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Comparison of blood culture contamination and true positivity between Emergency treatment unit, Intensive care units and General wards in a Tertiary care centre in Southern Sri Lanka: A cross-sectional study

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Abstract. Isolation of the bacterial pathogens in blood is a gold standard test to diagnose bloodstream infections. The contamination of the sample during collection is the main drawback leading to the mismanagement of patients. This study aimed to compare blood culture contamination (BCC) and true positivity (TP) between Emergency Treatment Unit (ETU), Intensive Care Units (ICUs) and general wards (GWs) and to assess contaminants and true pathogens. A cross-sectional study was performed from January 2022 to April 2022 in Teaching Hospital Karapitiya (THK). All consecutive peripheral blood culture reports from patients admitted to the ETU, ICUs and GWs wards in THK were included. We analyzed proportions of contaminant and true positive blood cultures, and variables associated with BCC. Of all 1859 blood cultures, the total positives were 396 (21.3%). The overall BCC rate was 8.5% and the BCC rate in ETU (12.5%) is significantly higher than GWs (7.8%) and ICUs (6.5%)(OR 2.3; 95% CI 1.2-4.3). The overall TP was 12.8%. The time-to-positivity of the true pathogen was significantly less compared to the contaminant (16.3 vs 24.6 hours). The predominant contaminant and true pathogen were coagulase-negative *Staphylococcus* spp. (83.6%) and *E.coli* (25.2%) respectively. The BCC was significantly higher in patients above 65 years ($p<0.001$). The blood culture contamination was significantly higher in ETUs compared to ICUs and GWs. Gram-positive bacteria were the predominant contaminant, and the majority of true pathogens were Gram-negative. Adherence to protocols and education and training of staff are mandatory to reduce contamination.

Keywords. Blood culture contamination, Emergency treatment unit, Intensive care units, General wards, True positivity

INTRODUCTION

Bloodstream infections are known to be a major cause of mortality and morbidity in hospitalized patients despite advances in therapeutic management (1). Therefore, rapid and accurate detection of bacteraemia by blood culture is essential for a better outcome of septic patients as it helps to commence the most appropriate antibiotics (2). Blood cultures (BCs) are considered as the “gold standard” for the diagnosis of bacteraemia, yet contamination is a major concern for both clinicians and microbiologists (3).

Contaminated blood cultures can cause difficulties in interpreting an actual positive blood culture, and this subsequently can lead to unnecessary treatment of the patient and can expose him to the side effects of a drug that he does not need. Prolonged hospital stays and unnecessary and costly care are additional issues (4). The Clinical and Laboratory Standards Institute (CLSI) defines blood culture contamination (BCC) as a “microorganism isolated from a blood culture during specimen collection or processing that was not pathogenic for the patient from whom the blood was collected” (5). The contaminated blood culture sample usually isolates normal skin flora (coagulase-negative staphylococci, *Propionibacterium* spp., *Aerococcus*, *Micrococcus*, *Bacillus* spp., *Corynebacterium* spp. [diphtheroids], and alpha-hemolytic streptococci) (6). The current recommendation by the CLSI is to maintain a BCC rate of less than 3%, however, reported incidences differ significantly from countries including

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Australia, New Zealand and the United States of America identifying rates of between 3–12 %, with the highest rates in the Emergency departments (ED) (7-8). In a study done by Namalie et al in Sri Lanka, it was found that the overall BCC rate was 5.6% and the highest was noticed in medical wards (53.6%), followed by surgical Intensive Care Units (ICU) (7.9%), paediatric wards (7.1%), surgical wards and surgical ICU (6.8% each) (9). The rate of blood culture contamination is a recommended measurement of healthcare quality, and it should be continuously monitored to maintain it within the international standard rate (5).

The emergency treatment unit (ETU) remains the vital unit in identifying and initiating investigations for septic patients and finally preventing the emergence of antimicrobial resistance (7). ETU is a challenging patient care environment that contributes to higher BCC rates due to high staff turnover, lack of ongoing training, workload, critically ill patients that require resuscitation, and the perceived time pressure to collect blood cultures prior to antimicrobial administration (10-12).

