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Coagulase Negative Staphylococcus as a Cause for Bacteraemia: Mere a Contaminant or Potential Threat as Pathogen, an Observational Study

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ABSTRACT:

Introduction: Coagulase-negative Staphylococcus is considered as one of the most common skin commensal bacteria in human beings. Usually, patients with weakened immune system (with prosthetic devices, pacemakers and intravenous catheters) are prone to develop infections due to CONS. They were previously considered as culture contaminants, but in the past decades they are gaining significance as significant pathogen. A contaminant can be differentiated from a pathogen depending upon various clinical and microbiological aspects. Clinically, a true infection is generally considered in patients with fever with or without Leukocytosis or hypotension whereas growth of CONS in blood culture within 48 hours of inoculation along with multiple blood cultures (>2) positive for same organism with similar antibiograms favours microbiological part of infection related to that particular pathogen.

Materials and Methods: Data were collected prospectively over a period of 1 year (January 2023-December 2023) to determine the prevalence of significant CONS bacteraemia at our tertiary care setup by automated culture and sensitivity methods (Biomerieux vitek-2 compact) and to evaluate drug therapy in patients with repeated cultures positive for CONS by Kirby-Bauer disc diffusion method on Muller-Hinton agar. The study findings were interpreted on the basis of CLSI standards. Detection of methicillin resistance among CONS was performed using cefoxitin disk (30 µg) diffusion method. Data analysis was done using descriptive statistics. The study was approved by our Ethics committee (IEC no GBCM/IEC/2024/05-01)

Results: Blood culture positivity came out to be 35% at our tertiary care set up in a period of one year. Out of which majority of the isolated organisms were CONS (73.09%) and rest (other than CONS) were 26.01 %. Among blood isolates other than CONS, most common isolates were Staphylococcus aureus, Echerichia coli, Acinetobacter calcoaceticus baumanii complex and few were Klebsiells sp, Pseudomonas and Enterococcus spp. The interesting fact which came out as a result in our study that, out of 133 CONS isolates only 97(73%) were positive in multiple cultures proving others to be a mere contaminant (23%). The samples which were failed to come as repeat cultures due to unavoidable circumstances were not calculated in the study. Among all the CONS isolates, Staphylococcus hominis subspecies hominis predominated (62 %) followed by Staphylococcus epidermidis (25 %), Staphylococcus hemolyticus (08 %), Staphylococcus saprophyticus (04 %) one isolate was identified as Kockuria Kristae (1%). Most of the CONS isolates were resistant to penicillin (91 %) and azithromycin (94 %), followed by levofloxacin and clindamycin. Least resistance was shown to vancomycin and linezolid (3%) followed by teicoplanin. Cefoxitin resistance CONS was detected in 62 (64%) isolates.

Conclusion: To conclude, CONS bacteraemia is one of the most considerate reasons for nosocomial blood-stream infections and its crucial to look upon these isolates as pathogen, not mere contaminant. The relevance of repeated blood cultures is proved in our study and many studies prior to rule out contaminant from clinically significant pathogen. As they could put a profound impact on an institution's bloodstream infection rates therefore slight improvement in hand hygiene measures could decrease the blood culture contamination rates. which in return will restrict unnecessary antibiotic usage and decrease antimicrobial resistance.

KEYWORDS: Sepsis; Blood culture; Bacteraemia, Coagulase Negative Staphylococcus aureus, CONS, Hand Hygiene, Methicillin Resistant Staphylococcus, Contaminants

INTRODUCTION

Blood culture is one of the most common laboratory tests performed especially in critically ill patients and the contaminated ones are apparently increasing with in past few decades leading to increased cost and confusing case scenario. On clinical evaluation of these isolates, it is being observed that although there is a relative increase of CONS associated bloodstream infections in recent years, these microorganisms are still considered as the most common contaminants in blood cultures [1]. The significance of CONS in infectious medicine came in to picture when isolation of these bacteria from infections in humans were documented by various

