in our countries the disinfection type is focused on application of 70 % alcohol only.

The prevalence of bacterial contamination in the diverging method of the current study is higher than different countries finding such as New zealand done by Dickson M and Dinesh D was 0.04% (n= 2378/59461) (29). The difference may be the implementation of a compressive activity like proper donor screening, double disinfection, closed processing system and the existence of national haemovigilance programme in New zealand.

From this study; when we compared the bacterial contamination between blood donor collection methods of blood and blood components, the non-diverging method was higher than the diverging method one. In addition, the calculated adjusted odd ration became 7.8 so that the non-diverging blood donor collection method was likely more exposed to bacterial contamination by 7.8 times than diverging one.

Those blood and blood components which were collected in both method share same conditions like application of single disinfection method, the absence of bacterial contamination screening and active national haemovigilance programme except changing the direction of the first 30-40 ml of blood into the diverging pouch. So, switching the first flow of blood reduced the contamination rate by 5.8 %. This concept is also supported by the reviewed study in Italy by *Liumbruno G M et al*. The purpose of diverting the first 40-50 ml of donated blood to reduce the microbes or skin fragments especially comes from donor skin entering into the collection bag (28). In addition, other study in Japan by *Satake M et al* also maintained that the positivity rate of bacterial contamination were 36/21786 (0.17%) and 11/21783 (0.05%) without and with diversion method in that order. Even if this study done in platelet concentrates only, used different methodology, sample volume and include anaerobic bacteria detection (30).

As opposed to the current study, a study done in Zimbabwe by *Makuni N et al* the finding of the highest contaminated among blood and blood components was platelet 10.3% (n= 4/36) followed by PRBC 1.3% (n= 2/149). There was no unit of whole blood was contaminated by bacteria. The difference may be they took unequal amount of from each blood and blood components by *Makuni N et al* (18).

In General, to reduce the bacterial contamination among collected blood and blood components integrated activities should be implemented before and after blood is collected from blood donors. Such activities are proper donor session recording, serious blood donor health interview, application of double skin disinfection, diverting the first 30–40 ml aliquot of blood, closed system collection and processing, establishing bacterial contamination screening and national haemovigilance program (28).

7.2 Antimicrobial Susceptibility Pattern of bacterial contamination

The emergence of antibiotic resistant in the management of infections is a serious public health problem in the globe; particularly in the developing world (49). The present data pointed out that 5.23% of tested antibiotics were resistant to the isolated bacteria. Some of the antibiotics that showed resistant were Gentamicin, Clindamycin, Cefoxitin, Trimethoprim-sulphamethoxazole, Penicillin and Erythromycin. But the majority of the antibiotics became sensitive for most of the isolates.

Among the gram-positive bacteria which were tested for the antibiotics in the currents study, most of the isolates became sensitive to Gentamicin, Ceftriaxone, Ampicillin, Tobramycin and Cefotaxime. Those isolates that showed intermediate for the antibiotics like Gentamicin, Clindamycin Amikacin and Trimethoprim-sulphamethoxazole were *Staphylococci aureus*. Moreover, Chloramphenicol also became intermediate only for *Listeria monocytogenes.* In contrast, half of *Staphylococci epidermidis* became resistant to Cefoxitin, Trimethoprim-sulphamethoxazole and Erythromycin. A study from Nigeria is nearly agreed with this study and they also reported that gram-positive bacteria were sensitive for Gentamicin and Ceftriaxone but the majority of the antibiotics were resistant (17).

The only antibiotic that showed resistant to the *Pseudomonas aeruginosa* was Gentamicin. The remaining gram-negative isolates like *Klebsiella spp* and *Proteus mirabilis* were sensitive for Gentamicin, Ciprofloxacin, Ceftriaxone, Amikacin, Meropenem, Cefepime, Piperacillin-tazobactam, Trimethoprim-sulphamethoxazole, Amoxicillin+ clavulanic acid and Tobramycin. But a study from Debremarko indicated that all the gram-negative organisms isolated were resistant to Cotrimoxazole and susceptible to Ciprofloxacin and Cefoxicitin (12).

From the seventeen bacterial isolated, 5(29.4%) were shown to be Multi drug resistant for level one grouped drug spectrum (MDR $\geq$ 2 drugs). In our study gram-positive bacteria showed MDR for chemically different drugs whereas gram-negative bacteria did not. Most of the isolated bacteria had high resistant level on Erythromycin (29.5%) and Trimethoprim-sulphamethoxazole (29.5%). Our finding was lower than Study done here in Gondar (66.7%), Ethiopia by *Wondimu H et al* (11).

8 Strength and limitation of the study

8.1 Strength

The strength of the present study:

- ❖ Has showed the prevalence of bacterial contamination among blood and blood components collected with diverging method for the first time in Ethiopia

8.2 Limitation

This study was

- ❖ Couldn't identify anaerobic bacteria.

9 Conclusion and recommendation

9.1 Conclusion

Our study showed that the prevalence of bacterial contamination of stored blood and blood components was higher in those collected with non-diverging method than diverging one. So, there was a difference between bacterial contamination in blood and blood components collected between diverging and non-diverging blood collection methods.

Furthermore, *Staphylococci epidermidis* became the most commonly isolated bacteria followed by *Staphylococci aureus*. Whole blood and PRBC were the highest bacterial contaminated blood and blood components. Those blood and blood components which have blood type O+ became the first to be contaminated by bacteria. A considerable level of resistant bacteria and MDR organisms were observed.

9.2 Recommendations

Bacterial contamination of blood and blood components comes from not only a gap of single activity but also a gap of a number of coordinating activities. Therefore, the following recommendation should be carried out for its prevention.

- ❖ Improving donor skin disinfection from single to double disinfection.
- ❖ Improving blood donor collection method from non-diverging to diverging method.
- ❖ Establishing bacterial screening procedure for culturing of donor blood for bacterial contamination before transfusing the blood and blood components.
- ❖ Establishing a haemovigilance system as a nation in the country.
- ❖ Further study should be carried out to know the outcome of bacterial contamination on the hospital clients at post transfusion.

10 References

1. American Association of Blood Banks [internet]. Transfusion medicine: Highlights of transfusion medicine history; 2018 [Cited 2017 Aug 12]. Available from: http://www.aabb.org/tm/Pages/highlights.aspx.
2. Tobler L H and Busch M P. History of post transfusion hepatitis. ClinChem. 1997; 43 (8): 1487-1493.
3. SieJ N, Achila Okoth O, Issac J, Menghisteab E, Hailemariam M, Hagos S, et al. Seroprevalence of transfusion-transmissible infections among blood donors at National Blood Transfusion Service, Eritrea: a seven-year retrospective study. BMC infectious disease. 2018; 18:1-9.
4. Jain R, Jain P, Mahadik V, Choudhury N. Prevalence and Incidence of Transfusion Transmitted Infections among VNRBDs in Central India. Natl J Integr Res Med. 2015; 6 (4): 82-85.
5. Aloysius G B M, Joel B, Apecu R, Yap B and Byarugaba F. Bacterial Contamination of Blood and Blood Products at Mbarara Regional Blood Bank in Rural South Western Uganda. Scientific research journal. 2013; 3: 205-209. doi:10.4236/aid.2013.33030.
6. Mcdonald, C. P. Interventions Implemented to Reduce the Risk of Transmission of Bacteria by Transfusion in the English National Blood Service. 2011; 38: 255–258. https://doi.org/10.1159/000330474.
7. Bihl F, Castelli D, Marincola F, Dodd Roger Y and Brander C. Transfusion transmitted infections. Journal of Translational Medicine. 2007; 5 (25): 1-44. doi:10.1186/1479-5876-5-25.
8. Adjei A A, Kuma G K, Tettey Y, Ayeh-kumi P F, Opintan J, Apeagyei F, et al. Bacterial Contamination of Blood and Blood Components in Three Major Blood Transfusion Centers, Accra, Ghana. Jpn. J. Infect. Dis. 2009; 62: 265–269.
9. Norfolk D, Handbook of Transfusion Medicine: 5th edition. United Kingdom: The stationary office (TSO); 2013.
10. Lee C C. Bacterial contamination of blood products. Hong Kong Red Cross Blood Transfusion Service, Hong Kong SAR, China. ISBT Science Series. 2011; 6, 427–431.

11. Wondimu H, Addis Z, Moges F, and Shiferaw Y. Bacteriological Safety of Blood Collected for Transfusion at University of Gondar Hospital Blood Bank, Northwest Ethiopia. International Scholarly Research Notices. 2013; 2013:7 doi:10.1155/2013/308204.
12. Esmael A, Dagnew Z, and Degu G. Bacterial Contamination of Stored Blood Ready for Transfusion at a Referral Hospital in Ethiopia. J Clin Res Bioeth. 2014; *5* (2):2–5. https://doi.org/10.4172/2155-9627.1000176.
13. Samia A. Girgis, Ghada A. Ismail, Fatma El-Zahraa H. Bahgat, Ibrahim K. Ali, Samar S. Rashad, and Salwa F Ahmed. Rapid Detection of Bacterial Contamination in Platelet Concentrates, by Polymerase Chain Reaction and DNA Sequencing in Comparison to Conventional Automated Culture Cairo, Egypt. Int.J.Curr.Microbiol.App.Sci (2014) 3 (4): 38-52.
14. Ofori O K A. Transfusion transmitted malaria and bacterial infections in a malaria endemic region: Research gate [unpublished dissertation]. Liverpool for the degree of Doctor in Philosophy; 2012.
15. Boye A, Daniel D, Samuel A, James A, Peter Mate-Siakwa. Bacterial Contamination of at-Point-of Transfusion Blood in a Tertiary Hospital in Ghana. EC Bacteriology and Virology.2016:121-128.
16. Hassall O, Maitland K, Pole L, Mwarumba S, Denje D, Wambua K. et al. Bacterial contamination of pediatric whole blood transfusions in a Kenyan hospital. Transfusion Complication.2009; 49:2594-2598. doi:10.1111/j.1537-2995.2009.02344.x.
17. Bolarinwa Rahman A, Aboderin Oladipo A, Odetoyin Babatunde W, Adegunloye Aramide B. Bacterial contamination of blood and blood components in a tertiary hospital setting in Nigeria. International journal of infection control. 2011; 7:1-6. doi: 10.3396/ijic.V7i1.001.11.
18. Makuni N, Simango C, and Mavenyengwa Rooyen T. Brief Original Article Prevalence of bacterial contamination in blood and blood products at the National Blood Service Zimbabwe. 2015; 9: 421-424. doi:10.3855/jidc.5428.

