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Facultative Anaerobic Bacterial Profile of Bacteremia and Septicemia among ICU Patients and its Antibiotic Susceptibility Pattern in Central Nepal

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ABSTRACT

Introduction

Bacteremia and septicemia is a life threatening condition resulting in major cause of morbidity and mortality in patients. The aim of this study was to determine prevalence of bacteremia and septicemia among surgical ICU patients and its antibiotic susceptibility pattern.

Methods

A cross-sectional study was carried out among the suspected cases from surgical ICUs of COMS-TH from July 2017 to December 2020. Blood samples were collected, processed, isolated and identified according to standard methodology. Multidrug resistance in Gram negative bacterial (MDR) and methicillin resistant *S.aureus* (MRSA) screening was done by following the standard protocol.

Results

A total number of 450 samples were processed, 48(10.7%) bacterial isolates from patients' blood sample showed positive by culture from department of surgical ICUs, College of Medical Sciences Teaching Hospital, Nepal. This study showed more incidences of gram negative isolates which are responsible for septicemia as compared to gram positive isolates.

Most frequently used drugs like Ciprofloxacin (83.9%), Gentamycin (74.2%), Ceftrixone and Ampicillin (71.0%), Cefazolin and Chloramphenicol (67.7%), Ofloxacin (67.7%), Amikacin (64.5%), Amoxyclave (61.3%), showed high rate of resistance among the isolates. Cefotaxime and Co-Trimoxazole (58.1%) showed second highest resistance pattern among GNB isolates from ICU patients. The least resistance pattern among the GPC was found in drugs like Amikacin and Azithromycin (47.1%) and Vancomycin (35.3%) Meropenem (29.4%). Coagulase negative *Staphylococcus* (CONS) had shown MDR 66.6% showed highest resistance pattern among *Enterococcus* spp, CoNS and *S.aureus*. This indicates most of the organisms were either moving towards resistance or already acquired resistance against antibiotics.

Conclusions

Blood culture positive rate of the isolates from surgical ICUs of COMS-TH was 48(10.7%) of the total 450 samples. *Pseudomonas aeruginosa* and *S.aureus* were most common pathogen causing bacteremia and septicemia. None of the antibiotics were 100% sensitive for *P.aeruginosa,* even Meropenem showed reduced sensitivity. Among all antibiotics Ampicillin and Amikacin followed by Ciprofloxacin has lowest sensitivity towards the organisms.

Keywords: bacteremia and septicemia; ICU patients; bacterial isolates; AST.

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INTRODUCTION

Bloodstream infections (BSI) are associated with high rates of morbidity and mortality worldwide, with mortality rate ranging from 20% to 70%.[1] Overall, early about 750,000 patients develop bacterial, viral or fungal BSI in the United States, resulting in 215,000 patient deaths. Recent year published data estimated that the most dangerous clinical manifestations of BSI, sepsis and septic shock, are the 10th leading cause of mortality in the United States and other developed countries, accounting for 6% of all deaths (50.4% deaths per 100,000 individuals in the overall population).[2] In Asia, an estimated data shows that 135,000 patients die each year because of sepsis and sepsis-associated complications, with an overall incidence of sepsis of 3 cases per 1,000 cases.[3] A national prospective multicenter research performed in Germany demonstrated that sepsis is the third most common cause of death in that country, with an overall prevalence of 22.4%, almost equally distributed between Severe Sepsis (SS) (12.5%) and Septic Shock (SS) (11.3%).[4]

Bacteremia is the presence of bacteria in the blood as confirmed by blood culture which is may be transient or associated with sepsis leading to the organ failure in a patient. Septicemia is the presence of microbes or their toxins in the blood with clinical evidence of infection and its systemic response manifested by two or more of the following aspects. Severe sepsis along with hypotension despite of giving adequate fluid resuscitation is called Septic shock.[5]

- Temperature >100.4°C (38°C) or <96.9°C (36°C)
- Heart rate >90 beats/ minute
- Respiratory rate >20 breaths/ minute or arterial CO_2 tension <32mmHg (3.4kPa)
- White Blood Count (WBC) >12,000 cells/ml, 4000cells/ml or >10% immature forms (band cells)