The aims of this study were to determine and compare the rates of BCC and true positivity (TP) at ETU, intensive care units (ICUs), and general wards (GWs) in THK, to determine common contaminants and true pathogens isolated in these blood cultures including their antibiotic sensitivities and to analyze the factors associated with BCC. Unfortunately, there was no published data on the BCC rate in ETUs in Sri Lanka. Therefore, this would be a preliminary study which we can use for the implementation of guidelines to minimize contamination rates, especially in low-resource healthcare settings.

MATERIAL AND METHODS

Study setting. A retrospective cross-sectional study was carried out from January 2022 to March 2022 in the largest public tertiary care centre in the Southern Province, Sri Lanka. This 1560-bed hospital has an ETU, 5 ICUs and 54 GWs and provides basic as well as specialized medical, paediatric, surgical, and critical care services to patients, free of charge. All the relevant data were obtained from the Microbiology laboratory of the Teaching Hospital, Karapitiya (THK), Sri Lanka. Blood cultures were processed using automated blood culture and manual methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Study population. All the consecutive blood cultures received from ETU, ICUs and GWs during the study period were included. The first peripheral blood culture from each patient was included, whereas repeated blood cultures of the same patient for the same clinical condition and blood drawn from the central venous lines of the patients were excluded. The details of the patients in request forms and the culture results including identification of the organism and antibiotic susceptibility patterns of true pathogens were recorded.

Data collection. Sociodemographic details of patients were obtained through a review of the request forms such as age, gender, and clinical diagnosis of the patients. We defined the organism as a skin contaminant if only one bottle out of two or three grew any of the normal flora of the skin. Before considering it as a contaminant, clinical details of the patients (e.g. presence of central venous lines and prosthetic material, immunocompromised state) were sorted out to exclude any possibility of pathogenic role of commensal microorganisms.

Ethical considerations. Ethical approval for the study was obtained from the Ethical Review Committee of Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka (Ref.no. 123.07.2022) on 19. 10. 2022. Administrative clearance to conduct the study was obtained from the Director, THK, Sri Lanka.

Statistical analysis. The proportions or rates of BCC and TP were calculated according to the following formula: the number of contaminated blood cultures or the number of BC with true pathogens × 100% to the number of total BC (including all true positive BC, contaminant, and negative BC). The variables explored as possible associated factors were age, gender, place of blood sample collected (e.g. ETU, ICUs, GWs) and method of processing. Data analysis was performed using SPSS version 26. Categorical variables were reported in frequency and percentages and continuous variables in mean (±SD). The chi-square test was used to assess the association between categorical variables with statistical significance at $p<0.05$. An Independent sample t-test was used to see the mean

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differences. Logistic regressions were performed for each of the exposures of interest. Odds ratio (OR) with 95% confidence intervals (CIs) were calculated.

RESULTS

Demographic data of patients. Over the study period, a total of 1859 consecutive blood cultures were sent to the Microbiology lab from patients admitted to the ETU (n=352), ICUs (n=232) and general wards (n=1275) in THK. Data are summarized in Figure 1.

The demographic details of the patients (n=1859) whose blood cultures were sent for processing were included in Table 1. In this study population, the majority of the patients were males (63.2%) and the mean age was 48.0 (±25.9) years. The minimum and maximum ages were 1 and 103 respectively. When considering the clinical diagnosis, the commonest presentation was sepsis with unknown aetiology (203 cases), followed by urosepsis (43 cases), infection of the lungs (27 cases) and CLABSI (25 cases).