authors [2]. Although various guidelines are there to define or differentiate between true pathogen and blood culture contaminant but still a true gold standard is lacking till date. The most common contaminants which we commonly encountered during positive blood cultures are coagulase-negative staphylococci, aerobic and anaerobic diphtheroids, Micrococcus spp., Bacillus spp., and viridans group streptococci. Among all, CONS are considered as one of the commonest ones. Microorganisms that usually represent true bacteraemia or fungemia include Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli and other members of the Enterobacteriaceae, Pseudomonas aeruginosa, and Candida albicans [3]. Single blood culture is usually not considered significant until otherwise their sensitivity is being asked by clinicians. Additional blood cultures if came positive within 48 hrs of previous one with same pathogen with the help of clinical history these pathogens can be taken significant. The clinical significance of blood culture isolates is although a tedious task to be done but by accurately identifying the contaminants the associated costs, the irrational antibiotic usage and antibiotic resistance can be lowered down in near future.

MATERIALS AND METHODS

A prospective, observational study was conducted at a tertiary care hospital after approval from our Institutional ethical committee ((IEC no GBCM/IEC/2024/05-01) to evaluate the rate of contamination in blood cultures. Data was collected prospectively over a period of 1 year (January 2023-December 2023) to determine the prevalence of significant CONS bacteraemia at our tertiary care setup by automated culture and sensitivity methods (Biomerieux vitek-2 compact). The paired specimens of blood which came positive for CONS bacteria using automated culture methods were identified by standard methodology. Microbiological parameters along with clinical data, laboratory indices, and patient characteristics were analysed. The single positive blood cultures were excluded from the study. These patients were either symptomatically better and discharged or due to financial reasons, second blood cultures could not be obtained. Evaluation of drug therapy in patients with repeated cultures positive for CONS were also studied to see the antibiotic resistance pattern. Antibiotic susceptibility was determined by using the Kirby Bauer's disk diffusion method as per Clinical and Laboratory Standards Institute (CLSI) recommendations using the Mueller Hinton agar (Hi-Media, Mumbai, India) and commercially available 6 mm antimicrobial disks of penicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg), nitrofurantoin (300 µg), cotrimoxazole (1.25/23.7 µg), ciprofloxacin (5 µg), amikacin (30 µg), linezolid (30 µg). Detection of methicillin resistance among CONS was performed using cefoxitin disk (30 µg) diffusion method. Diameter of the circular zone of inhibition ≥25 mm was interpreted as sensitive and ≤24 mm as resistant for CONS, except for S. lugdinensis for which zone diameter ≤21 mm was considered as resistant.

RESULTS

Total 182 blood cultures came positive out of total 528 cultures received at Microbiology department (34.46%). Among 182 culture positive isolates, CONS recovered were 133 (73.07%) and other than CONS were 49 (26.92%). Out of 133, single blood culture positive for CONS isolates were 36 (27.06%) and double/multiple blood culture positive were 97(72.93%). Single blood culture CONS isolates were considered as contaminant (clinical basis and negative second blood culture) and not included in further study. Out of 97 isolates females predominated over males with ratio being 1.4:1. Maximum CONS isolates belonged to age group < 20 years (n=61, 63%) followed by 41-60 years of age group (n= 16, 16.49%) and >60 years age group (n=12,12.37%) and least belonged to age group 21-40 (n=11,11.34%) [Table and Fig.1]. The peak months of CONS isolates recovered were August and September. The growth time in our study for culture positive patients were 48-72 hrs for 123 out of 133 CONS isolates. Growth time for rest isolates were 24-48 hours. Among all the CONS isolates, Staphylococcus hominis subspecies hominis predominated (62 %) followed by Staphylococcus epidermidis (25 %), Staphylococcus hemolyticus (08 %), Staphylococcus saprophyticus (04 %) one isolate was identified as Kockuria Kristae (1%) [Table 3]. Besides CONS, other blood culture isolates identified were 49 (34%). Out of 49 isolates, Staphylococcus aureus predominated being (25%) followed by Echerichia coli (16%), Acinetobacter calcoaceticus baumanii complex (14%), Klebsiella pneumoniae (12%), Enterococcus species and Candida species (9%), Citrobacter freundii and Pseudomonas spp. and Streptococcus spp. (5%). Sphingomonas paucimobilis and Proteus mirabilis were the least common species isolated being 2% [Table 4]. Maximum resistance among CONS isolates was shown to Azithromycin (94%), Penicillin (91%), Levofloxacin (86%), clindamycin (73.2%), Amoxicillin-clavulanic acid and tetracycline (66%). Majority of CONS isolates were Cefoxitin Resistant (64%). Minimum resistance was shown to Vancomycin and Linezolid (3%), Teicoplanin (14%) and Doxycycline, Gentamycin being 27% and 29%. [Table 5].MRS was detected among 62 (64%) CONS isolates by using Cefoxitin Disk diffusion method.