Bloodstream infection are important cause of serious morbidity and leading cause of mortality, among the most common hospital acquired infection.[6,7] They are associated with the syndrome requiring admission to intensive care unit such as sepsis and septic shock.[8] Research data also revealed that, septic shock are not only considered as leading cause of death, but also causing disability, worsening of quality of life especially for millions of people in developing countries like in Nepal and India.[9] Blood culture is done to reveal the presence of microorganism which is a highly specific indicator of blood infection and the results of antimicrobial susceptibility testing may assist in choice of appropriate antimicrobial therapy for such patient. Therefore, early and rapid administration of specific antimicrobial therapy to such patient shown to reduce mortality and morbidity.[10] The resistance pattern is related with series of social, environmental and technological changes in technology.[11] The surveillance of bloodstream microbes in a hospital setting is important in monitoring the spectrum of bacteria that invades the bloodstream and the type of pathogens associated with a particular clinical discipline. Such analytical research data is often used to determine empiric antibiotic therapy and also to alert Doctor regarding emerging and reemerging pathogen that may be a possible threat to the community.

METHODS

Specimen collection

A total of 450 blood samples from the suspected cases of bacterimia and septicemia that were admitted in different ICUs of College of Medical Science-Teaching Hospital (COMS-TH), Bharatpur, Nepal between July 2019 and December 2020 were included in this prospective study. All specimens were collected according to standard protocol by the American society

of Microbiology (ASM).[12] Blood volume were different according to age group as mentioned follows:

a. Child of 1 year : 1-3ml.
b. Children aged above 8 years: 5-10ml, divided between two blood cultures.
c. Adult : 10-20ml, divided between two blood sample for culture.[13]

Collected blood volume was then inoculated aseptically in Brain Heart Infusion Broth (BHI) at the ratio 1:10 (sample: broth) without changing needle before infusing the blood into the blood culture bottle. Without opening the cap, blood was infused through the hole that is present on the cap after lifting the cello-tape. Needle was then withdrawn and tape was immediately replaced. Blood was then gently mixed. Then the needle was destroyed without recapping. Specimens were received in laboratory with requisition form which consists of patient's name, age, sex, bed number, date and time of collection and brief history. Information regarding duration of hospital stay, antibiotic history was also taken into consideration whenever possible during the processing of specimens.

Processing of specimens

Macroscopic examination of broth culture

The culture bottles were examined for any viable evidence of microbial growth, such as turbidity to make presumptive diagnosis of positive blood culture.

Subculture done on solid media

The culture bottles were incubated at 37°C aerobically and all the specimens were blindly subcultured after 18 hrs and 48 hrs on blood agar and MacConkey agar, incubated aerobically and in 5% CO_2 in a candle jar for 24-48hrs. The bacterial colonies grown on either of the media were identified as per the standard methods.[14] The blood culture specimens which did not show any growth were re incubated upto 7th day, at the end of which final subculture was carried out. The culture specimens not showing any growth after the final subculture were reported as sterile and discarded.

Microscopic Examination

After doing blind subculture positive cultures were examined by gram's staining method. During bacterial identification, various factors including morphology of the organism (size, shape, arrangement), gram's staining of microbe, uniformity of the strain, pure or mixed form of organism, number of organism whether plenty, moderate or scanty like features were noted.

Subculture of positive sample

Macroscopically and microscopically positive broth were sub-cultured on 5% Sheep Blood agar (BA), MacConkey agar (MA) (HiMedia) plates. Then, these plates were incubated aerobically at 37°C for 24 hours. If there were no visible growth colony than culture plates were further incubated at 72hours at 37°C for suspected slow growing organisms.

Identification of isolates

Identification of significant isolates was done by following standard microbiological techniques which involved morphological appearance of the colonies; gram's staining reaction, catalase test, coagulase test, oxidase test with other biochemical properties. Blood agar was observed for hemolysis, MacConkey for lactose-fermenter or non-fermenter.

Biochemical tests used for the identification of pathogen

Different biochemical tests will be done for the identification of the bacterial isolates. At first, pure culture will be obtained from the primary culture and then, it will be processed for biochemical tests. The biochemical media,

employed will be triple sugar iron agar (TSI) media, sulphide indole motility (SIM) media, simmon's citrate media, chirstensen's urea media, methyl red/voges proskauer (MR/VP) media and other as per requirement.