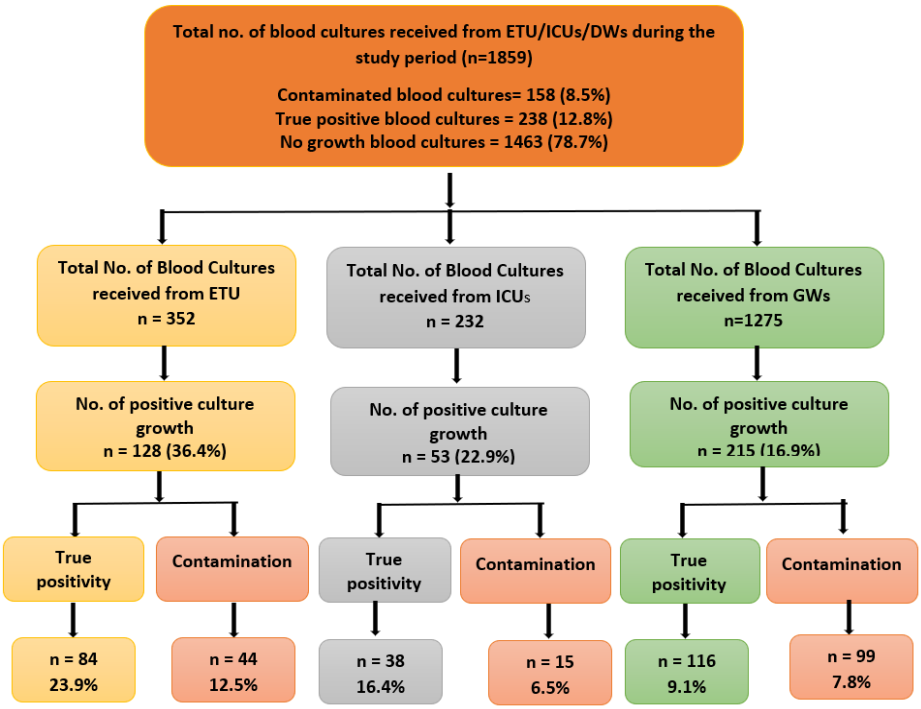

Figure 1. Strobe diagram- outline of the results

Prevalence of blood culture contamination and true positivity. When analyzing unit-specific rates of BCC and TP for ETU, ICUs and GWs, the number of blood cultures sent from each unit was considered into the denominator and the results are shown in Table 2. A total of 396 (21.3%) blood cultures were positive during the study period and overall BCC rate and TP rates were 8.5% and 12.8% respectively. The unit-specific BCC rates in ETUs, GWs and ICUs were 12.5%, 7.8% and 6.5% respectively. Whereas TP rates in ETUs, ICUs and GWs were 23.9%, 16.4% and 9.1% respectively. The highest rates of both BCC and TP were observed in ETU. The lowest rate of BCC was seen in ICUs and the lowest TP rate was in GWs. The time-to-positivity of blood cultures and prior usage of antibiotics results were analysed to differentiate between BCC and true positivity (Table 3). When considering the mean value of time-to-positivity of blood cultures, the growth of the true pathogen took significantly less time compared to the time taken by the contaminant (16.3 hours versus 24.6 hours, $p<0.001$).

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Table 1. Demographic data of the study sample

	ETU (n=352)	ICUs (n=232)	GWs (n=1275)
Gender			
Male (n=1159)	220 (62.7%)	150 (64.7%)	789 (62.1%)
Female (n=700)	132 (37.3%)	82 (35.3%)	487 (37.9%)
Age			
Mean (±SD)	54.4 (±24.9)	41.2 (±24.5)	47.5 (±25.9)
Clinical Diagnosis			
Urosepsis	20 (46.5%)	1 (2.3%)	22 (51.2%)
Lung infection	12 (44.4%)	4 (14.8%)	11 (40.7%)
CNS infection	8 (33.3%)	0 (0.0%)	16 (66.7%)
Skin & soft tissue infection	3 (33.3%)	2 (22.2%)	4 (44.4%)
Abdominal infection	3 (50.0%)	0 (0.0%)	3 (50.0%)
CLABSI	1 (4.0%)	9 (36.0%)	15 (60.0%)
Sepsis (Unknown aetiology)	70 (34.5%)	32 (15.8%)	101 (49.8%)

Abbreviations: CNS= Central nervous system, CLABSI= Central line associated blood stream infections, ETU= Emergency Treatment Unit, ICUs= Intensive Care Units, GWs= General wards

Table 2. Proportions of contamination and true positivity of blood cultures in ETU, ICUs and GWs