Table 1: Age & Gender Wise Distribution of Blood Culture Isolates

S. N.	CONS (n=97)		OTHERS (n=49)	
	MALE	FEMALE	MALE	FEMALE
0-20	28	33	18	09
21-40	03	08	03	02

41-60	06	10	06	02
>60	03	06	06	03
Total	40	57	33	16

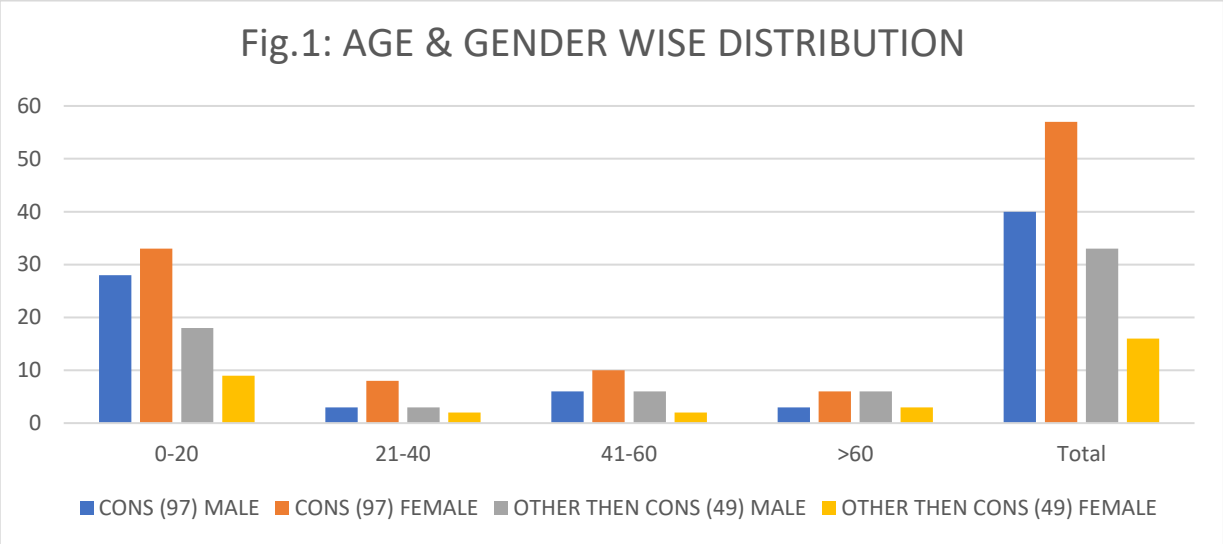

Table 2: Month-Wise Distribution Of Blood Culture Isolates (N=146)

MONTH (2023)	**SAMPLE**	**Total Culture**	**CoNS (n=97)**	**%**	**OTHER THAN CoNS (n=49)**	**%**
JANUARY	BLOOD	62	09	9.27	08	16.32
FEBURAY	BLOOD	31	04	4.12	03	6.12
MARCH	BLOOD	34	09	9.27	01	2.04
APRIL	BLOOD	33	06	6.18	04	8.16
MAY	BLOOD	38	08	8.24	03	6.12
JUNE	BLOOD	34	09	9.27	02	4.08
JULY	BLOOD	25	09	9.27	03	6.12
AUGUST	BLOOD	52	**14**	14.43	09	18.36
SEPTEMBER	BLOOD	72	**17**	17.52	06	12.24
OCTOBER	BLOOD	74	07	7.21	08	16.32
NOVEMBER	BLOOD	49	01	1.10	08	16.32
DECEMBER	BLOOD	24	04	4.12	01	2.04
Total		**528**	**97**	**100**	**49**	