19. Ineke G H, Rood Dirk D K, Sandra R A, Paul H M. Savelkoul and Annika P. Distribution, origin and contamination risk of coagulase-negative staphylococci from platelet concentrates. Journal of Medical Microbiology. 2011; 60: 592-599. doi:10.1099/jmm.0.023176-0.
20. Opoku-Okrah C, Feglo P, Amidu N, Dakorah M P. Bacterial contamination of donor blood at the Tamale Teaching Hospital, Ghana. African Health Sciences. 2009; 9 (1):13-18.
21. Müller T H, Montag T, Seltsam AW. Laboratory evaluation of the effectiveness of pathogen reduction procedures for bacteria. Transfusion Medicine and Hemotherapy. 2011; 38 (4):242-250.
22. McDonald CP. Bacterial risk reduction by improved donor arm disinfection, diversion and bacterial screening. Transfusion Medicine 2006; 16(6):381-396.
23. Centers for Disease Control and Prevention (CDC). Bacterial Contamination of Platelets [Internet] March 11, 2013[March 21, 2013; cited 2017/06/12].Available from:https://www.cdc.gov/bloodsafety/bbp/bacterial-contamination-of-platelets.html.
24. Michael R J, Caryn E G, Hillard M L, and Roslyn A Y. Relationship between Bacterial Load, Species Virulence, and Transfusion Reaction with Transfusion of Bacterially Contaminated Platelets. CID. 2008; 46:1214-1220. doi: 10.1086/529143.
25. Ibánez-Cervantes G, Bello-Lópeza J M, Fernández-Sáncheza V, Domínguez-Mendozab C A and Acevedo-Alfaro L I. Prevalence of bacterial contamination in platelet concentrate at the National Center of Blood Transfusion in Mexico. (2017), *24*, 56–61. https://doi.org/10.1016/j.tracli.2017.03.003.
26. Korte D De, Curvers J, De Kort W L. A. M, Hoekstra T, van der Poel L C, Beckers A.M E et al. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. 2006; 46: 477-485. doi:10.1111/j.1537-2995.2006.00746.x.
27. Korte D De, Marcelis J H. Platelet concentrates: reducing the risk of transfusion-transmitted bacterial infections. International Journal of Clinical Transfusion Medicine. 2014; 2: 29–37. doi:10.2147/IJCTM.S40037.

28. Liumbruno G M, Catalano L, Piccinini V, Pupella S, and Grazzini G. Reduction of the risk of bacterial contamination of blood components through diversion of the first part of the donation of blood and blood components, 2009; 7: 86-93. https://doi.org/10.2450/2008.0026-08.
29. Dickson M and Dinesh D. Bacterial contamination of platelet concentrate produced in New Zealand The New zeland medical journal.2013; *126* (1374):12–21.
30. Satake M, Mitani T, Oikawa S, Nagumo H, Sugiura S, Tateyama H. et al. Frequency of bacterial contamination of platelet concentrates before and after the introduction of diversion method in Japan. 2009; *49:* 2152–2157. https://doi.org/10.1111/j.1537-2995.2009.02243.x.
31. Jumaah N, Joshi S R, Sandai D. Prevalence of Bacterial Contamination when using a Diversion Pouch during Blood Collection : A Single Center Study in Malaysia. 2014; *21*(13): 47–53. [Cited 2017 Aug 12]. Available fromhttp://www.mjms.usm.my/notice/CURRENT%20ISSUE%20MJMS%2021%283%29/%20mjms-21-3-047.pdf.
32. Latifi M, Issazadeh K and Majid M R. Bacterial contamination of blood products in a different region of Iran. Cibtech JournalJournal of Zoology. 2015; 4 (1): 52-56. ISSN: 2319–3883.
33. Vadlavalli A K and Yeedulamudi R. Prevalence of Bacterial Contamination in Stored Blood, Experience At Tertiary Care Teaching Hospital. 2016; *15* (12): 58–60. https://doi.org/10.9790/0853-1512085860.
34. Barot T, Kothari F, Dighe M, Patel S. Prevalence of Bacterial Contamination of Stored Blood at Blood Bank S.S.G. Hospital, Vadodara. Int J Res Med. 2017; 5 (4): 77-80.e ISSN: 2320-2742.
35. Gibson, T., and W. Norris. Skin fragments removed by injection needles. Lancet [PubMed]. 1998; 2:983-985.
36. Tipple, M. A., L. A. Bland, J. J. Murphy, M. J. Arduino, A. L. Panlilio, J. J. Farmer III, M. A. Tourault, et al . Sepsis associated with transfusion of red cells contaminated with *Yersinia enterocolitica*. Transfusion. [PubMed]. 1990; 30:207-213.

37. In a conversation with a Tesfaye T, Laboratories coordinator of DHMD (December 9, 2017).
38. Whitley E, Ball J. Statistics review four: Sample size calculations. 2002; 6 (4): 335-341.
39. Tadesse A, Alem M. Medical Bacteriology. Gondar University: The Carter Center, USA; 2006.
40. Clinical and Laboratory Standards Institute guideline. Performance standard for antimicrobial susceptibility test. 27th ed.2017; 37 (1).
41. Yusuf M A, Abdul T H, Hamid T A. Isolation And Identification Of Bacteria Associated With Neonatal Septicemia At The Specialist Hospital, Bauchi. OSR Journal of Pharmacy and Biological Science.2012; 2(6):47-50. ISSN: 2278 -3008.
42. Monica C, District Laboratory Practice in Tropical Countries, Cambridge University Press, Cambridge, UK, 2nd edition, 2006.
43. World Health Organization. Blood transfusion safety. [Accessed date 07th Nov 2017]. Available from http://www.who.int/bloodsafety/haemovigilance/en/.
44. Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee. [Accessed date 24th Sep 2018]. https://www.transfusionguidelines.org/transfusion-handbook/3-providing-safe-blood/3-3-blood-products.
45. Binkhamis K, Forwarda K. Effect of the Initial Specimen Diversion Technique on Blood Culture Contamination Rates. Journal of Clinical Microbiology.2014; 52 (3):980-981.
46. Macopharma. Blood bags and accessories. [Accessed date 21th Nov 2017]. Available from https://www.macopharma.com/category/transfusion/blood-collection/collection-line/
47. Black Jacquelyn G. Principle and Explorations. Schreiber L, Brake D K, Corey P F, Bozik T,Dilernia B,Schiaparelli K,KinneL,Welchans J, editors. Microbiology.4th edn.1999:377-786.
48. Elbossaty W F. Antibiotic Drugs and Multidrug Resistant Bacteria. Int J Pub Health Safe.2017: 2 :(3):1-3.

49. Prestinaci F, Pezzotti F, Pantosti A. Antimicrobial resistant: a global multifaceted phenomenon. Pathogens and Global Health Journal.2015; 109 (7): 309-318.

11 Annexes

11.1 Annex I: Result interpretation for Disc diffusion method from CLSI 2017

Zone Diameter Interpretive Standards for *Enterobacteriaceae*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test = 12
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}C ,ambient air 16-18 hr

R.No	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
1	Meropenem	10 µg	≤ 19	20-22	≥ 23	
2	Amikacin	30 µg	≤ 14	15-16	≥ 17	Do not report for Salmonella and Shigella spp.
3	Gentamicin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
4	Tobramycin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
5	Ceftriaxone	30 µg	≤ 19	20-22	≥ 23	
6	Cefepime	30 µg	≤ 18	-	≥ 25	
7	Trimethoprim+Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
8	Ciprofloxacin (breakpoint for non-Salmonella)	5 µg	≤ 15	16-20	≥ 21	
9	Amoxicillin+clavulanic acid (PO only)	20/10 µg	≤ 13	14-17	≥ 18	
10	Piperacillin-tazobactam	100/10 µg	≤ 14	18-20	≥ 21	

Zone Diameter Interpretive Standards for *Pseudomonas aeruginosa*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test: 6
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}C ,ambient air 16-18 hr

R.No.	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
1	Amikacin	30 µg	≤ 14	15-16	≥ 17	
2	Gentamicin	10 µg	≤ 12	13-14	≥ 15	
3	Tobramycin	10 µg	≤ 12	13-14	≥ 15	
4	Meropenem	10 µg	≤ 15	16-18	≥ 19	
5	Cefepime	30 µg	≤ 14	15-17	≥ 18	
6	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
7	Piperacillin-tazobactam	100/10 µg	≤ 14	15-20	≥ 21	

Zone Diameter Interpretive Standards for *Staphylococcus spp*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test: 5
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 33-35°c (testing at above 37°c may not detect MRSA), ambient air.
Incubation time: 16-18 hr EXCEPT for Coagulase-negative staphylococci and cefoxitin: 24 hours.