	Total Positives No. (%)	BCC No. (%)	TP No. (%)	No growth No. (%)	Total Blood Cultures received No. (%)
ETU (No, %)	128 (36.4)	44 (12.5)	84 (23.9)	224 (63.6)	352 (100.0)
ICUs (No, %)	53 (22.9)	15 (6.5)	38 (16.4)	179 (77.1)	232 (100.0)
GWs (No, %)	215 (16.9)	99 (7.8)	116 (9.1)	1060 (83.1)	1275 (100.0)
Total (No, %)	396 (21.3)	158 (8.5)	238 (12.8)	1463 (78.7)	1859 (100.0)

Contaminants in blood cultures. Of all BCC (n=158), the predominant isolate was coagulase-negative Staphylococcus (CoNS) (83.6%, n=132). The other contaminants were diphtheroid 8.2% (n=13), *Bacillus* spp. 6.3% (n=10) and *Micrococcus* 1.9% (n=3) all of which are considered as commensal microorganisms of skin.

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Table 3. Comparison of blood culture contamination and true positivity in relation to time to positivity and prior antibiotic exposure

	Contamination (n=158)	**True positivity (n=238)**	**Significance**
Time to get positive results in hours (mean, ±SD)	24.5 (±17.6)	16.3 (±18.4)	*p<0.001* (95% CI; 4.56-11.84)
Prior antibiotic usage (No, %)	50 (31.6)	98 (41.2)	*p*=0.48

Table 4. Factors associated with blood culture contamination

	Contamination (n=158)	No Contamination (n=1463)	Significance	COR (95%CI)	AOR (95%CI)
Place of blood culture					
ETU *vs* ICU					
ETU	44 (16.4)	224 (83.6)	x^2=7.6, df=1	2.34 (1.26-	**2.34** (1.26-4.36)
ICU	15 (7.7)	179 (92.3)	***p*=0.006**	4.35)	***p*=0.007**
ICUs *vs* GW					
ICUs	15 (7.7)	179 (92.3)	x^2=0.15, df=1	0.89 (0.51-	-
GW	99 (8.6)	1058 (91.4)	*p*=0.7	1.58)	
ETU *vs* GWs					
ETU	44 (16.4)	224 (83.6)	x^2=14.9, df=1	2.10 (1.43-	**2.10** (1.43-3.08)
GWs	99 (8.5)	1060 (91.5)	***p*=0.0001**	3.08)	***p*=0.0001**
Method of processing					
Automated	77 (10.4)	665 (89.6)	x^2=0.62, df=1	1.14 (0.82-	1.2 (0.86-1.68)
Manual	81 (9.2)	798 (90.8)	p=0.4	1.58)	
Gender					
Male	102 (10.2)	898 (89.8)	x^2=0.94, df=1	1.12 (0.84-	1.24 (0.88-1.77)
Female	54 (8.7)	567 (91.3)	*p*=0.33	1.68)	
Age (in years)					
≤ 64	87 (8.0)	1003 (92.0)	x^2=14.3, df=1	1.89 (1.35-	**1.89** (1.35-2.64)
≥ 65	70 (14.1)	427 (85.9)	***p*=0.0001**	2.64)	***p*=0.0001**

Abbreviations: AOR= adjusted odds ratio, COR= crude odds ratio

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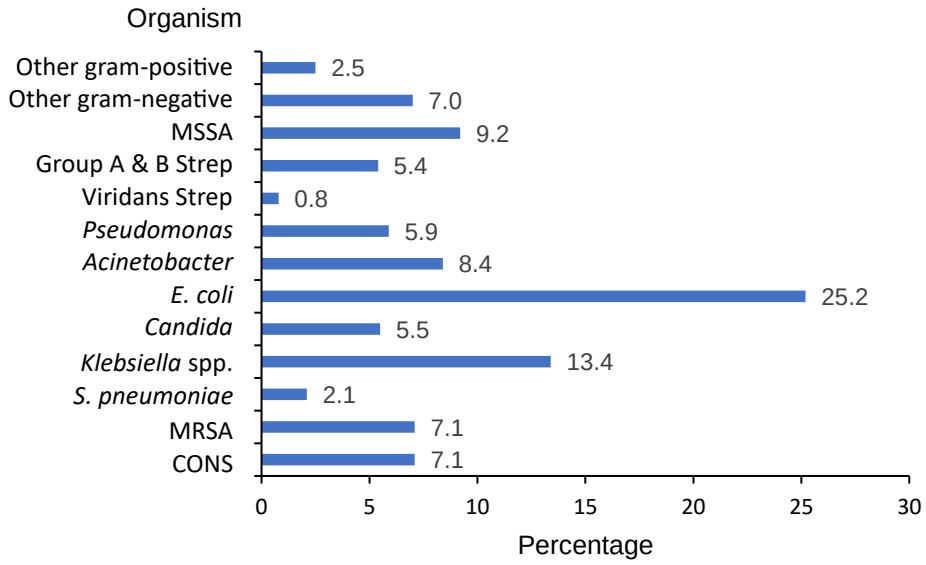

