



Original article

Antibacterial resistance pattern of transfusion transmissible bacteria from blood donors attending Federal Medical Centre, Birnin Kebbi

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ARTICLE INFO

Article history:

Received 7 December 2022

Received in revised form 13 January 2023

Accepted 14 January 2023

Keywords:

Antibacterial resistance
Transfusion-transmissible bacteria
Blood donors
Federal Medical Centre
Birnin Kebbi

ABSTRACT

Background: Patient safety is seriously threatened by bacterial infections that spread through blood transfusions (TTBI). It has been noticed that a number of infectious viral, bacterial, and parasitic pathogens are involved as barriers to the patient's blood safety. This study aimed to determine the antibiotic resistance pattern of bacteria in the donors' blood attending Federal Medical Centre, Birnin Kebbi, Kebbi State, Nigeria. **Methods:** The study adopted a cross-sectional research design. A structured questionnaire was administered to 120 blood donors to obtain bio-demographic information and their blood was collected accordingly. The blood samples were processed using standard bacteriological analysis. The isolated bacteria was confirmed using standard biochemical tests. The identified bacteria were subjected to antibiotic testing using the Kirby-Bauer disc diffusion method. **Results:** Twenty-four 24(20.0%) blood samples were found contaminated with bacteria. The main bacteria isolates were Gram-negative organisms namely- *Klebsiella oxytoca* (20.8%), *Citrobacter diversus* (16.7%), *Enterobacter aerogene* (16.7%), *Shigella sonnei* (12.5%), *Shigella dysenteriae* (4.2%), *Morganella morganii* (4.2%), *Escherichia coli* (4.2%) with only *Staphylococcus aureus* (20.8%) as Gram-positive bacteria. The prevalence rate of TTBI was high within the age group 36-49 years 12(50.0%). *Morganella morganii* was (100%) resistance to all the antibiotics used in this study. **Conclusions:** the study revealed that, donors' blood was contaminated with Gram-negative bacteria which are capable of causing sepsis and which may result in death. Therefore, it is recommended that screening for bacterial infections should be done routinely because of the threat it posed as seen in Federal Medical Centre, Birnin Kebbi, Kebbi state, Nigeria.

Introduction

Blood transfusion is the transfer of blood or blood products from one person (donor) into another

person's bloodstream (recipient). This is usually done as a lifesaving maneuver to replace blood cells or

DOI: 10.21608/MID.2023.178690.1422

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blood products lost through severe bleeding, during surgery when blood loss occurs or to increase the blood count in an anemic [1]. Since the starting of blood transfusion scientifically in the early 1940s, an integrated strategy for blood safety is required for the elimination of Transfusion transmitted infections (TTIs) and for the provision of safe and adequate blood transfusion services to people. Both Gram-positive and Gram-negative bacteria have been linked to transfusion-transmitted bacterial infections, however Gram-negative bacteria are more frequently associated with significant morbidity and mortality [2].

Blood transfusion is one of the most important tools in modern medical therapy, and saving patients is its aim. If the safe blood supply is not considered, it can be life-threatening. However, the blood has its potential risks causing serious side effects in the recipients. It is known that bacteria, viruses and parasites can be transmitted through blood transfusions [3]. Choosing healthy donors with low risk of blood contamination is one of concerns around the world. Bacterial contamination of donated blood is defined as the presence of bacteria in the blood or blood components which are collected and/or processed for transfusion [4]. A ready to be transfused blood should be free from microbial contaminants including bacteria [5]. For this blood should be collected and processed following aseptic technique [5]. However, bacterial contamination of donated blood may occur as a result of endogenous (from the donor) or exogenous (during collection and processing) route [6].

Globally, the exact prevalence of bacterial contamination of blood and blood components is unknown [7]. However several studies showed that bacterial contamination of donated blood was, 0.2%, 0.15%, and 0.1% in the United States of America, UK, and France, respectively [7]. It was indicated in various studies that the factors that promote bacterial contamination of donated blood include; touching disinfected phlebotomy site, the improper use of disinfection, double puncture at the same hand or both hands of a donor, improper storage of blood and donor bacteremia [7]. In Nigeria, there is paucity of data on bacterial contamination of blood and blood products, despite the high demand for these created by children with severe anaemia secondary to malaria, victims of road traffic accidents or in obstetrics emergencies amongst other indications [8]. Bacterial contamination of whole blood and its various components can occur at several points

including production of blood bags, donor venepuncture, blood donor bacteraemia, blood component separation or at the time of transfusion [8].