R.No.	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
1	Penicillin G (only S. aureus)	10 unit	≤28	-	≥ 29 NB→	If ≥ 29 mm examine zone edge: report as R if sharp ("cliff"); report as S if fuzzy ("beach") Always R if cefoxitin R.
2	Cefoxitin (For S.aureus or S.lugdunensis)	30 µg	≤21		≥22	Do not report; report oxacillin and other beta-lactams based on cefoxitin result (see comments below)
3	Cefoxitin (For CoNS except for S.lugdunensis)	30 µg	≤24		≥25	Do not report; report oxacillin and other beta-lactams based on cefoxitin result (see comments below)
4	Erythromycin (PO only)	15 µg	≤13	14-22	≥23	DO NOT TEST IN URINE OR CSF ISOLATES
5	Clindamycin (PO only)	2 µg	≤14	15-20	≥ 21 NB→	DO NOT TEST IN URINE OR CSF ISOLATES Place erythromycin disc and clindamycin disc 12-20mm apart (edge to edge). Report clindamycin as R if "D-phenomenon" is seen (inducible clindamycin resistant) (EUCAST)
6	Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≤ 10	11-15	≥ 16	
7	Amikacin	30 µg	≤ 14	15-16	≥ 17	
8	Gentamicin	10 µg	≤ 12	13-14	≥ 15	DO NOT TEST IN CSF UNLESS REQUESTED
9	Tobramycin	10 µg	≤ 12	13-14	≥ 15	Tobramycin

Source: the tables are adopted from CLSI guideline 2017

11.2 Annex II: Data Collection format

ADDIS ABABA UNIVERSITY
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Type: Registration log for blood and blood component	Version: 01	Effective date: 01,Jan,17
Sub type: Pre-examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-001	

R.No.	Types of sample	Unit no.	Sample collection date	Remark

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Type: Blood and blood components collected with Diverging system data recording log	Version: 01	Effective date: 01,Jan,17
Sub type: Pre-examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-002	

Specimen ID	Types of blood and blood components	Unit no.	Date of collection	Date of expire date	Blood groups	Transfused date	Storage time	Storage T°C	Any leakage of blood bags		Remark
									Y	N	

Keys: Y= Yes N= No Storage time = date of transfused – date of collected

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Type: Blood and blood components collected with non-diverging system data recording log	Version: 01	Effective date: 01,Jan,17
Sub type: Pre-examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-003	

Specimen ID	Types of blood and blood components	Unit no.	Date of collection	Date of expire date	Blood groups	Transfused date	Storage time	Storage T°C	Any leakage of blood bags		Remark
									Y	N	

Keys:

Y= Yes **N= No** **Storage time = date of transfused – date of collected**

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Type: Inoculated BHI result recording	Version: 01	Effective date: 01,Jan,17
Sub type: examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-004	

Specimen ID	Types of sample	Unit no.	Volume sample	Date of Inoculation	Observe macroscopically presence of T.H,G and DC turbidity, hemolysis, gaseousness, or pellicle formation								Remark
					D 1	D2	D 3	D4	D5	D6	D 7		

Keys:

T= turbidity **H= hemolysis** **G = gaseousness**
DC = pellicle formation **D = Day**

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Type: Blood culture work sheet	Version: 01	Effective date: 01,Jan,17
Sub type: examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-005	

Specimen ID		Unit No.		Test done by		
Tested date		Specimen type		Result verified by		
Gram stain result ________________________						
Culture observation and work up						
Date	Tech	Observation and work up	Reviewed by		Remark	
			Initial	Date		
Final culture result:						

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Type: Biochemical Test	Version: 01	Effective date: 01,Jan,17
Sub type: examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-006	

Specimen ID		Unit No.		Test done by	
Tested date		Specimen type		Result verified by	

Gram stain result ________________________

Gram positive				
Test types	Result		Interpretation	Remark
	P	N		
Catalase test				
Coagulase test				
Slide method				
Tube method				
MRS				
Gram-negative				
Lactose				
Glucose				
Sucrose				
H_2S				
Gas				
Citrate				
Indole				
Oxidase				
Urease				
Motility				

Identified species :

Keys P= Positive N= Negative

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Type: Antimicrobial susceptibility test	Version: 01	Effective date: 01,Jan,17
Sub type: examination data	Revision Date: 01,Dec,18	
Section: Microbiology	Document Number: Micro -LOG-007	

Isolates	Gentamicin	Ciprofloxacin	Ceftriaxone	Clindamycin	Amikacin	Cefoxitin	Ampicillin	Meropenem	Cefepime	Piperacillin-tazobactam	trimethoprimsulphamethoxazole	Amoxicillin+ clavulanic acid	Penicillin	Erythromycin	Tobramycin

Keys: S= Sensitive I = Intermediate R = Resistant

11.3 Annex III: Gram-positive and Negative Biochemical Identification chart

11.3.1 Gram-positive bacteria

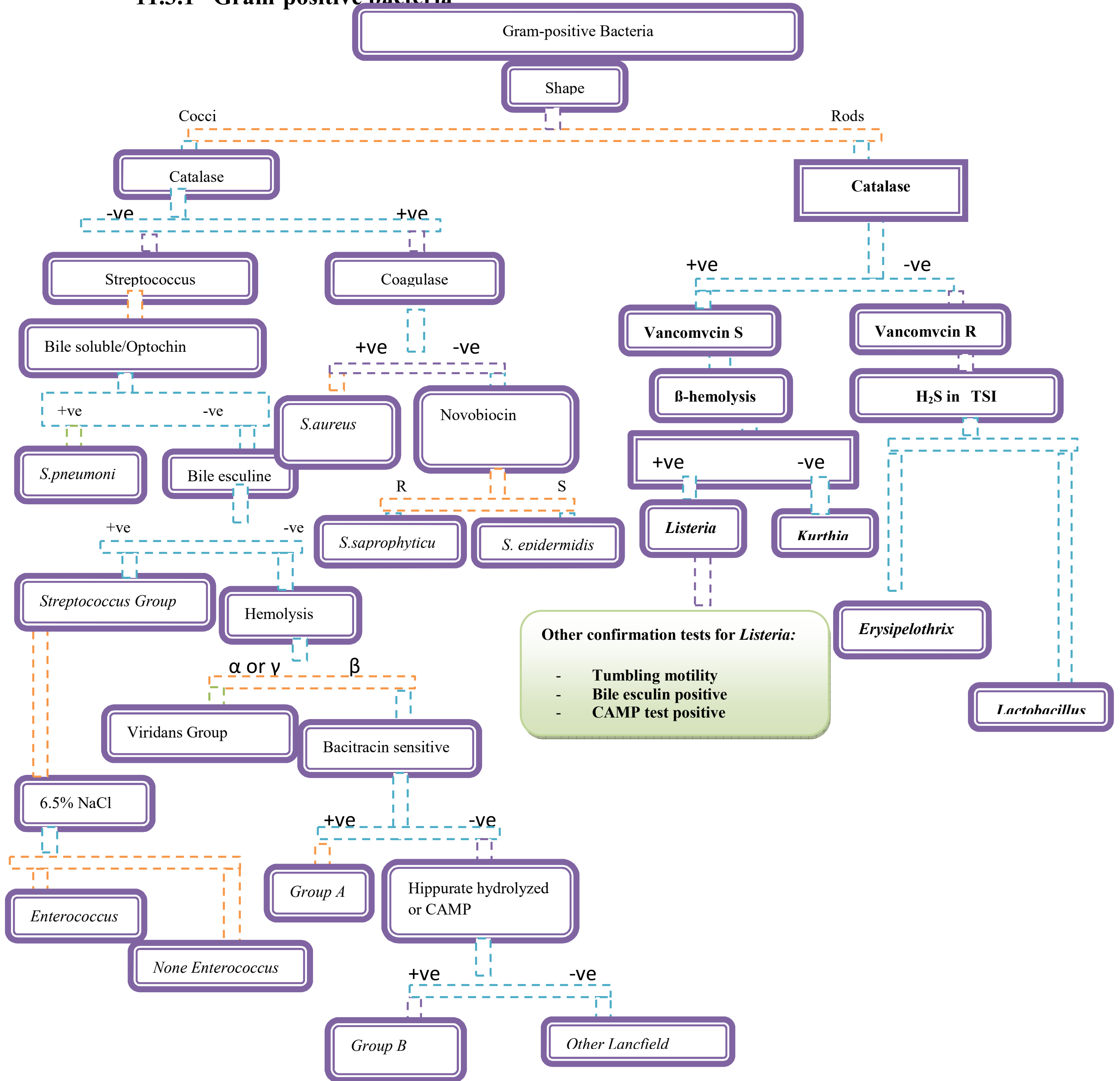


Figure 10.7: Gram-positive bacteria identification chart

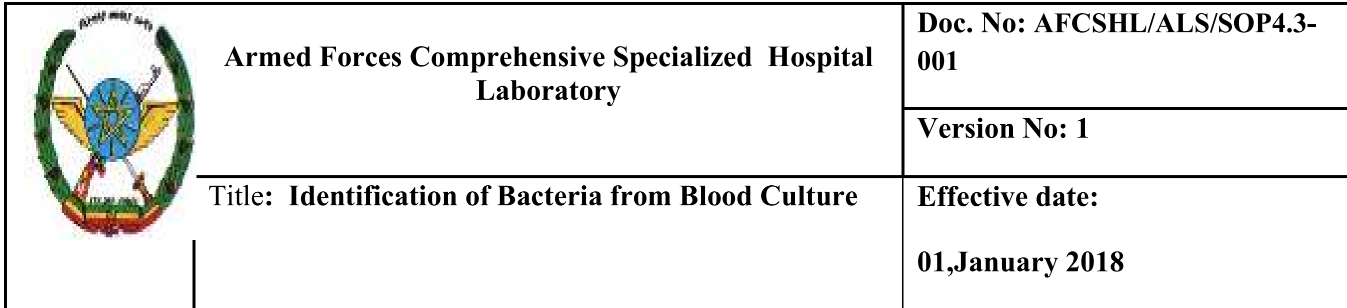
11.3.2 Gram-negative rods bacteria biochemicals identification