Antibiotic Susceptibility Testing

The antibiotic sensitivity tests of the pathogen isolated from clinical specimen against different antibiotic was done using mueller hinton agar (MHA) (HiMedia) by the standard disk diffusion technique of Kirby-Bauer method.[15] At least three to five well isolates colonies of the same morphotypes were selected from the MHA plate. The base of each colony was touched with an inoculating wire and the growth were transferred into a tube containing 5ml of nutrient broth or required ideal broth for fastidious organisms and then incubated at 37°C (usually 2 to 6 hours) until it achieved turbidity.

For testing *Staphylococcus* spp direct colony suspension method was employed by making a direct broth or saline suspension of isolated colonies selected from a 16-24 hour growth on culture plate. A sterile cotton swab was dipped into broth and the swab rotated several times and pressed firmly on the inner side of the tube above the fluid level to remove excess inoculums from the swab. Then the dried surface of a MHA plate was inoculated by streaking the swab over the entire agar surface three times, turning the plate 60°C between streaking.

The predetermined battery of the antimicrobial disks was placed on the surface of the prior inoculation agar plate such that there are 24 mm distances from disk to disk. The disks were slowly pressed down to ensure complete contact with the Mueller Hinton agar surface. For other 15 minutes of applying disks, the plates were left at room temperature to allow antimicrobial to diffuse from the disk. Then they are incubated aerobically at 37°C overnight.

After overnight incubation, the diameter of zone of inhibition (ZOI) of each disk was measured (including the diameter of the disk) and recorded in millimeter. In the case of blood agar plates, the zone was measured from upper surface of the agar illuminated with refracted light, with the cover removed. For *Staphylococcus* spp and *Enterococcus* spp 24 hour of incubation was followed and transmitted light was used to examine the Oxacillin and Vancomycin zone of light within the apparent ZOI.[16]

The result was then compared with standard chart developed by Hi-media, determine bacterial susceptibility towards different antimicrobial agents in term of 'Sensitive-S', and 'Resistant-R. The measurements was made with a ruler on the under surface of the plate without opening the lid. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 will be also tested, in every set of experiment, in parallel, as a part of quality control.

For disk susceptibility testing, in our study, Ampicillin (10mcg), Cefotaxime (30mcg), Co-Trimoxazole (25mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Cefazolin (30mcg), Ceftriaxone (30mcg), Amikacin (30mcg), Ofloxacin (5mcg), Meropenem (10mcg), Gentamycin (120mcg), Amoxyclav (30mcg), Piperacillin/Tazobactam (100/10mcg), Carbenicillin (100mcg), Ampicillin/Sulbactam (10/10mcg), Penicillin (10units), Clindamycin (2mcg), Oxacillin (1mcg), Azithromycin (15mcg) and Vancomycin (30mcg).

Tests for MRSA

One microgram Oxacillin disk test was used for disk diffusion method to find out MRSA, recommended by CLSI was put up and agar plates were incubated at 37°C. Oxacillin disk containing plates were read at 24 hours incubation. The diameter of the zone of inhibition of growth was recorded

and interpreted as susceptibility or resistant by criteria as CLSI guide lines. *S.aureus* ATCC 25923 was used as negative and positive controls respectively. Organisms were shown Methicillin resistant when zone of inhibition was < 10mm for *S.aureus* with Oxacillin disk and < 21 mm for *S.aureus*.[17]

Statistical Analysis:

All data will be analyzed by appropriate statistical tool, statistical package for social science (SPSS version 16). It was compared by T-test for continuous variables and chi square test for categorically variables. The statistical significance was defined as 'P' value less than 0.05. Analysis was carried out using Microsoft excel, SPSS software version 16 and others as per requirement.

RESULTS

Case distribution according to the culture positivity

During the study period from dated: July 2017 to December 2020 a total number of 450 samples were processed where we found blood culture positive in only 48/450 (10.7%) of cases. Among those 48 blood culture positive cases 30 (62.5%) cases were observed in male patients, whereas, 18 (37.5%) episodes were observed in female patients. Age of the patients ranges from 1 to above 80 years. Among 48 patients, 28 (58.3%) patients had single and 20 (41.7%) patients had multiple episodes of septicemia (4 had 1 episodes and 1 had 2 episodes of septicemia). A total of 48/450 (10.7%) bacterial isolates from ICU patient blood sample showed positive by culture from different ICU, College of Medical Sciences Teaching Hospital, Nepal.