Figure 2. Bloodborne true pathogens (CoNS= Coagulase-negative *Staphylococcus*, MRSA=Methicillin-resistant *Staphylococcus aureus*, MSSA=Methicillin-sensitive *Staphylococcus aureus*)

Table 5. Prevalence of antibiotic resistance among *E. coli*, *Klebsiella* spp, *Acinetobacter* spp., *Pseudomonas* spp., and other Gram-negatives (n = 143*)

	E. coli (n= 60)	*Klebsiella* spp *(n=32)*	*Acinetobacter (n= 20)*	*Pseudomonas spp. (n=14)*	*Other Gram-negatives (n=17)*
	+ R / n (%)	+ R / n (%)	+ R / n (%)	+ R / n (%)	+ R / n (%)
Ampicillin	53/59 (89.8)	32/32 (100.0)	-	-	-
Co-amoxiclav	47/60 (78.3)	19/32 (59.4)	-	-	6/16 (37.5)
Cefuroxime	39/55 (70.9)	22/31 (70.9)	-	-	6/11 (54.5)
Cefotaxime	35/60 (58.3)	20/31 (64.5)	12/13 (92.3)	-	2/11 (18.2)
Ceftazidime	-	-	12/20 (60.0)	1/13 (7.7)	-
Ciprofloxacin	43/59 (72.9)	24/32 (75.0)	15/20 (75.0)	1/14 (7.1)	-
Cefepime	25/45 (55.6)	15/26 (57.7)	14/20 (70.0)	1/12 (8.3)	3/13 (23.1)
Gentamicin	14/59 (23.7)	10/32 (31.3)	11/20 (55.0)	4/14 (28.6)	3/14
Amikacin	1/59 (1.7)	3/31 (9.7)	10/20 (50.0)	0/14 (0.0)	3/12 (25.0)
Polymyxin B	1/50 (2.0)	1/29 (3.4)	0/29 (0.0)	2/14 (14.3)	3/13 (23.1)
Imipenem/Meropenem	4/39 (10.3)	4/24 (16.7)	9/10 (90.0)	0/8 (0.0)	0/6 (0.0)
Piperacillin / tazobactam	12/59 (20.3)	15/32 (46.9)	15/19 (78.9)	1/13 (7.7)	0/16 (0.0)
Cefoperazone /sulbactam	7/53 (13.2)	11/29 (37.9)	10/20 (50.0)	1/14 (7.1)	0/15 (0.0)

+ R = resistant isolates; **n** = number of isolates evaluated for each antibiotic varies

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Factors associated with blood culture contamination. The factors associated with BCC were analysed by comparing total contaminant blood cultures (n=158) with the total negative blood cultures which did not show any growth (n=1463). The risk factors assessed were the unit of the hospital where the blood culture was sent, the method of processing, and patient-related factors such as gender and age. Logistic regression was applied for the factors and the significance and odds ratios were evaluated.

Blood cultures from ETU showed a significantly higher contamination rate compared to both ICUs (16.4 versus 7.7) and GWs (16.4 versus 8.5) giving 2.1-2.3 odds compared to the other two units. Similarly, blood cultures from patients above 65 years showed significantly higher contamination risk than those from patients aged 64 years or younger, (14.1 vs 8.0) resulting in 1.89 odds compared to another age category. There was no significant association found between automated and manual blood cultures and the gender of the patients with BCC.