These transfusion-transmissible illnesses continue to be a danger in underdeveloped nations like Nigeria; multiple reports have identified transmissible viruses (HIV, HBV, HCV, and *Treponema pallidum*) in blood donors. Transmissible infections have significantly decreased in sophisticated nations as a result of significant national policy (better donor selection and newer screening tools) [9]. Although transfusion-transmitted bacterial infection was found to be the most frequent reason for transfusion-related problems, multiple research have shown that contaminating microorganisms, mostly skin flora, may be cultivated. Nigerian blood and blood components for transfusion are frequently contaminated with bacteria, which poses a danger for hospital-acquired infections [9]. The prevalence rate of bacterial infections of blood and blood products at Obafemi Awolowo University Teaching hospital Ife, Ile-Ife Nigeria was found to be 8.8% [9].

Antibiotic resistance poses a serious threat to global public health, causing at least 1.27 million deaths worldwide and over 5 million fatalities in 2019 [10]. Literature have shown that most of the bacteria isolated from blood ready for transfusion are resistance to commonly prescribed antibiotics. For instant, **Wondimu et al.** [11] reported multiple antimicrobial resistances from 66.7% of the bacteria isolated from Blood Collected for Transfusion at University of Gondar Hospital Blood Bank, Northwest Ethiopia. Similarly, **Tsegaye et al.** [12] reported 33.3% resistant *Pseudomonas aeruginosa* against gentamicin form Blood and Blood Components Using Divergent and Non-Divergent Collection Methods at Armed Forces Comprehensive Specialized Hospital, Addis Ababa, Ethiopia. A high resistance *Staphylococcus aureus* strains were reported against penicillin and cloxacillin [13]. In Nigeria, **Bolarinwa et al.** [9] also reported higher resistance bacterial species isolated from blood and blood components in a tertiary hospital setting. However, there is no organized system of bacterial detection from donors' blood in Nigerian's hospitals despite the risk and damage these bacteria may cause to the recipient's health. Therefore, this study was aimed at determining the antibacterial resistance pattern of bacteria isolated from donor's blood attending Federal Medical Center Birnin Kebbi.

Material and Methods

Study area

The study was carried out at Federal Medical Centre, Birnin Kebbi, Kebbi state. It's located in the Northwestern part of Nigeria. Kebbi state is situated at latitudes $10^{\circ} 8' N - 13^{\circ} 15' N$, and longitudes $3^{\circ} 30' E - 6^{\circ} 02' E$ with Sokoto and Zamfara states to the east, Niger state to the south, the Benin Republic to the west and the Niger Republic to the northern border. The population of the state was estimated to be 3,238,628 in 2006 and was projected to be 3,952,766 in 2012. The state occupies an area of about 36,229 square kilometres. Kebbi state experiences peak rainfall between July and August while harmattan (cold season) is usually from November to February and is characterized by strong winds. The mean annual temperature is about $27^{\circ}C$. However, during the harmattan season, the lowest temperature is $21^{\circ}C$. Temperature can go up to $40^{\circ}C$ from April to June. The average relative humidity during the wet season is 80%. The variation in relative humidity explains the hot, dry environment in the northern parts of Nigeria. The main occupation of people in this area are farming, business, civil servant and nomadic [14, 15].