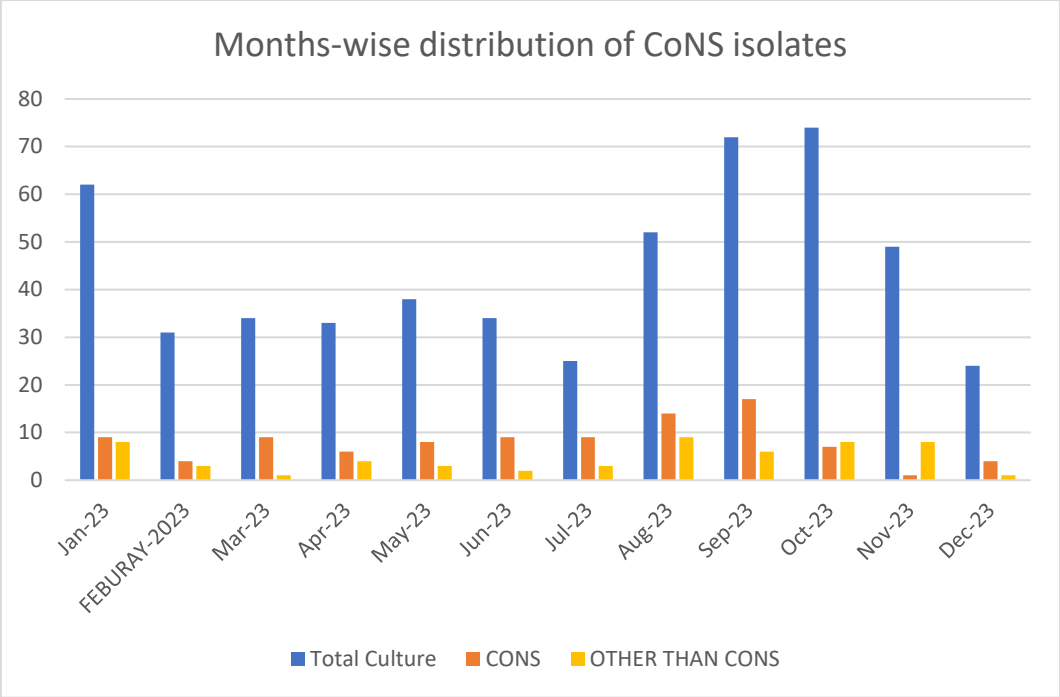

Table 3: Distribution of BLOOD CONS isolates (n=97)

S. N0.	ISOLATE	Number (n=97)	Percentage (%)
1	Staphylococcus hominis subspecies hominis	60	62
2	Staphylococcus epidermidis	24	25
3	Staphylococcus haemolyticus	08	08
4	Staphylococcus saprophyticus	04	04
5	Kocuria kristinae	01	01

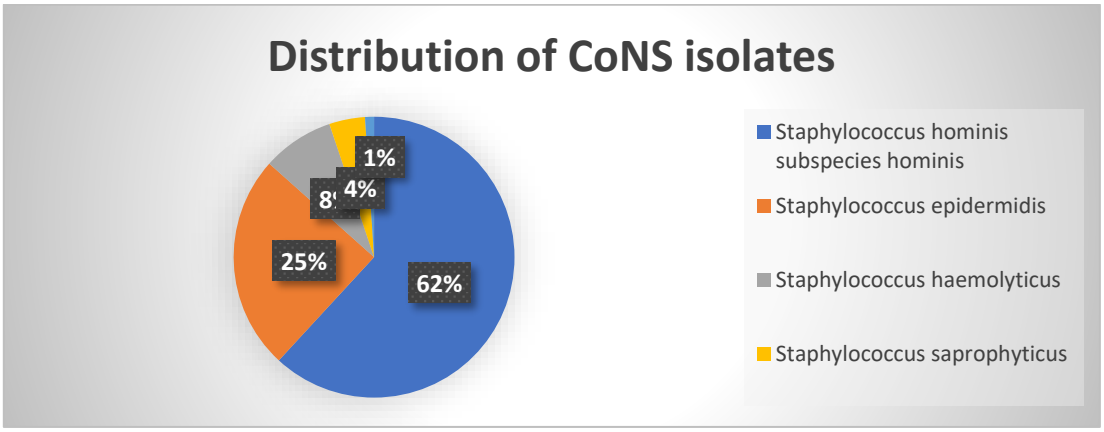

Table 4: Blood culture isolates besides CONS (n=49)