Bloodborne pathogens. All the isolated microorganisms considered as true pathogens were shown in Fig. 2. Of all (n=238), the predominant isolate was Escherichia coli (*E.coli*) (25.2%, n=60) followed by *Klebsiella* pneumoniae (13.4%, n=32), methicillin-sensitive *Staphylococcus aureus* (MSSA) (9.2%, n=22) and *Acinetobacter* spp. (8.4%, n=20).

Among the other pathogens, equal proportions (7.1%, n= 17) of methicillin-resistant *Staphylococcus aureus* (MRSA) and CoNS were isolated. The proportion of *Candida* spp. was 5.5% (n=13). Of all CoNS, 7 were isolated from patients suspected of CLABSI.

Antibiotic sensitivity patterns of bloodborne true pathogens. Of all Gram-negative true pathogens (n=143/238), 34 isolates were extended-spectrum-β lactamase (ESBL) producing organisms, including *E.coli* (28/60, 46.7%) and *Klebsiella* spp. (6/32,18.8%). The antibiotic resistance patterns among common Gram-negative bacteria are given in Table 5. Of all Gram-negatives, the majority of *Acinetobacter* spp. showed resistance to all antibiotics tested except polymyxin B. *E.coli*, *Klebsiella* spp. and *Pseudomonas* spp. showed less resistance to amikacin and polymyxin B compared to other antibiotics.

DISCUSSION

BCC refers to the isolation of a microorganism which has been introduced into the blood sample during specimen collection (pre-analytical) up to the processing of the sample (analytical) and is not considered as the causative pathogen of bacteraemia of the patient (13). In the current study, data were collected from a tertiary care hospital with a single Microbiology laboratory. The staff in the lab and the technique of processing the blood cultures were the same throughout the study, thereby keeping the analytical and post-analytical factors that could contribute to blood culture contamination, constant. Therefore, in the current study, we consider that BCC could be primarily due to pre-analytical factors, eg: contamination during blood culture collection from patients.

The prevalence of BCC varies from 1% to 17% of BCs performed (14). Such variations may be explained by the sampling conditions and the definition of contamination itself. Factors influencing BCC rates depend on the hospital, the staff, and the type of patients. In this study, a total of 396 (21.3%) blood cultures were positive and the overall BCC rate was 8.5% BCC rate in ETU (12.5%) is significantly higher than GWs (7.8%) and ICUs (6.5%) giving 2.1-2.3 odds compared to these two units. The overall BCC value as well as unit-specific BCC values are higher than the recommended rate given by CLSI, which should be <3% (5). In the previous study done in Sri Lanka (9), the overall BCC was 5.6%. Even though, it was less than the rate in our study, surprisingly a higher rate was present in medical wards (53.6%). Comparison of BCC rate in ETU was not possible, as data were not available. Similarly, in a study conducted in the United Kingdom by Raja et al, the overall BCC contamination rate was 7.4% (15). Moreover, Lee et al. 2012 and Halverson et al. 2013, found that the higher rates reported in

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teaching hospitals, especially in emergency departments and rapid staff turnover, lack of ongoing training and workload may contribute to this phenomenon. Furthermore, Choi et al. in Singapore showed higher BCC rates in ED comprising 4% when compared to medical wards which was 0.5% (16). However, on the contrary, in a study by Ramirez et al., reported a higher BCC rate in ICU (31%) when compared to ED (20%) (13).

In the current study, the overall true positivity rate was 12.8% and, rates in ETU, ICU and GWs were 23.9%, 16.4% and 9.1% respectively. In comparison, to other studies worldwide, in which the true positivity ranges from 1.4% to 12.2% in ETU populations (17), these are better results. Similarly, in a study in Singapore, the percentage of positive blood cultures was 13.5% in the ETU group, compared with 6.0% in the GWs group (16). There was a lower rate of positive blood cultures in the general ward group. They have suggested that the reasons for the lower rate of TP in GWs may be due to prior antibiotics in the GWs reducing the sensitivity of blood cultures and a lower threshold for performing blood cultures (16). When considering the time-to-positivity of blood cultures, in this study, the growth of true pathogens took significantly less time (16.3 hours) compared to that of the contaminant (24.6 hours). When a CoNS is isolated, time to get positive for more than 20 hours is usually considered in favour of a contaminant (18). This fact will guide a clinician to decide the true pathogenicity of microorganisms, especially in the absence of other supportive evidence.