Sample collections

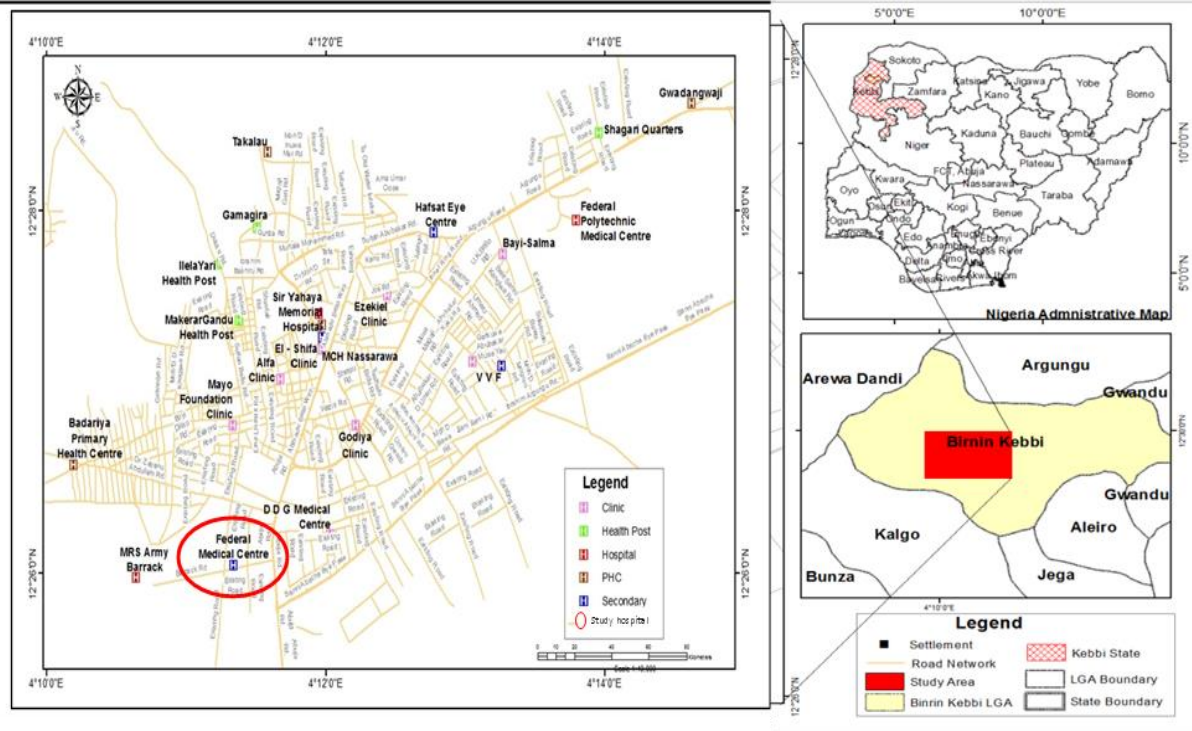
One hundred and twenty (120) clinical blood samples were collected from healthy male blood donors at Federal Medical Centre, Birnin Kebbi. Female donors were excluded. After obtained consent from each donor. They were allowed to sit on the chair or examination couch while the arm were tight with a tourniquet for the easy access to the vein. The site of prominent vein was properly cleaned with antiseptic agent (75 % methylated spirit), while new syringe and needle was inserted into the vein to withdraw 5ml of blood which was transferred into a sterile specimen bottle containing cooked meat broth.

Isolation of bacteria

The inoculated five milliliters (5ml) of blood sample cooked meat broth was mixed properly and incubated at $37^{\circ}C$ for 2 – 7 days. After incubation period all samples were sub-cultured on to freshly prepared blood agar, and MacConkey agar media [7]. The Plates were incubated at $37^{\circ}C$ for 24hrs. The morphological characteristics and presence or absence of growth on the media were observed and recorded. The observed bacterial growth were sub-cultured on to freshly prepared nutrients agar medium to have a pure culture. The purified colony was transferred into prepared slant bottles containing nutrient agar and stored in the refrigerator at $4^{\circ}C$ for further analysis [16]. The pure isolates of the cultured bacteria were identified using Gram staining and confirmed using standard biochemical tests such as the catalase test, coagulase test, hydrogen sulphide production (H_2S), indole test, citrate utilization, gas production, and carbohydrate metabolism [11,16].

Antibiotics sensitivity test

A freshly prepared Muller Hinton agar (MHA) plate was inoculated with the test bacterium adjusted to 0.5 McFarland standard turbidity standard using a sterile swab stick. Antibiotics discs of Augmentin 30 μ g, ciprofloxacin 5 μ g, cefuroxime 30 μ g, ofloxacin 5 μ g, chloramphenicol 3 μ g, azithromycin 15 μ g, ceftriaxone 30 μ g, gentamycin 10 μ g, clindamycin 2 μ g and meropenem 10 μ g were placed on the surface of the inoculated plate using sterilized forceps. The plates were incubated for 24 hours at $37^{\circ}C$. The results were interpreted according to European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standard Institute for Antimicrobial Susceptibility Testing [17].

Figure 1. Map of Kebbi State showing the Federal medical center Birnin Kebbi, Kebbi State Nigeria [1].