Distribution of the bacterial Isolates

Distribution of gram positive cocci 17(35.4%) and gram negative bacilli 31(64.6%) from different cases of bacteremia and septicemia showed highest percentage of gram negative bacilli is responsible for the cause of disease.

Distribution of individual bacterial isolates

A total of 48 strains were isolated from blood samples with true septicemia from ICU patients. Distribution of aerobic bacteria from cases of septicemia identified as gram negative bacteria were *P.aeruginosa* 8(16.6%), *Acinetobacter* spp 7(14.5%), *Escherichia coli* 4(8.3%), *Proteus mirabilis* 4(8.3%), *Klebsiella pneumoniae* 3(6.3%), *Enterobacter* spp 2(4.2%), *Citrobacter* spp 2(4.2%), *Serratia* spp 1(2.1%) and gram positive bacteria were *S.aureus* 5(10.4%), Coagulase negative *Staphylococcus* (CoNS) 4(8.3%), *Streptococcus pneumoniae* 3(6.3%), *Streptococcus pyogenes* 3(6.3%), and *Enterococcus* spp 2(4.2%) as shown in Table 1.

Table 1. Shows list of an organism isolated from surgical ICU patients.

Bacterial Isolates	Number	Percentage
P. aeruginosa	8	16.6
Acinetobacter spp	7	14.5
Escherichia coli	4	8.3
Proteus mirabilis	4	8.3
Klebsiella pneumoniae	3	6.3
Enterobacter spp	2	4.2
Citrobacter spp	2	4.2
Serratia spp	1	2.1
GNB Total	**31**	**64.6**
S. aureus	5	10.4
CoNS	4	8.3
Streptococcus pneumoniae	3	6.3
Streptococcus pyogens	3	6.3
Enterococcus spp	2	4.2
GPC Total	**17**	**35.4**
Total	**48**	**100**

Susceptibility pattern of GPC and GNB for different antibiotics

Blood culture and susceptibility report showed

out of 450 patients, 48(10.7%) were confirmed as culture positive aerobic bacteria. The overall rate of bacterial isolation reduced with increasing age but the types of organisms cultured did not vary with age of the patients. The most frequent gram negative bacilli (GNB) isolates were *P.aeruginosa* 8(16.6%) and gram positive cocci (GPC) were *Staphylococcus aureus* 5(10.4%). According to the given table 2 and 3, in total antimicrobial resistant rate of GPC and GNB were found to be 57.3%. The sensitivity rate of GNB is (43.4%) and resistance rate is (56.6%) and isolated GPC showed sensitive of (41.4%) and resistance of (58.6%). We did not isolate anaerobes. Our laboratory technique is not sensitive enough to detect obligate anaerobes.

Percentage resistance of gram negative isolates to different antimicrobials

Present research shows most of the drugs with high resistance rate among all GNB isolates (56.6%). Among GNB isolates *P.aeruginosa*, *Acinetobacter* spp., and *Klebsiella* spp. showed average of (60.5%) multidrug resistance (MDR). Most frequently used drugs like Ciprofloxacin (83.9%), Gentamycin (74.2%), Ceftrixone and Ampicillin (71.0%), Cefazolin and Chloramphenicol (67.7%), Ofloxacin (67.7%), Amikacin (64.5%), Amoxyclave (61.3%), showed high rate of resistance among the isolates. Cefotaxime and Co-Trimoxazole (58.1%) showed second highest resistance pattern among isolates from ICU patients. Drugs like Co-Trimoxazole and Chloramphenicol among five multidrug resistance isolates showed susceptible to clinically neglected least prescribing drugs. Whereas least commonly used preserved drugs resistance pattern was shown by Carbenicillin and Ampicillin/Sulbactam (32.3%), Piperacillin/Tazobactam (29.9%), Meropenem (22.6%) among the isolates as shown in Table 2.

Table 2. Shows susceptibility pattern of GNB (n=31) of different antibiotics.