R/No	Organisms	Lactose	Oxidase	Catalase	MIU			Triple sugar Iron				Simon citrate	Special character
					Motility	Indole	Urease	Butt	Slant	Gas	H_2S		
1	*E. coli*	+	-	+	+	+	-	Y	Y	+	-	-	
2	*Klebshella*	+	-	+	-	+	+	Y	Y	+	-	+	Mucoid at culture media
3	*Enterobacter Spp*	+	-	+	+	-	-	Y	Y	+	-	+	
4	*Citrobcter*	+	-	+	+	-	d	Y	Y/R	+	d	+	
5	*Salmonella Typhi*	-	-	+	+	-	-	Y	R	-	+	-	
6	*Salmonella Paratyphi A*	-	-	+	+	-	-	Y	R	+	-	-	
7	*Salmonella typhimurium& others*	-	-	+	+	-	-	Y	R	d	+	d	
8	*Shigella Spp*	-	-	+	-	d	-	Y	R	-	d	-	
9	*Proteus*	-	-	+	+	v	+	Y	R	+	+	v	Swarming in blood agar
10	*Pseudomonas Spp*	-	+	+	+	-	d	R	R	-	-	+	
11	*Vibrio cholera*	-	+	+	+	+	-	Y	Y	-	-	d	
12	*Paraheamolyticus*	-	+	+	+	+	-	Y	Y	-	-	d	
13	*Serratia mercescus*	-	-	+	d	-	d	Y	R	-	-	+	
14	*Yersinia enterocolitica*	-	-	+	+	d	+	Y	R	-	-	-	Biopsy staining(Gram)
15	*Providencia*	-	-	+	+	+	-	Y	R	-	-	+	Fruity smell an orange enter colony in DCA

Key: Y= Yellow (Acidic reaction) **R= Red pink (Alkaline reaction)** **d= Different strain give different results**

Source:
https://www.google.com/search?q=biochemicals+identification+table+for+Gramnegative+rods+bacteria&tbm=isch&tbo=u&source=univ&sa=X&ved=2ahUKEwjvyNGk_djeAhWDLFAKHV8ZC3YQsAR6BAgFEAE&biw=1708&bih=744#imgrc=pQrPW2jhq4eoNM.

11.4 Annex IV: Customized SOP of AFCSH Microbiology laboratory



Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-001
	Version No: 1
Title: **Identification of Bacteria from Blood Culture**	**Effective date:** 01,January 2018

Principle	Blood specimens are submitted for culture from blood sample source. After Overnight incubation at 35 – 37 ^{0}c the broth media are blindly subculture and continue up to 7 days incubation to determine for bacterial growth.

Quality Control

Control	Stability	Frequency	Preparation (y/n)
E.coli ATCC 25922 strain	Blood culture broth is stable for 6 month	Every batch of media prepared	-Inoculate the control QC organisms in 0.85% saline to get a cell density comparable to 0.5 McFarland standard -Dilute 1:100 in normal saline -Using a syringe take 0.5ml saline and inoculate in the broth - Incubate at 37°C for overnight - Subculture to BAP and incubate at 37°C under 5% CO_2

Procedure

Step	Action
1.	Disinfect the venepuncture site using 70% ethanol for 1 minutes & 2% tincture of iodine for 3 minutes.
2.	Using a sterile syringe and needle, withdraw about 20 ml of blood from an adult* or about 2 ml from a young child.
3	Insert the needle through the rubber liner of the bottle cap and dispense 10–12 ml of blood into the culture broth medium bottle containing 25 ml of broth.

4	Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth and mix the blood in the EDTA container.
5	Clearly label each bottle with the name and number of the patient, and the date and time of collection.
6	Incubate inoculated Blood agar and Chocolate agar with 5-10 % CO_2 and MaCconkey agar aerobically at 35–37°C for up to 7 days.
7	Perform gram stain and Blind subculture after overnight incubation.
8	Sub-culturing a blood culture broth if any sign of growth like turbidity, gas formation, clot, pellicle formation and hemolysis.

Result Interpretation Sign of growth like Turbidity, hemolysis, clotting and pellicle formation may be seen.

Reference N/A

Principle

The dissociated crystals violate positive and Chloride negative ions come from the aqueous solution of crystal violate able to enter into the cell wall and membrane of Gram-positive and Gram-negative cells. The negative charged bacterial cell components interchange with the crystal violate positive ion which blots the cells purple. Iodine makes a complex with crystal violate positive ion which is not lost its color by the decolorizing agent in case of gram-positive bacteria. When the decolorizing agent is added, the cell wall of gram-negative leaks and allow the large complex of crystal violate -Iodine to be washed from the cell and retained the counter stain color.

Quality Control

Control	Frequency	Preparation (y/n)
Gram positive*: S.aureus* Gram negative*: E. coli*	Each day of use.	N

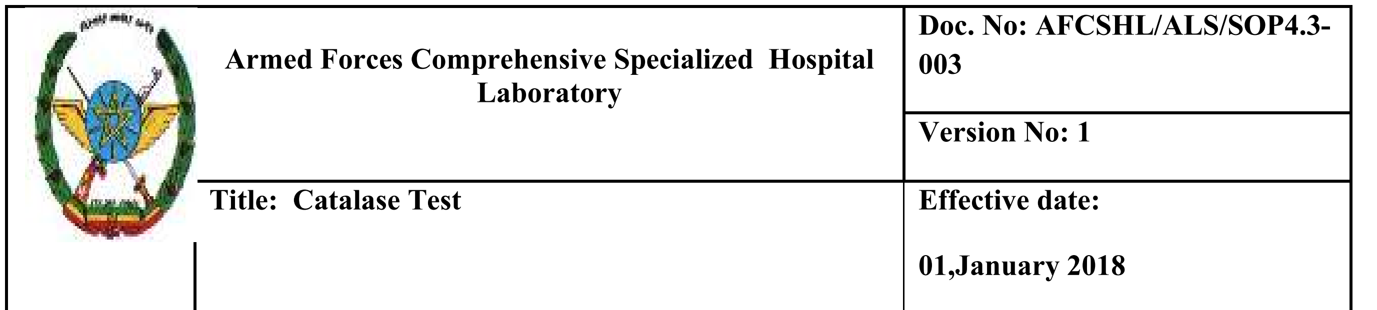
Procedure

Step	Action
1.	Label a glass microscope slide with the laboratory accession number.
2.	Draw one ml blood sample from BHI and dispense one drop on slide and then spread it.
3	Airs dry the slide.
4	Heat fixation

	Pass air-dried smears through a flame two or three times. Do not overheat.
5	Flood the prepared slide with crystal violet for one minute.
6	Rinse the slide gently with tap water.
7	Flood the slide with Gram's iodine for one minute.
8	Rinse the slide gently with tap water.
8	Working with one slide at a time, flood the slide with decolorize for 15 seconds and rinse with tap water.
9	Flood the slide with safranin for one minute.
10	Rinse the slide gently with tap water.
11	Drain the slide in an upright position. Blot the back of the slide and place on a slide warmer or heating block to completely dry.
12	Scan 20-40 fields using oil immersion.

Result Interpretat ion	Gram-positive bacteria and yeast will stain dark purple. Gram-negative bacteria will stain pink to red.

Reference	Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-003
		Version No: 1
	Title: Catalase Test	Effective date: 01,January 2018

Principle Catalase is an enzyme which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

Quality Control

Control	Frequency	Preparation (y/n)
Positive: *S.aureus* ATCC Negative: *Streptococci Pyogene*	3% H_2O_2 preparation	N

Note: If the results are out of expected value, repeat the test and take corrective action.

Procedure

Step	Action
1	Pour 2 – 3 ml of the hydrogen peroxide solution in to a test tube.
2	Using a sterile wooden stick or a glass rod, remove several colonies of the test organism & immerse in the hydrogen peroxide solution.
3	Look for immediate active bubbling.

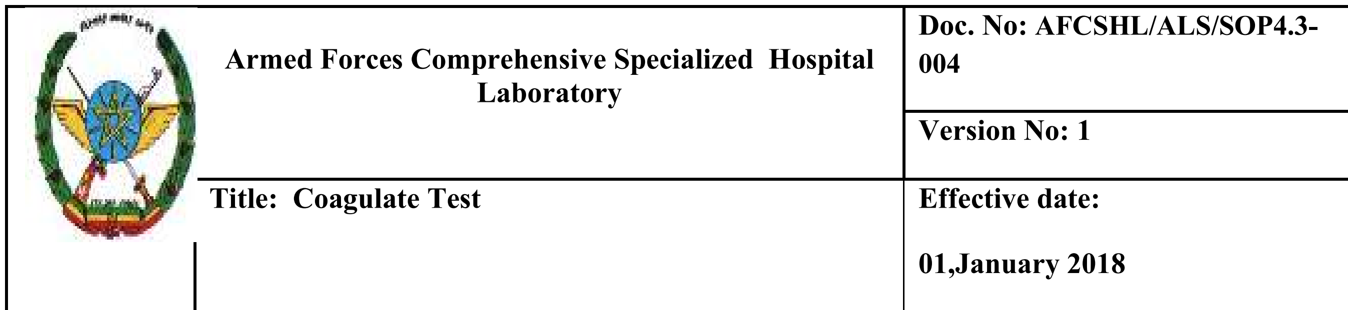
Result Interpretat Active bubbling ………………….. Positive Catalase test

ion

No bubbling ……………………… Negative Catalase test

Reference

1. Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties
2. Baron's medical microbiology, 4th eds. 2000



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-004
		Version No: 1
	Title: Coagulate Test	Effective date: 01,January 2018

Principle

Coagulase is an enzyme which causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of S.aureus (free coagulase & bound coagulase).