Results

Socio-demographic characteristics of blood donor's participants are shown in **table (1)**. The overall prevalence of TTBI in this study is 24(20.0%). Prevalence of bacterial infections in relation to age group was high 12 (50%) in 36-49 years, followed by 22-35 years and below 21 years with 6(25%) each. There is a significant association between the age and transfusion transmissible bacterial infections in this study ($\chi^2= 17.62, p= 0.0099$). Prevalence of TTBI 18(75%) was observed in the rural area and 6(25%) in the urban area. There is a significant association between place of residence and transfusion transmissible bacterial infections ($\chi^2= 4.60, p= 0.0273$). A prevalence of transfusion transmissible bacterial infections of 50% was observed from farmers, while businessmen and civil servants had 33.3% and 16% respectively. The TTBI prevalence was high 10(41.7%) among participants with non-formal education, followed by primary school leavers 8(33.3%), secondary school leavers 6(25%) and none were infected among the people who attended advanced schools. There is a significant association between the level of education of the donors and transfusion transmissible bacterial infections ($\chi^2= 6.46, p=0.0412$) (**Table 1**).

The present study has shown that the most bacteria isolates were *Klebsiella oxytoca* 5(20.8%),

Citrobacter diversus 4(16.7%), *Enterobacter aerogene* 4(16.7%), *Shigella sonnei* 3(12.5%), *Shigella dysenteriae* 1(4.2%), *Morganella morganii* 1(4.2%), *Escherichia coli* 1(4.2%) and *Staphylococcus aureus* 5(20.8%) (**Table 2**).

The results of antibiotics susceptibility test showed that *E. coli* was 100% sensitive to all antibiotics tested while *Morganella morganii* was resistance to all antibiotics tested (**Table 3**).

Table 1. Socio-demographic characteristics of blood donors' participants and prevalence of TTBIs.

Variables	Frequency n (%)	Prevalence n (%)	p value
Age			0.0099*
Below 21	36 (30.0)	6(25.0)	
22 – 35	72 (60.0)	6(25.0)	
36 – 49	12 (10.0)	12(50.0)	
Place of residence			0.0273*
Rural area	34 (28.3)	18(75.0)	
Urban area	86 (71.7)	6(25.0)	
Occupation			0.0416*
Farming	62 (51.7)	12(50.0)	
Business	30 (25.0)	8(33.3)	
Civil servant	28 (23.3)	4(16.7)	
Education			0.0412*
Non-formal education	45 (37.5)	10(41.7)	
Primary	20 (16.7)	8(33.3)	
Secondary	37 (30.8)	6(25.0)	
Tertiary	18 (15.0)	0 (0.0)	

Key: TTBIs- Transfusion Transmissible bacterial infections, *Statistically Significance.

Table 2. Frequency of occurrence of bacterial species isolated from blood donors attending Federal medical Centre, Birnin Kebbi.

Bacterial isolate	Frequency n (%)
<i>Citrobacter diversus</i>	4 (16.7)
<i>Klebsiela oxytoca</i>	5 (20.8)
<i>Enterobacter aerogene</i>	4 (16.7)
<i>Shigella dysenterae</i>	1 (4.2)
<i>Morganella morganii</i>	1 (4.2)
<i>Shigella sonnei</i>	3 (12.4)
<i>Escherichia coli</i>	1 (4.2)
<i>Staphylococcus aureus</i>	5 (20.8)
Total	24 (100)

Table 3. Antibiotic resistance pattern of bacteria isolated from blood donors from FMC Birnin Kebbi.

Bacterial isolates	Antibiotics Tested									
	AMC (%)	CIPR (%)	CXM (%)	OFX (%)	CHLR (%)	AZITH (%)	CRO (%)	CN (%)	CLIN (%)	MERO (%)
<i>C. diversus</i> (n=4)	0 (0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	-	-	2(50.0)	-	0(0.0)
<i>K. oxytoca</i> (n=5)	2(40)	3(60)	3(60.0)	-	3(60.0)	-	-	2(40.0)	-	2(40.0)
<i>E. aerogene</i> (n=4)	2(50)	2(50)	-	3(75.00)	2(50.0)	-	-	0(0.0)	-	0(0.0)
<i>S. dysentrae</i> (n=1)	0(0)	1(100)	-	0(0.0)	0(0.0)	-	-	-	-	0(0.0)
<i>M. morgani</i> (n=1)	1(100)	1(100)	1(100)	-	1(100.0)	-	-	-	-	1(100.0)
<i>S. sonnei</i> (n=3)	1(25)	1(25)	1(25)	2(75.0)	1(25.0)	-	-	1(25.0)	-	0(0.0)
<i>E. coli</i> (n=1)	0(0)	0(0)	-	-	0(0.0)	-	-	0(0.0)	-	0(0.0)
<i>S. aureus</i> (n=5)	-	0(0)	-	3(60.0)	-	2(40.0)	0(0.0)	-	0(0.0)	-