S.NO.	Isolate	No. of isolates (n=49)	Percentage (%)
1	Staphylococcus aureus	09	18.38
2	Escherichia coli	08	16.34
3	Acinetobacter species	07	14.28
4	Klebsiella pneumonaiae	06	12.24
5	Enterococcus species	04	08.16
6	Candida species	04	08.16
7	Citrobacter freundii	02	04.08
8	Pseudomonas species	02	04.08
9	Streptococcus species	02	04.08

10	Enterobacter aerogenes	02	04.08
11	Proteus mirabilis	01	02.04
12	Sphingomonas species	02	04.08

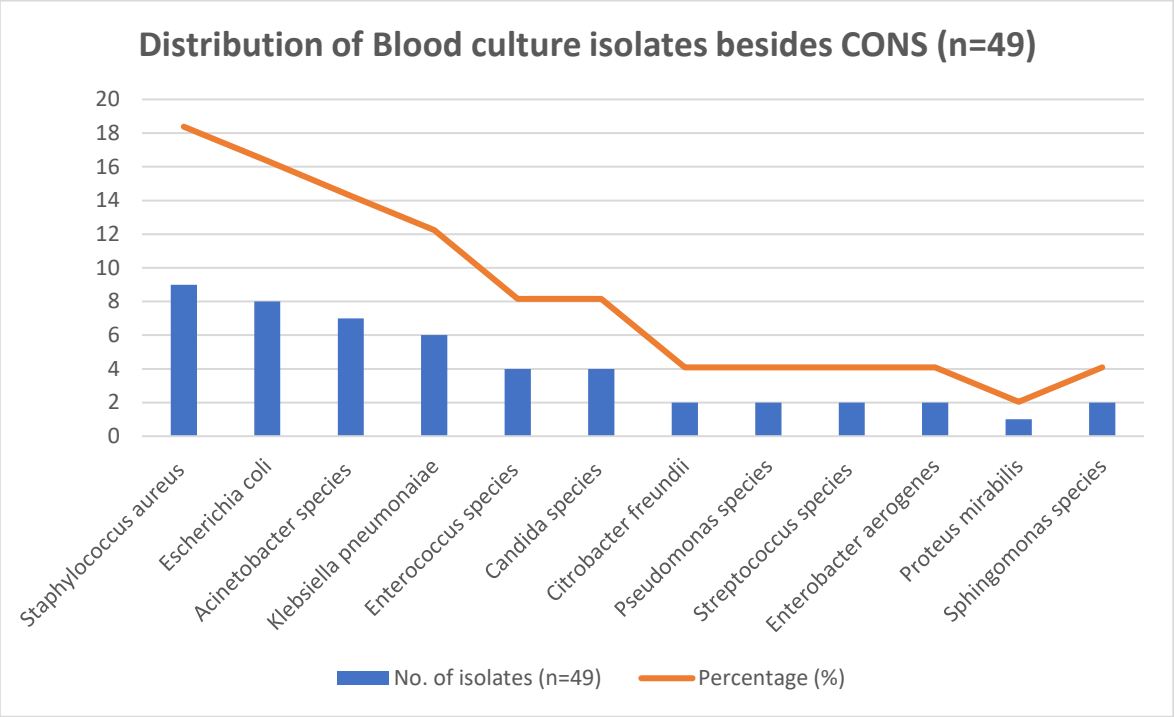

Table 5: Antibiotic resistance & sensitivity in CONS isolates (n=97)