A tube test must always be performed when the result of a slide test is not clear, or when the slide test is negative.

Quality Control

Control	Frequency	Preparation (y/n)
Positive: *S.aureus* (ATCC 25923) Negative: *S.epidermidis*	Every plasma preparation	N

Note: If the results are out of expected value, repeat the test and take corrective action

Procedure

1.Slide coagulase test method (detects bound coagulase)

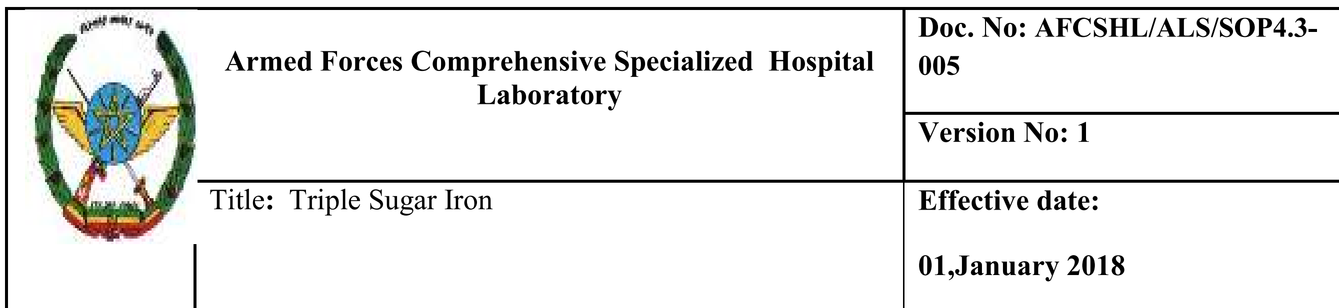
Step	Action
1	Place a drop of distilled water on a slide.
2	Emulsify the test strain to obtain homogeneous thick suspension.
3	Add a loopful of plasma, & mix gently.
4	Look for clumping of the organism within 10 seconds.

2.Tube coagulase test method (detects free coagulase)

Step	Action
1	Pipette 0.2 ml of plasma into a test tube.
2	Add 0.8 ml of the test broth culture to the test tube.

	3	After mixing gently, incubate the test tube at 35 – 37 ^{0}c.
	4	Examine for clotting after 4 hour.

Result Interpretation	Clumping within 10 seconds/ after 4 hour …………………… *S.aureus* No Clumping within 10 seconds/ after 4 hour ……………….. No coagulase
Reference	1. Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties 2. Baron's medical microbiology, 4th eds. 2000



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-005
		Version No: 1
	Title: Triple Sugar Iron	Effective date: 01,January 2018

Principle

Triple Sugar Iron (TSI) Agar is a combination of three sugars which are Lactose, Sucrose, and Glucose. The amount of the concentration levels of these sugars are 1% for lactose and sucrose and 0.1 % for glucose. When bacteria ferment the lactose/sucrose, enough amount of acid produced and both the slant and button became yellow which shows the fermentation of either lactose or sucrose. If only glucose is fermented, the slant becomes red due to the produced acid is converted to oxidized by the environmental oxygen acid and only the butt be yellow. The ferrous sulfate detects the presence of hydrogen sulphide. The pH is detected by phenol red; in acidic condition, it will be yellow and in alkaline one became red. It also has peptone which is used as the source of nitrogen when it is utilized under the aerobic condition the ammonia is produced. Sucrose is used up by coliforms more than lactose and also it is used to detect certain gram-negative bacteria that utilized sucrose but not lactose.

Quality Control

Control	Stability	Frequency
• ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline.
- Open the seal and aseptically add 1ml of broth or Saline.
- Inoculate on TSI media.

● incubate at 37ºC incubator.

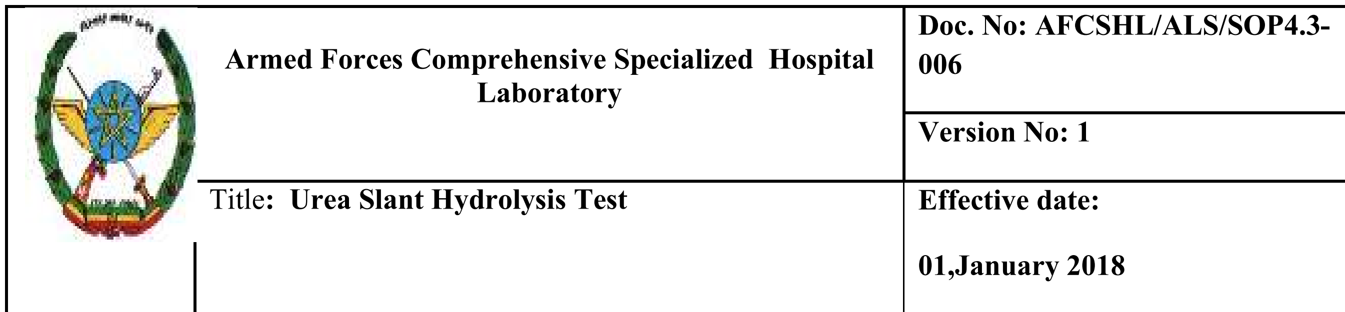
● After overnight incubation (18-24hr) observe the colony and perform Biochemical tests.

Salmonella enteritidis ATCC 13076 - Alkaline slant, acid butt (K/A); H_2S +; Gas+
E. coli ATCC 25922 – Acid butt, acid slant (A/A); no H_2S; Gas +
*P. aeruginosa A*TCC 27853 – Alkaline slant and butt (K/K); no H_2S; no gas
Note: If the colony is not pure re-culture from the stock

Procedure	**Step**	**Action**
	1	With a sterilized straight inoculation needle touch the top of a well-isolated colony.
	2	Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
	3	Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 to 24 hours.
	4	Using wire loop take a heavy inoculum of growth from an 18-24 hour pure culture.

Result Interpretation	
	Yellow butt and red slant becomes = only ferments glucose.
	Yellow slant and butt indicates = ferments dextrose, lactose and/or sucrose.
	Red slant and butt = non-fermentor for lactose, glucose, and sucrose.
	Black precipitate in the butt = H_2S production positive.
	Splitting and cracking of the medium = Gas production positive.

Reference Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties



Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-006
	Version No: 1
Title: **Urea Slant Hydrolysis Test**	Effective date: 01,January 2018

Principle

The urea medium of Rustigian and Stuart3 is particularly suited for the differentiation of *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea; The complete Urea Agar contains 15.0 g/L of agar in addition to the ingredients in the base medium. When organisms utilize urea, ammonia is formed during incubation which makes the reaction of these media alkaline, producing a pink-red color. Consequently, urease production may be detected by the change in the phenol red indicator.

Quality Control

Control	Stability	Frequency
● ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

● Reconstitute the lyophilized sample by TSY broth or Normal saline.

● Open the seal and aseptically add 1ml of broth or Saline.

● Inoculate on media (Blood agar & MacConkey agar).

● incubate at $37^{o}C$ incubator.

● After overnight incubation (18-24hr) observe the colony and perform Biochemical tests.

Proteus mirabilis ATCC 49565 pink red slant and butt.

Escherichia coli ATCC 25922 yellow slant.

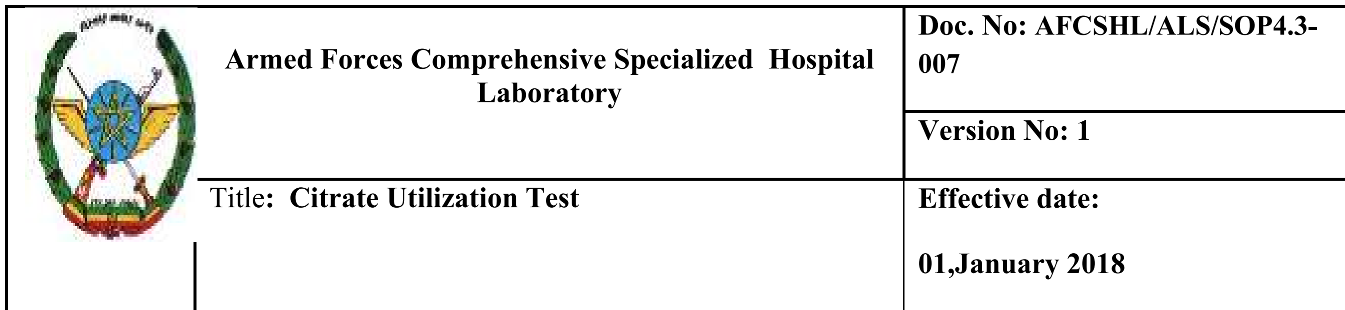
Note: If the colony is not pure re-culture from the stock.

Procedure	Step	Action
	1	Take nutrient Broth tube.
	2	Label the tube.
	3	Sterilize wire loop using the Bunsen burner.
	4	Using wire loop take a heavy inoculum of growth from an 18-24 hour pure culture.
	5	Suspend in Nutrient Broth.
	6	Vortex the suspension.
	7	Incubate at 37ºC incubator.
	8	When the suspension becomes turbid takes drop of suspension aseptically and add drops into urea slant.
	9	Incubate the inoculated media at 37ºC incubator for overnight.
	10	Observe change of color on the media.