Antimicrobials	P.aeruginosa (n=8)		Acinetobacter spp (n=7)		Escherichia coli (n=4)		Proteus spp (n=4)		Klebsiella spp (n=3)		Enterobacter spp (n=2)		Citrobacter spp (n=2)		Serratia spp (n=1)		Total (n=31)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AMP	2	6	2	5	2	2	1	3	1	2	-	2	-	2	1	-	9(29.0)	22(71.0)
CTX	5	3	2	5	2	2	2	2	1	2	-	2	-	2	1	-	13(41.9)	18(58.1)
COT	3	5	2	5	2	2	2	2	2	1	1	1	1	1	-	1	13(41.9)	18(58.1)
C	2	6	2	5	2	2	2	2	1	2	-	2	-	2	1	-	10(32.3)	21(67.7)
CIP	1	7	2	5	1	3	1	3	1	2	-	2	-	2	-	1	6(19.4)	26(83.9)
CZ	1	7	-	7	3	1	3	1	2	1	1	1	-	2	-	1	10(32.3)	21(67.7)
CTR	4	4	2	5	1	3	1	3	1	2	-	2	-	2	-	1	9(29.0)	22(71.0)
AK	2	6	2	5	2	2	2	2	1	2	1	1	1	1	-	1	11(35.5)	20(64.5)
OF	3	5	3	4	1	3	1	3	1	2	-	2	-	2	1	-	10(32.3)	21(67.7)
MRP	5	3	3	4	4	-	4	-	3	-	2	-	2	-	1	-	24(77.4)	7(22.6)
GEN	3	5	3	4	2	2	2	2	1	2	1	1	1	1	1	-	14(45.2)	23(74.2)
AMC	3	5	2	5	2	2	2	2	1	2	1	1	1	1	-	1	12(38.7)	19(61.3)
PIT	5	3	3	4	3	1	3	1	3	1	2	-	2	-	1	-	22(70.1)	9(29.9)
CB	3	5	2	5	4	-	4	-	3	1	2	-	2	-	1	-	21(67.7)	10(32.3)
A/S	6	2	4	3	3	1	2	2	2	1	2	-	1	1	1	-	21(67.7)	10(32.3)

Table 3. Shows susceptibility pattern of GPC (n=17) of different antibiotics.

Antimicrobials	*S. aureus* (n=5)		CoNS (n=4)		*Streptococcus pneumoniae* (n=3)		*Streptococcus pyogens* (n=3)		*Enterococcus* spp (n=2)		Total (n=17)	
	S	R	S	R	S	R	S	R	S	R	S (n / %)	R (n / %)
AMP	1	4	1	3	1	2	1	2	-	2	4(23.5)	13(76.5)
CTX	2	3	1	3	1	2	1	2	2	-	7(41.2)	10(58.8)
COT	2	3	1	3	1	2	2	1	1	1	7(41.2)	10(58.8)
C	2	3	1	3	1	2	1	2	-	2	5(29.4)	12(17.5)
CIP	2	3	1	3	1	2	1	2	2	-	7(41.1)	10(58.8)
CZ	1	4	-	4	-	3	1	2	-	2	2(11.8)	15(88.2)
CTR	1	4	1	3	1	2	1	2	2	-	6(35.3)	14(82.4)
AK	3	2	2	2	1	2	1	2	2	-	9(52.9)	8(47.1)
OF	1	4	1	3	2	1	2	1	2	-	8(47.1)	9(52.9)
MRP	3	2	3	1	2	1	2	1	2	-	12(70.6)	5(29.4)
A/S	2	3	1	3	2	1	2	1	-	2	7(22.5)	10(58.8)
P	1	4	-	4	2	1	2	1	-	2	9(52.9)	11(64.7)
CD	1	4	-	4	1	2	1	2	-	2	5(29.4)	12(70.5)
OX	2	3	-	-	-	-	-	-	-	-	-	-
AZM	2	3	1	3	2	1	2	1	2	-	9(52.9)	8(47.1)
VAN	2	3	3	1	2	1	2	1	2	-	11(64.7)	6(35.3)

Percentage resistance of gram positive isolates to different antimicrobials

As shown in Table 3, susceptibility pattern of GPC against different isolates shows most of the commonly used drugs were (58.6%) resistant. Drugs like Cefazolin (88.2%), Ceftriaxone (82.4%) Ampicillin (76.5%), Chloramphenicol and Clindamycin (70.5%), showed highest resistance pattern among *Enterococcus* spp, CoNS and *S.aureus*. (66.6%) Second highest resistance pattern was found for Penicillin (64.7%), Co-Trimoxazole, Ciprofloxacin, Cefotaxim and Ampicillin/ Sulbactam (58.8%), and Ofloxacin (52.9%). The least resistance pattern among the GPC was found in drugs like Amikacin and Azithromycin (47.1%) and Vancomycin (35.3%) Meropenem (29.4%). Among all isolates of *S.aureus* MRSA was (60.0%) whereas VRSA was also found to be (60.0%). Coagulase negative *Staphylococcus* (CONS) had shown MDR 66.6% of isolates. This indicates most of an antibiotic were either moving towards resistance or already acquired resistance against antibiotics.