S. N.	Antibiotics	Resistant	Sensitive	INTERMEDIATE
1	Cefoxitin	62	35	Nil
2	Penicillin G	89	08	
3	Vancomycin	03	94	Nil
4	Linezolid	03	94	Nil
5	Clindamycin	71	26	Nil
6	Azithromycin	91	06	Nil
7	Teicoplanin	14	83	Nil
8	Gentamycin	28	68	01
9	Doxycycline	26	71	Nil
10	Amoxicillin-clavulanic acid	64	33	Nil
11	Levofloxacin	83	14	Nil
12	Tetracycline	64	33	Nil

DISCUSSION

Blood culture is done routinely at each and every setup to investigate the cause of fever/sepsis/infection in almost all hospitalized patients. The importance of finding true pathogen from blood culture and providing necessary antibiotic treatment is needed foremost. Isolation of CONS always creates confusion among clinicians and Microbiologists and requires either paired specimens or other routine lab investigations to consider it as significant pathogen [3-6]. True bacteraemia includes growth within 48 hrs and more than 2/multiple blood cultures positive for the same microorganism. Contaminated cultures can be assumed by increased growth time (72 hrs usually) and repeated bacterial growth for skin commensal flora during antibiotic treatment [1-2,7]. In our study, total 182 blood cultures came positive out of total 528 cultures. Among which CONS isolates were 133 and rest were 49. Out of 133 CONS isolates, 97(73%) was considered as true pathogen. Single blood culture CONS isolates were considered as contaminant (clinical basis and negative second blood culture) and not included in further study.

Total bacteriological culture yield in our study was 27.8 % excluding the contaminants grown in blood culture This is consistent with the findings by Prabhu K et al [44%] ,33 % by Kokku and Sharma et al, 19 % by Oyakale et al [8-11]. In contrast with, study by Birru et al and Arora et al, stated low positivity rate in their studies being 9.8% [6]. The reason might be their short duration study and low blood cultures received. Majority of isolates recovered in our study were CONS recovered were 73.07% followed by Staphylococcus aureus (18.4 %), Escherichia coli (16.3%), Acinetobacter (14.3%), Klebsiella spp. (13.2%), Enterococcus and candida (8.2%) and the least were Pseudomonas, Proteus and Citrobacter spp. (2%). Similarly, a study by Kwarlosky et all showed maximum blood culture isolates to be CONS (42.1%) followed by Staphylococcus aureus (16.3%), Enterococcus faecalis (8.3%), Escherichia coli (7.2%), Klebsiella pneumoniae (3.6%), and Enterococcus faecium (3.5%) and rest of isolates to be similar except few [12]. Another study by Kokku et al also found CONS to be most common isolate being 27%. Male to female ratio in our study was found to be [1:2] for both CONS and other than CONS isolates whereas Kokku et al and sharma et al showed male preponderance with male to female ratio being [3:2]. Another study by Singh et al showed female (74.8%) preponderance in their study [13]. With respect to age blood culture positivity was found to be higher in children < 20 years, followed by 40-60 years and least age group involved was >60 years in our study. Prevalence of bloodstream infections varied among different age groups. Higher incidence of patients was at the lower extreme of ages in this study, which was comparable to studies carried out by Zosangliani et al, Pradhan et al. [14,15]. Age group of 20-29 years showed highest isolation of CoNS (28.9%) while no isolate was recovered from the age group of 70-79 years in study by singh et al [13]. Similar parameters were reported by Alex AM et al [16]. On the contrary, Asangi SY et al., and Baddour LM and David L found majority of the CoNS isolates in males and above the age group of 40 years [17,18]. In contrast Roopa C and Biradar S revealed maximum number of isolates in the age group of 61- 70 years with no gender predominance [19,20]