Result Interpretation	The production of urease is indicated by an intense pink-red color on the slant. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis. A negative reaction is no color change. The agar medium remains pale yellow.

Reference

Acharya T. Microbe online [Internet]. TSI Test: Principle, procedure and interpretation. [Updated 2013; cited on 07th Nov 2017]. Available from Source: http://microbeonline.com/triple-sugar-iron-agar-tsi-principle-procedure-and-interpretation/.



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-007
		Version No: 1
	Title: **Citrate Utilization Test**	**Effective date:** 01,January 2018

Principle

Ammonium Dihydrogen Phosphate is the sole source of nitrogen in Simmons Citrate Agar. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the sole source of carbon in this medium. Magnesium Sulfate is a cofactor for a variety of metabolic reactions. Bromthymol Blue is the pH indicator. Organisms that can utilize Ammonium Dihydrogen Phosphate and Sodium Citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline).

Quality Control

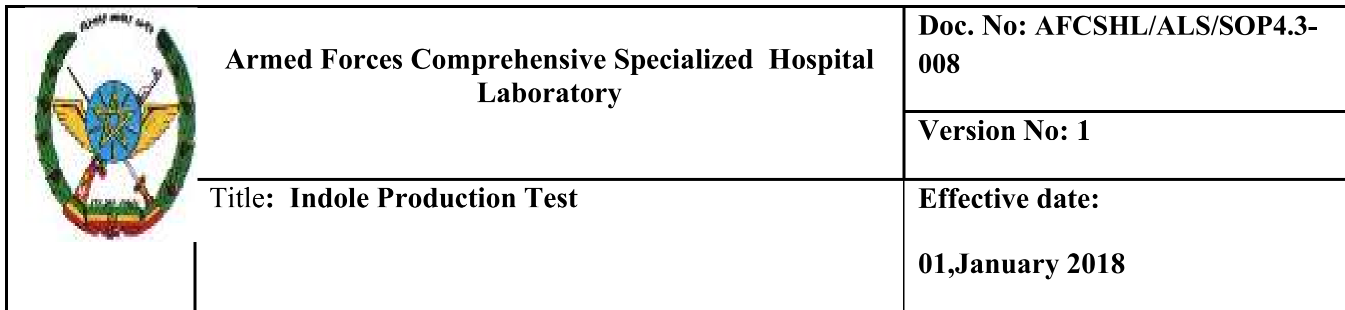
Control	Stability	Frequency
• *Klebssiella pneumonia* ATCC strains	6 weeks at room temperature	Weekly subculture

Procedure

Step	Action
1	Prepare slopes of the medium in test tubes as recommended by the manufacturer (store at 2–8^{0}C).
2	Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
3	Incubate at 35°C for 48 hours. Look for a bright blue colour in the medium.

Result Interpretation	A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by inhibition to poor growth without change in color (medium remains green)

Reference	Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-008
		Version No: 1
	Title: **Indole Production Test**	Effective date: 01,January 2018

Principle

The indole test determines the ability of an organism to produce indole from the degradation of the amino acid tryptophan. Tryptophan is hydrolyzed by tryptophanase to produce three possible end products – one of which is indole. A colored product is produced when the indole is combined with certain aldehydes (kovacs Reagent)

Quality Control

Control	Stability	Frequency
● ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

● Reconstitute the lyophilized sample by TSY broth or Normal saline

● Open the seal and aseptically add 1ml of broth or Saline

● Inoculate on medias (BAP and MAP)

● incubate at 37ºC incubator

● After overnight incubation(18-24hr) observe the colony and perform Biochemical tests

Escherichia coli ATCC 25922 -dark pink color develops

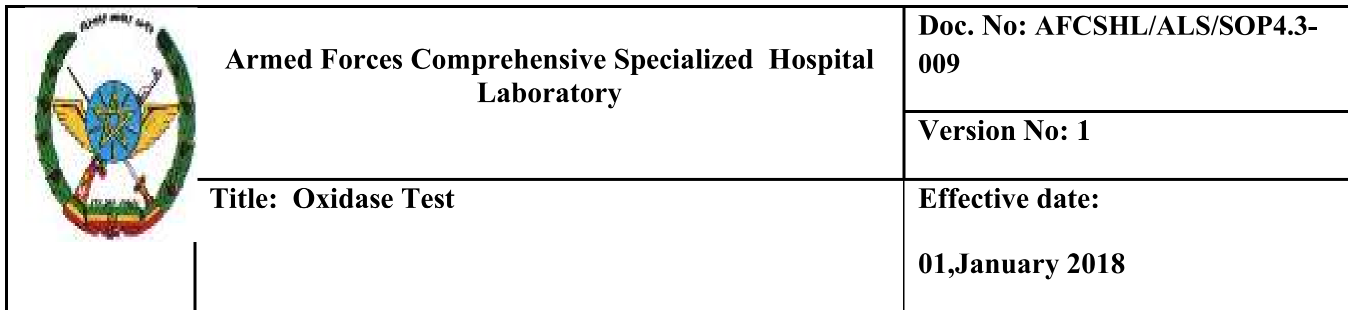
Enterobacter aerogenes ATCC13048 -fair to good growth blue

Note:

If the colony is not pure re-culture from the stock

Result Interpretation	Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethylaminobenzaldehyde) is added to a broth with indole in it, a dark pink color develops.

Reference	Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties



Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase – producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

Quality Control

Control	Frequency	Preparation (y/n)
Pseudomonas aeruginosa ATCC strains	Every oxidase reagent preparation	N

Note:

If the results are out of expected value, repeat the test and take corrective action.

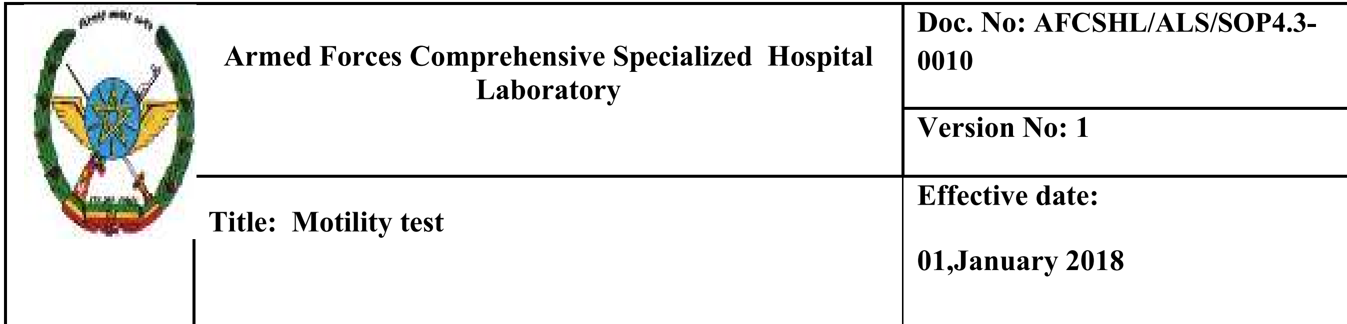
Procedure

1.Filter paper method

Step	Action
1	Soak a piece of filter paper in the oxidase reagent solution
2	Scrap some fresh growth from the plate with a disposable loop or stick & rub on to the filter
3	Examine for blue color within 10 seconds

Result Interpretation	Deep purple colour . . . . Positive oxidase test (within 10 seconds) Colorless colour . . . . . Negative oxidase test (within 10 seconds)

Reference	1. Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties 2. Baron's medical microbiology, 4th eds. 2000



Principle

Bacterial motility can be observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of non motile organisms only occurs along the stab line.

Quality Control

Control	Stability	Frequency
● ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

● Reconstitute the lyophilized sample by TSY broth or Normal saline

● Open the seal and aseptically add 1ml of broth or Saline

● Inoculate on medias (Blood agar & MacConkey agar)

● incubate at 37ºC incubator

● After overnight incubation(18-24hr) observe the colony and perform **Biochemical tests**

Escherichia coli control strain ATCC 25922 is Motile

Shigella is Non motile

Note:

If the colony is not pure re-culture from the stock

Procedure

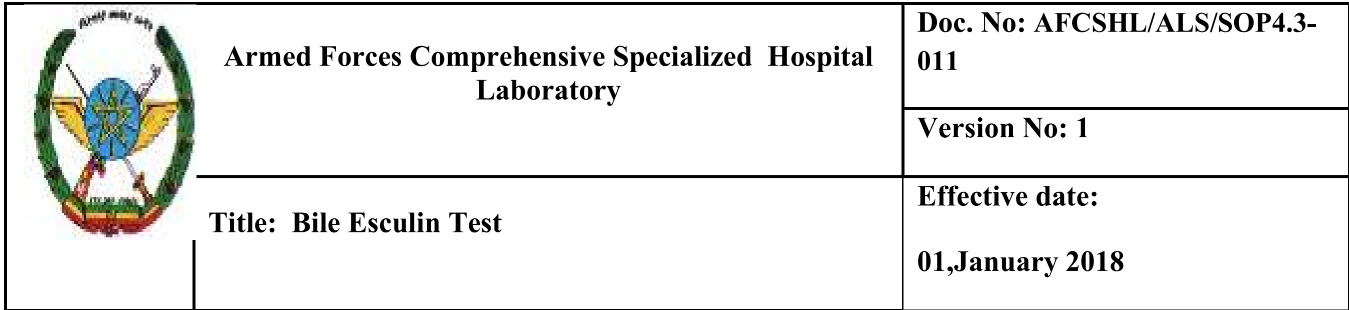
Step	Action
1	Take nutrient Broth tube
2	Label the tube
3	Take pure colony on MacConkey Agar Plate near Bunsen burner
4	Suspend in Nutrient Broth
5	Vortex the suspension
6	Incubate at 37ºC incubator
7	When the suspension become turbid take drop of suspension aseptically and stab the medium not drop the broth
8	Incubate the inoculated media at 37ºC incubator for overnight
9	Observe change of color (diffusion of bacteria) on the media

Result Interpretation

Motility is observed visually by diffuse growth spreading from the line of inoculation. Certain strains of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Reference

Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties



Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-011
	Version No: 1
Title: Bile Esculin Test	Effective date: 01,January 2018

Principle

Bile suppresses most gram-positive bacteria. Esculin in the culture media is hydrolyzed to esculetin and dextrose. The esculetin reacts with ferric chloride in the media to form a black brown color.