BCC occurs following the transfer of microorganisms from the immediate environment of the patient to the collected sample (19). In the current study, the predominant BC contaminant was CoNS (83.6%) and other contaminants were diphtheroids, *Bacillus* spp. and *Micrococcus* which are commensal flora of skin. Similarly, there are other studies with similar results including the previous study done in Sri Lanka (9, 20-22).

Regarding the true pathogens in blood cultures, we have isolated mainly Gram-negative organisms including *E. coli* (25.2%), *Klebsiella* spp. (13.4%), *Acinetobacter* spp. (8.4%). Among the Gram-positives, were MSSA (9.2%), MRSA (7.1%), and CoNS (7.1%). Of all CoNS, 7 were isolated from patients suspected of CLABSI which were diagnosed by repeated positive blood cultures with the same organism. Similarly, Chang et al, Gram-negatives and Gram-positives pathogens, most commonly encountered were *Escherichia coli* and *Klebsiella* spp. and *Staphylococcus aureus* and *Streptococcus* spp respectively (21). The true pathogen can be predicted along with the clinical diagnosis as it is the causative agent of sepsis. In this urosepsis was the commonest among known aetiologies, hence Gram-negatives should be the commonest isolate. Commensal flora of the skin eg. CoNS are an increasing source of CLABSI (23).

In the current study, there were factors associated with blood culture contamination other than the unit or the place of blood culture collection. A significantly higher contamination rate was seen in patients aged 65 years or older than those who were aged 64 years or younger, (14.1 vs 8.0) resulting in 1.89 odds. Similarly, in the study carried out by Chang et al, older patients (>65) (IRR: 1.02 per year) were more likely to be associated with BCC (21). The assumption was that the frequent visits of these patients to hospitals might have led to colonization with larger numbers of skin commensals with antimicrobial resistance as well as the presence of poorly accessible veins leading to difficult venipuncture (21). On the contrary, in a study in Nigeria, it showed that the BCC rate was higher in children than in adults (11% versus 8%) (20). There was no significant association between the gender of the patients and the method of processing of blood cultures, automated versus manual blood cultures with BCC. Similar to our study, Chang et al. 2015, also did not find any association with the gender of the patient (21).

The main strength of the present study was that this is the first attempt in Sri Lanka that compared BCC and true positivity between ETU, ICUs and GWs and determined factors

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associated with BCC. Data that we gathered here will greatly help in reducing BCC rates in those units which seem to be a neglected area at present. The reduction of BCC is a vital component not only to uplift the quality of the healthcare system but also to reduce the total cost incurred by the Government. Activities such as training of staff involved in the collection of blood culture samples, educational interventions, conducting regular audits and feedback are recommended to reduce blood culture contaminations in local settings. Based on regular surveillance, updating current guidelines and strict adherence to protocols will further improve the situation.

The limitations of this study were being retrospective in nature, some data were not available or incomplete e.g. comorbidities of the patients, which might have contributed to the study. Data related to antiseptic solutions used for skin cleaning prior to blood culture collection were not evident in our study.

CONCLUSION

BCC is a global problem irrespective of the availability of resources. In this study, the BCC was significantly associated with older age (≥65 years). The ETU recorded the highest BCC rate compared to those of ICUs and GWs and the estimated overall prevalence of BCC is higher than the global acceptable rate in all these units which highlights the necessity of adhering to strict aseptic conditions while drawing blood from patients.

Declarations. Ethics approval and consent to participate study were performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Review Committee of Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka (Date19. 10. 2022 /No123.07.2022). Informed consent was not sought as the details of patients were obtained retrospectively from the records available in the Microbiology laboratory.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. PV collected all the data and analyzed and wrote the initial draft. SW analyzed and major contributor to writing the manuscript. BP sorted and interpreted the data in the microbiology lab. MR collected and generated tables and figures. All authors read and approved the final manuscript.

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