Key: AMC-Augmentin, CIPRO-Ciprofloxacin, CXM-Cefuroxime, OFX-Ofloxacin, CHLR- Chloramphenicol, AZITH-Azithromycin, CRO-Ceftriaxone, CN-Gentamycin, CLINDA-Clindamycin and MERO-Meropenem

Discussion

Globally, the exact prevalence of bacterial contamination of blood is unknown [9]. In the African continent, there is no comprehensive data to show the magnitude of the infection. In this study, the prevalence of bacterial blood contamination is 20.0%. This is higher compared to the prevalence reported from several studies 0.2%, 0.15% and 0.1% in the United States of America, the UK and France, respectively [7,9]. The results of bacterial contamination from these developed countries are low this may be a result of their well-organized Blood transfusion services and implementation of effective quality control systems. In Africa, **Adjei et al.** [18] reported a 9% prevalence of bacterial blood contamination from three major blood transfusion centers in the Greater Accra Region of Ghana. **Wondimu et al.** [11] reported a 15.33% prevalence of bacterial blood contamination from the University of Gondar Hospital Blood Bank, Northwest Ethiopia. Similarly, **Agzie et al.** [7] reported 9.2% of bacterial blood contamination from stored blood and blood components ready for transfusion at blood banks in Mekelle, Northern Ethiopia. However, **Tsegaye et al.** [12], reported a 4.5% prevalence of bacterial blood contamination at the Armed Forces Comprehensive Specialized Hospital, in Addis Ababa, Ethiopia. **Rukundo et al.** [19], reported a 3% prevalence of bacterial

contamination of blood for transfusion at Mbarara Regional Referral Hospital, Southwestern Uganda. In Nigeria **Bolarinwa et al.** [9], reported an 8.8% prevalence of bacterial blood contamination from Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.

The results of this study showed that all bacteria isolated were Gram-negative with exception of *Staphylococcus aureus* which was Gram-positive. The results showed that *Klebsiella oxytoca* and *Staphylococcus aureus* had a higher frequency of occurrence (20.8%) each. Another study by **Agzie et al.** [7] reported bacterial contamination from the donor's blood as *Staphylococcus aureus*. Coagulase-negative *staphylococcus*, *Escherichia coli*, which is closely related to this present finding. **Rukundo et al.** [19], also a reported higher prevalence of *Staphylococcus aureus* (66.7%) of blood for transfusion at Mbarara Regional Referral Hospital, Southwestern Uganda. In a study done by **Boye et al.** [20], also reported the highest total number of blood samples contaminated by Gram-negative and Gram-positive bacteria, at-point-of transfusion blood in a tertiary hospital in Ghana, this agreed with the present study. This could be a result of the inoculation of bacteria from the donor's skin into the blood samples collected. However, it was reported by **Bolarinwa et al.** [9] that all the bacterial blood contaminant identified in

their study were Gram-positive which is contrary to the finding of the present study. The difference may likely come from donor types and the frequency rate of the donations because the more the individual donates blood the risk of being infected by the bacteria species. The study also reported the occurrence of *S. dysenteriae* and *S. sonnei* which are rare bacterial causing bacteremia. **Morduchowicz et al.** [21], **Drews et al.** [22], and **Carretero-Vicario et al.** [23] reported the occurrence of *Shigella sonnei*, and *Shigella dysenteriae* as causative agents of bacteremia. The presence of *S. dysenteriae* and *S. sonnei* in the blood could be the result of comorbidities in the patient that predispose to systemic invasion than the toxin or other virulence factors in specific strains [22].

The prevalence rate of bacterial infections among blood donor's age was found to be high 12(50.0%) between 36-49 years which agrees with the finding of **Prabhakar et al.** [24] who reported a prevalence of bacterial contamination (50.8%) 35-50 years old. This may be due to the fact that this age group possesses high-risk exposure to some prevailing infectious diseases as there in the productive stage of human development. Another finding by **Shaz et al.** [25] shows that the highest bacterial contamination of blood donors 62.8% was seen between the ages of 26-69 which is higher than the finding of this study. Most likely, the increase in the number of TTBI's could be caused by aged donors of 69 years who are more vulnerable to infections due to low body immunity. **Owusu-Ofori et al.** [26] reported that the majority of blood donors who were mostly infected with bacteria 39.7% in their study were aged between 18-25 years which is somehow lower than the result of this present study. **Yang et al.** [27] revealed that blood donors between the age 25 - 45 years old constituted higher bacterial (syphilis) infections in his study. This finding disagreed with this study when compared with the age profile where the prevalence of bacterial infections was high 12(50.0%) from 36-49 years. Any age group that is below 20 years may likely be vulnerable to TTBI's as their immune system is not fully developed. **Chang et al.** [28] shows that the highest TTBI's 52.4% were from 26-55 years a margin that is similar to the finding of this present study, this may be due to the fact that people with this age bracket are believed to be in their active productive life and have their body immune system overwhelming which make them more vulnerable to TTBI's.