DISCUSSION

The present research was conducted to isolate the aerobic bacteria causing blood stream infection and their antimicrobial susceptibility patterns among the ICU patients visited CMS-

TH. Infection is one of the silent features of life, and antibiotic therapy has not yet reduced the incidence of infection with complicating septicemia.[18] This study was conducted among the patients attending COMS-TH, Chitwan to find out the current trend of the bacterial spectrum causing bacteremia and septicemia and to find out their susceptibility pattern of antibiotics used against them. The study also emphasized on the incidence of MDR among different isolates from surgical ICU.

It shows that, according to case distribution to the culture positivity among patients, during study period of dated: July 2017 to December 2020 a total number of 450 samples were processed where we found blood culture positive in only 48/450 (10.7%) of cases. Among those 48 blood culture positive cases 30 (62.5%) cases were observed in male patients, whereas, 18 (37.5%) episodes were observed in female patients. The different age of patients ranges from 1 to >80 years. Among 48 patients, 28 (58.3%) patients had single and 20 (41.7%) patients had multiple episodes of septicemia (4 had 1 episodes and 1 had 2 episodes of septicemia). A total of 48/450 (10.7%) bacterial isolates from ICU patient blood sample showed positive by culture from different ICU, College of Medical Sciences Teaching Hospital, Nepal.

Only about one fourth of all positive blood cultures represented true blood stream infection. These findings contrast markedly with this observations in earlier study, in which approximately one third of all positive blood cultures represented contamination.[19] Although several recent studies have indicated that the current single needle technique for obtaining blood and inoculating cultures is not associated with increased rates of contamination[20-22] Spitalnic *et al* [23] in a meta analysis, have suggested otherwise. Alternatively, it may be that in hospitals such as ours, in which medical officers, sisters and paramedical students obtain the most of blood culture samples, failure to apply antiseptic solutions (alcohol, iodophors and alcohol) properly and to let them exert their antimicrobial effect over 1-2 minutes as recommended results in increased contamination.

Distribution of gram positive cocci 17(35.4%) and gram negative bacilli 31(64.6%) from different cases of bacteremia and septicemia showed highest percentage of gram negative bacilli is responsible for the cause of disease as shown by Table 1. The bacteriological profile of blood stream infections remained almost similar throughout the year. Septicemia produced by gram negative bacteria has become a progressively frequent and serious complication of surgical practice. During the past 12 years and 2 months, 398 cases of gram negative septicemia were observed at University of Cincinnati Medical Centre.[24] The increasing number of elderly patients in the seventh and eighth decades requiring operations and their apparent decreased resistance has been reflected in a progressively greater number of gram negative and other infection.

In the present study, gram negative bacteria constituted the major group of isolates comprising 64.6%. This was lower with the results reported by Mehdinejad *et al* [25] as 86.5% and by Mehta *et al* [19] as 80.96%. But it was higher than previous study in 1999 by Banjade,[26] who reported only 57.8%, while SENTRY surveillance in United States and Canada in 1997 reported higher 94% of gram negative isolates,[27] while Mehta *et al* [19] reported 100% growth of gram negative isolates.

Out of total 450 suspected cases of septicemia 45(10.7%) showed culture positive, where 31(64.6%) were confirmed gram negative bacilli as shown in Table 2. The rate of isolation of gram negative bacilli was highest in *P.aeruginosa* 8(16.6%) followed by *Acinetobacter* spp 7(14.5),

Escherichia coli 4(8.3%), *Proteus mirabilis* 4(8.3%), *Klebsiella pneumoniae* 3(6.3%), *Enterobacter* spp 2(4.2%), *Citrobacter* spp 2(4.2%) and *Serratia* spp 1(2.1%). The present study revealed only 8(16.6%) of the total isolates as *P.aeruginosa* which was quite similar with study by Mehta *et al* [19] who reported 19.7% from India. But it was higher than EI-Jabha *et al*[28] who reported 5.8% from Gaza City and Mehdinejad *et al* [25] who reported 9% from Iran.