Quality Control

Control	Stability	Frequency
● ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline
- Open the seal and aseptically add 1ml of broth or Saline
- Inoculate on bile esculin media
- incubate at 37ºC incubator
- After overnight incubation(18-24hr) observe the media

E. faecalis ATCC 29212 – Growth, blackens agar
E. coli ATCC 25922 – No growth, no blackening of the media

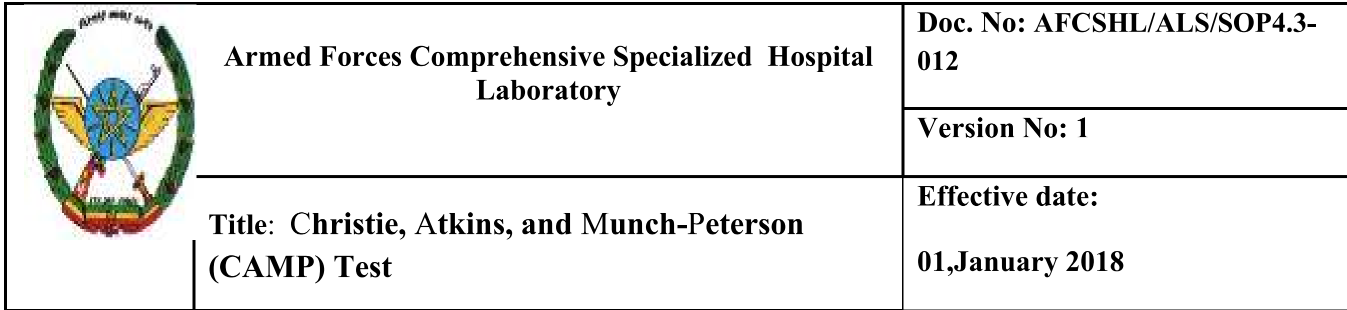
Note:

If the colony is not pure re-culture from the stock

Procedure	Step	Action
	1	Using sterile loop, pick one or two colonies from an 18-24 hours culture.
	2	Inoculate onto the surface of slant of bile esculin medium contained in tube with an S-shaped motion
	3	Incubate the inoculated tube at 35-37°C for 24 hours.
	4	Observe the result.

Result Interpretation	**Positive:** Blackening of more than half of the agar slant, **Negative:** No blackening of medium.

Reference	Pokhrel P.Microbiology notes [Internet]. Bile Esculin Test-Principle, Procedure, Result Interpretation and Limitation [Updated 2015; cited on 07th May 218]. Available from Source http://www.microbiologynotes.com/bile-esculin-test-principle-procedure-result-interpretation-and-limitation/



Principle

S. agalactiae or *Listeria monocytogenes* produce CAMP factor that synergistically acts with the beta lysin of *Staphylococci aureus* and enhances the lysis of red blood cells.

Quality Control

Control	Stability	Frequency
● ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline
- Open the seal and aseptically add 1ml of broth or Saline
- Inoculate on 5-10% sheep blood media
- incubate at 37ºC incubator
- After overnight incubation(18-24hr) observe the media

S. agalactiae ATCC 12386 – Positive (Presence of arrowhead hemolysis)

S. pyogenes ATCC 19615 – Negative (Absence of arrowhead hemolysis)

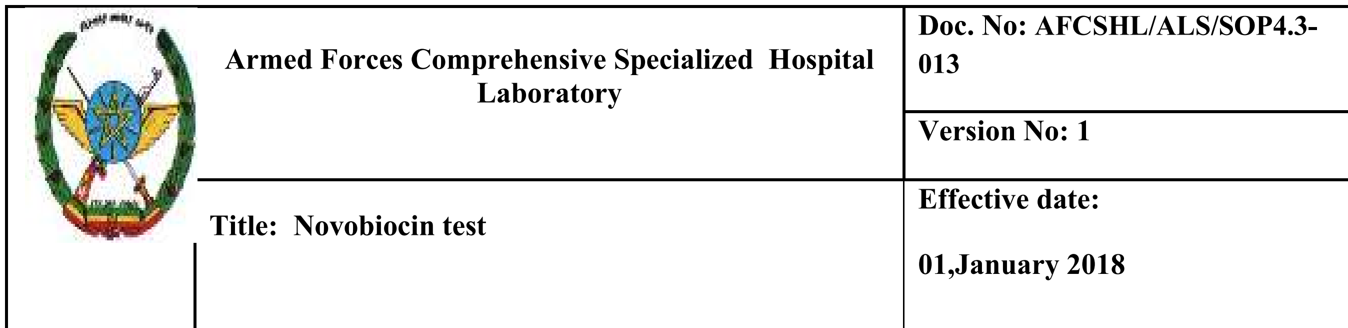
Note:

If the colony is not pure re-culture from the stock

Procedure	Step	Action
	1	Using an inoculating loop, streak a beta-lysin-producing Staphylococcus aureus (ATCC25923) in a straight line across the center of a sheep blood agar plate.
	2	Streak test organism in a straight line perpendicular to the *S. aureus* leaving 1cm space between the two streaks. (Multiple organisms can be tested on a single plate if they are 3 to 4mm apart).
	3	Incubate the plate at 37 degree Celsius in ambient air for 18-24 hours.

Result Interpretation	**Positive:** Enhanced hemolysis is indicated by an arrow head-shaped zone of beta- hemolysis at the junction of the two organisms. **Negative:** No enhancement of hemolysis.

Reference	Pokhrel P.Microbiology notes [Internet]. CAMP Test- Principle, Purpose, Procedure, Result and Limitation [Updated 2015; cited on 07th May 2018]. Available from Source http://www.microbiologynotes.com/camp-test-principle-purpose-procedure-result-and-limitation/



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-013
		Version No: 1
	Title: Novobiocin test	Effective date: 01,January 2018

Principle

Novobiocin test is antibiotic disc used for the differentiation of coagulase negative staphylococci species. The discs will be sensitive for *Staphylococcus epidermidis* and also resistant for *Staphylococcus saprophyticus.*

Quality Control

Control	Stability	Frequency
• ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline
- Open the seal and aseptically add 1ml of broth or Saline
- Inoculate the organisms each half on 5-10% sheep blood media
- Put the 5 µg novobicine disc on the inoculated media
- incubate at 37ºC incubator
- ●After overnight incubation(18-24hr) observe the media

S. saprophyticus ATCC 15305 – Zone of inhibition ≤15mm.

S. aureus ATCC 25923 – Zone of inhibition ≥ 22mm.

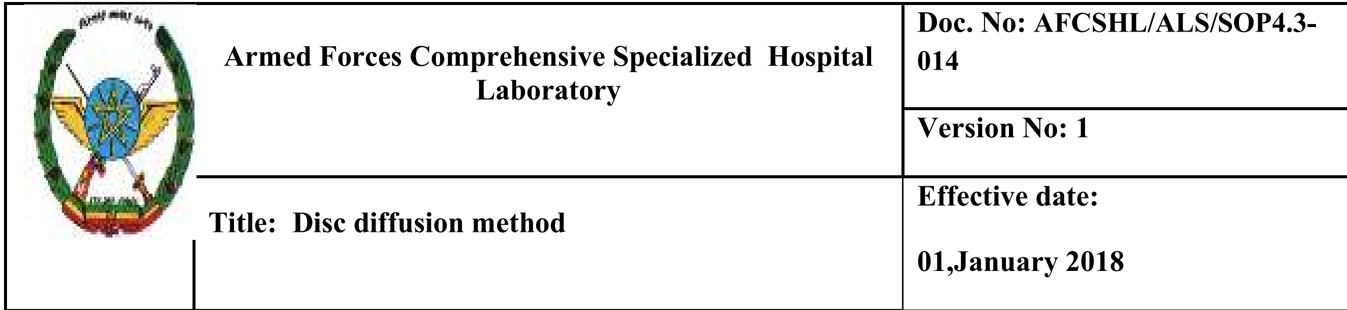
Note:

If the colony is not pure re-culture from the stock

Procedure	Step	Action
	1	Allow containers to come to room temperature before use.
	2	Using a pure 18-24 hour culture, prepare a suspension of the organism; equivalent to a McFarland 0.5 opacity standard; to be identified in Tryptic Soy Broth , Sterile Water, or Brain Heart Infusion (BHI) broth
	3	Inoculate Mueller Hinton Agar, 5% Blood Agar, or Tryptic Soy agar plate with a sterile swab to obtain confluent growth.
	4	Aseptically apply one 5μg novobiocin disk onto the inoculated agar surface and lightly press down to ensure full contact with the medium.
	5	Incubate plate aerobically for 18 to 24 hours at 35 to 37°C.
	6	Measure (in millimeters) the diameter of the zone of inhibition around the novobiocin disk, and record as susceptible or resistant.