There was no such specific seasonal pattern shown in culture positive isolates except peak season of blood cultures received and culture positivity rate both were maximum during months of August, September and October. The reason might be usual higher frequency of viral illnesses and infections during monsoon period. The frequency of Gram positive and Gram - negative bacteria isolated from blood culture in the present study was 72 % and 28 % respectively. These findings are analogous to studies done in Manipur (64.7 % versus 35.3 %), Mangalore (64.19 % vs 34.56 %), Hyderabad (54.3 % vs 45.7%), Cameroon (56.2 % versus 43.8 %) 11 and Tanzania (82.1 % vs 17.9 %) [13-14,21-22]. In contrast to the present finding, higher incidence of Gram - negative bacteria was reported by other studies done in Lahore (50.1 % vs 47.5 %) and Uganda (58% vs 42 %) [23,24]. The growth time (time duration for obtaining positive culture) in our study came out to be 48-72 hours for 123 out of 133 (92.4%) CONS isolates,

24-48 hrs for all isolates other than CONS. The reason might be lower generation time for pathogenic bacteria as compared with contaminants. This finding was in concordance with other studies like Sidhu SK et al [1]. Staphylococcus hominis subspecies hominis was the commonest species isolated in our study followed by Staphylococcus epidermidis Staphylococcus hemolyticus and Staphylococcus saprophyticus. Only one isolate was identified as Kockuria Kristae. A study by Weinstein et al stated most common species to be Staphylococcus epidermidis followed by Staphylococcus hominis and Staphylococcus haemolyticus [25]. Maximum resistance among CONS isolates was shown in descending order from Azithromycin, Penicillin, Levofloxacin, clindamycin, Amoxicillin-clavulanic acid and tetracycline to minimum resistance shown to Vancomycin, Linezolid, Teicoplanin, Doxycycline and Gentamycin. Majority of CONS isolates were Cefoxitin Resistant (64%). Study by Singh et al showed maximum resistance to penicillin (84.5%) and least to tigecycline (2.2%). No resistance to vancomycin and linezolid was seen. Studies by Usha MG, Asangi SY, Sharma V and Pedroso SHSP and Gunti R et al., have shown maximum resistance to penicillin, erythromycin, ciprofloxacin and cotrimoxazole with over 80% which correlate with the present study [13,26-27]. Alex AM et al., and Jayakumar R et al., noted uniform susceptibility to vancomycin and linezolid in their study [16,28].

CONCLUSION

Despite numerous advances in blood culture techniques, many of the hospitals and laboratories have noted a significant rise in proportion of contaminated blood cultures in recent years. Possible explanations for this might be the newer continuously monitoring blood culture systems and media modifications have improved algorithms for detecting microbial growth and microorganisms in very low numbers that were previously missed [29-32]. Laboratory work up for blood culture pathogens: Richter et al validated, and implemented an algorithm to minimize the workup of blood culture contaminants according to which If two or more blood cultures are obtained and only one is positive, the isolate is reported as a probable contaminant and susceptibility testing is not done unless asked [7]. If only a single blood culture is obtained and grows one of the likely contaminants, clinical correlation is required to call it contaminant or pathogen. If two or more blood cultures are obtained and two cultures are positive within a 48-h period, with similar organism which appears to be similar on their antibiogram profile or biochemical/molecular testing, this is considered clinically significant bacteraemia. If different organisms are grown they are considered as probable contaminant.

The increased use of central venous access catheters for the purpose of obtaining blood samples to prevent patient from extra prick and pain also contributes in increasing blood culture contaminants [31,32].There is a crucial need for lower down the blood culture contamination to decrease the hospital cost, antibiotic usage which in turn leading to increase multidrug resistance by changing our collection methods a bit obtaining blood via venipuncture rather than from an intravascular catheter or using a two-needle rather than a single-needle technique and strengthening our infection control practices. Some evidences are there that proper use of antiseptic before taking blood samples (with at least 30 sec-1 min contact time) leads to significant drop in blood culture contaminants [33-36]. Trained phlebotomists or blood culture teams can also reduce contamination rates in individual institutions to a significant level [35,36].

LIMITATIONS

Due to lack of Infrastructure advanced molecular methods for molecular characterisation of CONS at the subspecies level could not be accessed and may be carried out in future for further reference.

ABBREVIATIONS

CONS: coagulase negative staphylococcus
GBCM: Gautam Buddha chikitsa Mahavidyalaya
IEC: Institutional Ethical Committee
MRS: Methicillin Resistant Staphylococcus
CLSI: Central Laboratory Standard Institute

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