Out of 450 suspected cases of septicemia showed 48(10.7%) culture positive whereas 17(35.4%) were gram positive cocci as shown in Table 1. The rate of isolation of gram positive cocci was highest in *S.aureus* 5(10.4%) followed by CoNS 4(8.3%) *Streptococcus pneumoniae* 3(6.3%), *Streptococcus pyogenes* 3(6.3%), and *Enterococcus* spp 2(4.2%). This result was higher than the study done by Mehdinejad *et al* [25] and Mehta *et al* [19] they reported 13.5% and 18.0% of gram positive isolates respectively.

The most frequent gram negative bacilli (GNB) isolates were *P.aeruginosa* 8(16.6%) and gram positive cocci (GPC) were *Staphylococcus aureus* 5(10.4%). According to the given table 2 and 3, in total antimicrobial resistant rate of GPC and GNB were found to be 57.3%. The sensitivity rate of GNB is (43.4%) and resistance rate is (56.6%) and isolated GPC showed sensitive of (41.4%) and resistance of (58.6%).

Present research shows most of the drugs with high resistance rate among all GNB isolates (56.6%). Among GNB isolates *P.aeruginosa*, *Acinetobacter* spp., and *Klebsiella* spp. showed average of (60.5%) multidrug resistance (MDR). Most frequently used drugs like Ciprofloxacin (83.9%), Gentamycin (74.2%), Ceftrixone and Ampicillin (71.0%), Cefazolin and Chloramphenicol (67.7%), Ofloxacin (67.7%), Amikacin (64.5%), Amoxyclave (61.3%), showed high rate of resistance among the isolates. Cefotaxime and Co-Trimoxazole (58.1%) showed second highest resistance pattern among isolates from ICU patients. Drugs like Co-Trimoxazole and Chloramphenicol among five multidrug resistance isolates showed susceptible to clinically neglected least prescribing drugs. Whereas least commonly used preserved drugs resistance pattern was shown by Carbenicillin and Ampicillin/Sulbactam (32.3%), Piperacillin/Tazobactam (29.9%), Meropenem (22.6%) among the isolates as shown in Table 2.

In this study, 62.5% of *P. aeruginosa* were sensitive to Meropenem, 25% of isolates were sensitive to Amikacin with the least activity to Ciprofloxacin with 12.5% sensitivity. Among the isolates 60.5% were MDR. *Acinetobacter* spp have been implicated in recent years as important nosocomial pathogen, especially in intensive care setting. Despite their low pathogenic potential they are being reported increasingly as the causal organism of numerous hospitals outbreaks in several countries. In a recent international multicentre study, *Acinetobacter* spp were ranked amongst 10 organisms most commonly causing septicaemia in 18 of 44 large European hospitals.[29] Therefore, this study was not the exception and showed 7(14.5%) growth for *Acinetobacter* spp of total isolates, which was higher than the study done by Arora *et al*[30] reported 6.6% from India. But it was quite higher than SENTRY[27] surveillance report as 1.3% from US and Canada, EI-Jadba *et al*[29] reported 1.2% from Gaza City and Mehdinejad *et al* [25] reported 4.5% from Iran.

As shown in Table 2 sensitivity was shown towards (42.8%) antibiotics like Gentamycin, Ofloxacin, Meropenem, Piperacillin/Tazobactam and (28.6%) for other broad and narrow spectrum antibiotics. MDR was 61.6% among *Acinetobacter* spp isolated from different cases of septicemia.

Escherichia coli and *Proteus* species were only 8.3%

of total isolates. This result was lower than other studies in Kanti hospital by Karki *et al*[31] reported 29.3%. These variations can be explained by the reason that they have included lower age groups like in Children. Only one positive isolate of 100% sensitivity was shown toward commonly used antibiotics *viz* Co-Trimoxazole, Cefazolin, Meropenem, Amikacin, Piperacillin/Tazobactam and Ampicillin/Sulbactam where as other drugs were resistance to *Escherichia coli* and *Proteus* species. In this study, *Klebsiella* 6.3%, *Enterobacter* spp and *Citrobacter* spp were 4.2% also recovered and accounted 64.6% of total gram negative isolates. This result was contradictorily higher than previous study done by Banjade reported[26] 0.1% in 1999.