Result Interpretation	*Staphylococcus saprophyticus* : growth < 12mm or uniform growth up to the edge of the disk *Staphylococcus epidermidis*: Zone of inhibition >16 mm or larger

Reference	Tankeshwar.Microbiology notes [Internet]. Novobiocin Susceptibility Test: Principle, procedure and interpretations. [Updated 2015; cited on 07th May 2018]. Available from Source https://microbeonline.com/novobiocin-susceptibility-test-principle-procedure-and-interpretations/



	Armed Forces Comprehensive Specialized Hospital Laboratory	Doc. No: AFCSHL/ALS/SOP4.3-014
		Version No: 1
	Title: Disc diffusion method	Effective date: 01,January 2018

Principle

Paper antimicrobial discs with a known volume and concentration of antibiotics are placed on sensitivity testing media consistently inoculated with the isolates. The antimicrobial diffuses from the disc into the medium and the growth of the organisms is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

Quality Control

Control	Stability	Frequency
• ATCC strains	6 weeks at room temperature	Weekly subculture

Control preparation:

- Reconstitute the lyophilized sample by TSY broth or Normal saline
- Open the seal and aseptically add 1ml of broth or Saline
- Inoculate the organisms each half on muller hinton media
- Put the antibiotic discs on the inoculated media
- incubate at 37ºC incubator
- After overnight incubation(18-24hr) observe the media

Note:

If the colony is not pure re-culture from the stock

Procedure	Step	Action
	1	Prepare pure colony suspension in to normal saline equivalent to 0.5 Mcfarland standards.
	2	Streak on appropriate media the entire surface.
	3	Select antimicrobial agents according to the CLSI guideline & Put the disc on the plate aseptically
	4	Incubate for 16 – 24 hrs at 35 +/- 2°C
	5	Measure zone of inhibition and interpret the result based on CLSI break point.

Result Interpretation	The interpretation of each selected antimicrobial agents will be interpreted based on CLSI guideline.

Reference

1. Monica cheesbrough, low – price eds. Part 2.District Laboratory Practice in Tropical Counties
2. Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement V. 28 No.1, CLSI

11.5 Annex V: Checklist for Calibration of Biological safety Cabinet

CLEAN ROOM MAINTENANCE

CK No.: 1987/028085/23 Vat. Reg. No.: 4150184333

CRM

18736

PO BOX 4193
HAMBERG
1726

WORK SHEET
16 Bertandina Street
Roodepoort
1724

Tel: +27 11-763-8820/8405
Fax: +27 11-763-2760
E-mail: crm@corpdial.co.za
Website: www.cleanroom.co.za

DATE: 26-06-2017

CUSTOMER:

DEPT: BACTERIOLOGY

CUST.ORDER No:

TYPE: Class 2 BSC

SERIAL No: 77782031100303

PRE-FILTER:

HEPA FILTER:

TEST EQUIPMENT:

CALIBRATION DATE:

TEST RESULT:

THE BSC IS SAFE TO USE.

SPARES	QTY	PRICE	CUSTOMER REF:
			Contact person:
			Telephone No:
			Fax No:
			PO Box:
			City:
			Code:
			Travel (km):

NEXT SERVICE DUE: June 2018

TECHNICIAN:

ACCEPTED BY:

SIGNATURE:

SIGNATURE:

11.6 Annex VI: English version of an information sheet for the study area

Principal Investigator: Wondwossen Tsegaye, Addis Ababa University, College of Health Sciences, School of Allied Health Science, Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

Title of the research project: Prevalence of Bacterial Contamination and Antimicrobial Susceptibility Profile among Transfusion Ready Blood and Blood Components at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

First of all, we would like to thank you in advance for your cooperation for the permission that the study to be conducted in your health facility. Please read the general information about the study. If you have any question regarding the study please ask freely.

Background information

Background: Transfusion of bacterial contaminated blood and blood components could be a cause of morbidity and mortality. Understanding the mechanism of blood contamination is important in developing infection control strategy.

Purpose

The purpose of this study was to determine the Prevalence of Bacterial Contamination and Antimicrobial susceptibility Profile among Transfusion Ready Blood and Blood Components at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia.

Benefits from the study

One laboratory technologists who did culture and sensitivity was paid 4,000.00 ETB as per the agreement made with the principal investigator. In addition, two cleaners also paid 1,200.00 ETB. However; based on the results, the study was benefited to select the donor blood collection methods that minimize the bacterial contamination from the non-diverging and diverging method. Hence you were indirect benefits other patients in this respect.

Risks associated with the study

There was no anticipated risk for those blood and blood components. Samples were collected from the blood bag tubing from where 5-10 cm away from the junction between the top end of blood bags and tubing then the tubing was sealed with sealer machine and detached 20-25 cm blood segment for whole blood and PRBC. For FFP and platelet the samples were collected in the Biosafety cabinet. These help to prevent the blood and blood components from being contaminated.

Confidentiality

Any information that was obtained in connection with this study remained confidential. The information collected about blood and blood components were coded using numbers. Information will be only disclosed for the study area, blood bank service and publication purpose.

11.7 Annex VII: Amharic version of an information sheet for the study area

<u>ጥናቱን የሚያጠናው</u>: -

ወንድወሰን ፀጋዬ፣ ከአዲስአበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት።

<u>አርዕስ</u>: -

የባክቴሪያዎች የመበከል ስርጭትና የፀረ ባክቴሪያዎች በባክቴሪያዎቸ ላይ ያላቸው የማጥፋት ምላሽ ለታካሚዎች ለመስጠት በሚቀርቡ ደምና የደም ተዋፅዎች በጦር ሐይሎች ኮምፕርሄንሲቭ እስፔሻላይዝድ ሆስፒታል አዲስ አበባ፣ ኢትዮጵያ ።

ጥናቱ በእናንተ ተቋም እንዲካሄድ ላሳያችሁት ትብብርና ለሰጣችሁት ፈቃድ በቅድሚያ ምስጋናችን የላቀ ነው። እባከሆ ስለ ጥናቱ አጠቃላይ መረጃ ከታች ስለቀረበልሆ በጥሞና ያንብቡና ማንኛውም ጥያቄ ካለዎት በነፃነት ይጠይቁ።

<u>አጠቃላይ መረጃ</u>: -

በባክቴሪያ የተበከሉ ደምና የደም ተዋፅዎቸ ለታካሚ በደም አማካኝንት ማስትላልፍ ለበሽታ እና ሞት መንስኤ ሊሆን ይችላል። መተላለፊያ መንገዶችን መረዳት በዚህ የሚመጣውን ህመም ለመቆጣጠር በሚደርገው እቅድ ለማዘጋጀት አስፈላጊ ይሆናል።

<u>የጥናቱ ዓላማ</u>: -

የዚህ ጥናት ዓላማ የባክቴሪያዎች የመበካል ስርጭት እና የፀረ ባክቴሪያዎች በባክቴርያዎቸ ላይ ያላቸው የማጥፋት ምላሽ ለታካሚዎች ለመስጠት በሚቀርቡ ደምና የደም ተዋፅዎች በሁለት የለጋሾች ደም በተቀዱ ዘዴዎች ማነፃፀር ነው።

<u>የጥናቱ ጥቅም</u>: -

አንድ ላቦራቶሪ ቴክኖሎጂስቶች ካልቸር እና ሴንሲቲቪቲ ለሚሰሩ ከጥናቱ አድራጊ ጋር በተደረገው ስምምነት 4,000 ብር ክፍያ እንዲሁም ለሁለት ፅዳት ሰራተኞች 1,200 ብር

ተከፍሏል። ከጥናቱ በሚገኝ ውጤት ከሁለቱ የለጋሽ ደም አቀዳድ ዘዴዎች የትኛው ዘዴ የባክቴሪያ የመበከል በተሻለ ይቀንሳል የሚለዉን ለመምረጥ ይጠቅማል።በተጨማሪም እናንተ ይህንን ጥናት በተቋማችሁ እንዲሰራ በመፍቀዳችሁ በተዘዋዋሪ መንገድ ደም እና የደም ተዋፅዎ ለሚወሰዱ ታካሚዎች እየጠቀሙ መሆኑን እንገልፃለን።

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳት እና ተያያዝ ችግሮች፡-

በዚህ ጥናት ላይ የሚካተቱ ደምና የደም ተዋፅዎ ምንም አይነት ጉዳት አልደረሰባቸውም፡ ፡ ናሙና በተካተቱ የደም ከረጢቶች ከላይ ከ5-10 ሳ。 ሜ ከከረጢቶቹ አናት ከፍ ተብሎ የደም ከረጢቶች እንዳይበከሉ በማጣበቂያ ማሽን መልሰን አጣብቀናቸዋል: : ከጉዳትና ብክለት ለመከላከል ባዮሴፍቲ ካቢኔት ውስጥ ናሙናውን ወስደናል።

<u>የመረጃ ሚስጥራዊ አጠባበቅ</u>፡-

ከዚህ ጥናት የሚገኙ ማንኛውም መረጃ ሚስጥራዊነቱ የተጠበቀ ይሆናል። ደምና የደም ከረጢቶች የራሳቸው ኮድ ቁጥር ተሰቷል። ከጥናቱ የሚገኙ መረጃዎች ጥናቱ ለተደረገበት ተቋም፥ ለደም ባንክ አገልግሎት ተቋሞች እንዲሁም ጥናቱ ለማሳተም ብቻ ይፋ ይሆናል።

12 Declaration

The undersigned declared that this thesis complied with the regulations of the University and met the accepted standards with respect to originality and quality. Principal investigator also agreed to accept responsibility for the scientific ethical and technical conduct of the research project and for the provision of required progress reports.

M.Sc. candidate: Wondwossen Tsegaye (BSc)

Signature: ___________________

Date of submission: ___________________

This thesis submitted with our approval as advisors.

Advisor:

Adane Bitew (MSc, PhD)

Signature: _____________________

Date:__________________________