The current study also revealed that majority of blood donors infected with bacteria 18(75.0%) were residing in the rural area with

6(25.0%) from urban area. The reason why the prevalence of bacterial infections was high from the rural could be the nature of their poor nutritional status, lack of personal and environmental hygiene and low knowledge of precautionary measures of prevention and control of infections. Hence, there is significant association between the place of residence of the blood donors and transfusion transmissible bacterial infections.

The current study finding shows that TTBI's prevalence rate 12(50.0%) were observed from farmer's blood donor while lowest were observed from civil servants with 4(16.7%) this disagrees with the finding of **Alfouzan et al.** [29] which revealed that the highest rate of bacterial infections of blood donated was seen from civil employees (28.5%) and the lowest were from the others blood donors.

In this present study, the high prevalence of TTBI's 10 (41.7%) was recorded from the blood donors with non-formal education, primary school leavers 8 (33.3%), secondary school leavers 6(25.0%) and 0% from those that attended advanced schools Tab 4.1. A report by **Enawgaw et al.** [30] shows that the majority of the blood donors that were infected by bacteria 3(18.0%) had a secondary education certificate, which differs slightly from the result of this present study. The reason for disagreement may be a result of their social lifestyles. Another finding by **Siraj** [31] shows that bacterial infections of blood donation level were increasing steadily with the increase in the educational level. The mean rank for knowledge score was highest among tertiary institutions 17(95.2%) and lowest among non-formal education 2(0.8%). The level of education of the blood donors was sought to know whether they have adequate knowledge and a positive attitude towards blood donation. Although, some people might have possessed knowledge about blood donation previously because of perceptions of fear of pain, medically unfit, social stigmatization and lack of adequate information on how, where and when to donate blood has generated great fear among the intending blood donors. There is a significant association between the TTBI's and the educational status of blood donors.

The present study showed that *Morganella morganii* were 100% resistant to all antibiotics tested *Escherichia coli* were 100% sensitive to all the antibiotics used in this study, this agreed with **Liu et al.** [32], who reported that *Morganella morganii* was reported to be among the multidrug resistance bacteria which was recently ranked as 12th among the Gram-negative bacteria that cause

bloodstream infections. **Wondimu et al.** [11] also showed that all bacteria isolated were 100% sensitive to gentamicin, chloramphenicol, amoxicillin and doxycycline. More so, all the isolated organisms were also sensitive to Meropen 100%, except *Morganella morganii* which is resistant to all antibiotics. **Bolarinwa et al.** [9] showed that all the bacteria isolated were Gram-positive with 100% resistance to all the antibiotics used except gentamicin and ceftriaxone. This is also dissociated with a report of this present study.

Conclusion

This research revealed the prevalence rate (20.0%) of transfusion transmissible bacterial infections among the blood donors attending the Federal medical center Birnin Kebbi. The highest number of Gram-negative bacteria were isolated 7(87.5%) with only 1(12.5%) as Gram-positive bacteria. *Klebsiella oxytoca* 5(20.8%) and *Staphylococcus aureus* 5(20.8%) were the most isolated bacteria in this study. The prevalence rate of TTBI according to the donors age group was found to be high 12(50.0%) in the 36-49 age group. *Morganella morganii* was (100%) resistant to all the antibiotics used in this study.

Conflicts of interest: None.

Financial disclosure: None

Author's contribution

Usman Abubakar, conducted the laboratory work of this study. The first mentioned author and Adamu Almustapha Aliero, Daniel Dan-Inna Attah and Adesina Muibi Adefowope contributed equally to its content apart from the laboratory part. All authors read and approved the final version of this manuscript before submission.

Acknowledgments

We would like to thank the management of the Federal Medical Center Birnin Kebbi for given us the approval to carry out this research. Studied participants for participating in this study. Finally the staff of the microbiology laboratories for their kind support during this study.

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