As shown in Table 3, susceptibility pattern of GPC against different isolates shows most of the commonly used drugs were (58.6%) resistant. Drugs like Cefazolin (88.2%), Ceftriaxone (82.4%) Ampicillin (76.5%), Chloramphenicol and Clindamycin (70.5%), showed highest resistance pattern among *Enterococcus* spp, CoNS and *S.aureus*. (66.6%) Second highest resistance pattern was found for Penicillin (64.7%), Co-Trimoxazole, Ciprofloxacin, Cefotaxim and Ampicillin/ Sulbactam (58.8%), and Ofloxacin (52.9%). *S.aureus* remains an important cause of nosocomial blood stream infection. Present study showed *S.aureus* as 10.4% of all isolates. This result was lower with study by Cosgrove *et al* [32] who reported 15-60% of isolates of *S.aureus*, study by Rattanaphone *et al* [33]reported 19% of *S.aureus* from Laos in the year 200 to 2004, in the year 2007 to 2008 from Kanti Hospital showed 65% growth of *S.aureus*. Such lower yield of *S.aureus* in this laboratory might be due to predominance of gram negative bacteria.

Several gram positive bacteria showed significant rates of antibiotic resistance patterns. As shown in Table 3, *S. aureus* had 60% susceptibility towards Meropenam, Amikacin while Ciprofloxacin and Co-Trimoxazole had sensitivity of 40%. Amoxicillin and Penicillin showed reduced sensitivity. Among all isolates MRSA was 60.0% whereas VRSA was found to be 60.0%. Out of them some antibiotics indicates that sensitivity pattern of isolates is changing with time. The proportion of Methicillin resistance among *S.aureus* isolates were seen in eastern United States (Southeast, 38.5%; Northeast, 29.8%), and proportion observed in Southwest was (22.5%) and Northwest was (14.5%). The general surgery and cardiothoracic services had the highest proportions of Methicillin-resistant *S.aureus* (39.5% and 35.6% respectively)[34]

Methicillin resistant *S.aureus* (MRSA) as reported by Takeda *et al*[35] occurs in low levels in Nothern Europe less than 1%, increasing levels in middle European countries United States, New Zealand and Australia where the resistance ranged from 6 to 22% and very high levels in Southern European countries as well as in parts of the United States, Asia and South Africa where the resistance ranged from 28 to 63%.

There were only four spp of CoNS, which were sensitive to all commonly used antibiotics like Amoxicillin, Ciprofloxacin, Co-Trimoxazole, even they were sensitive to new antibiotic like Meropenem. CoNS had shown MDR 66.6% as indicated in Table 3. *Enterococcal* spp bacteremia were nosocomial in origin. Infection with *Enterococcus* spp tend to occur in more debilitated patients. In this study *Enterococcus* spp accounted only 4.3% of total isolates. This was lower than other studies like SENTRY[27] surveillance reported 9.5% from US and Canada and Mehdinejad *et al* [25] reported 9.7% from Iran. 100% of *Enterococcus* spp showed sensitivity to Meropenem, but rest of the antibiotics like Ampicillin, Cefotaxime, Amikacin were resistant. Coagulase negative *Staphylococcus* (CONS) had shown MDR 66.6% of isolates. This indicates most of an antibiotic were either

moving towards resistance or already acquired resistance against antibiotics.

CONCLUSIONS

From this study, the sensitivity pattern is constantly changing even in same set up and even geographical and ethnical variation undoughtably showed changed pattern of sensitivity. MDR isolates are increasing in most of the strains which is one of the major health problems. Novel types of resistance mechanisms have been encountered in Nepal as well as this situation is alarming and need prompt action before dreadful condition. Following the recommendations given by summit on antimicrobial resistance, like not indulging patient demands for unneeded antibiotics, educating patients on appropriate antibiotics use, identifying the pathogen, choosing short course, narrow spectrum antibiotics, encouraging patients to get vaccinated improving resistance surveillance system will help controlling this situation. Multidisciplinary approach and effort like clinician, Microbiologist, public health officer and patients themselves together can help to overcome such conditions.

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