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ORIGINAL ARTICLE

HIGH BURDEN OF NOSOCOMIAL INFECTIONS CAUSED BY MULTI-DRUG RESISTANT PATHOGENS IN PEDIATRIC PATIENTS AT HAWASSA UNIVERSITY COMPREHENSIVE SPECIALIZED HOSPITAL

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ABSTRACT

Background: *Pediatric patients are at increased risk of nosocomial infections with multi-drug resistant pathogens which are more prevalent in the hospital environment. The aim of this study was to determine the prevalence of nosocomial infections, antibiotic resistance pattern of bacterial isolates and associated factors in pediatric patients.*

Methods: *This was a cross-sectional study conducted from March to August, 2016. Data included socio-demographic and clinical variables in patients clinically suspected of having nosocomial infections and collected using a structured questionnaire. Bacterial identification and antimicrobial susceptibility test was done with standard microbiological methods. Data were analyzed with SPSS version 20 and p value < 0.05 was considered to be statistically significant.*

Results: *Out of 939 children admitted to the hospital, 384 patients (40.9%) were clinically suspected and had 462 nosocomial infections. Blood stream infection was the most common site of nosocomial infections. Culture confirmed nosocomial infections were reported in 82 patients (21.4%; 95% CI, 17.2-25.8%) with 88 isolates. Among the most frequently isolated bacteria, 21 (23.9%) were Klebsiella spp, and 16 (18.2%) were S. aureus, 62.5% of which were methicillin resistant. Among all bacterial pathogens, 88.9% were multi-drug resistant. Extremely high (97.9%) multi-drug resistance was associated with Gram negative bacteria. Among these, all isolates, except E. coli, were 100% multi-drug resistant. Long hospital stay and malnutrition were significantly associated with nosocomial infections.*

Conclusion: *The high prevalence of nosocomial infections with multi-drug resistant bacteria suggests the need for proper implementation of the nosocomial infections prevention and control measures.*

Keywords: *Nosocomial infection, multi-drug resistance, pediatrics, Ethiopia*

BACKGROUND

Nosocomial infection (NI), also called hospital acquired infections (HAI) is defined as localized or systemic infection contracted from hospital after 48 hours of stay or more by a patient, admitted for a reason other than that specific infection(1, 2). The increased use of antimicrobial agents and advance in life saving medical practices which expose the patients to invasive procedures, are associated with the ever increasing Nis, particularly in developing countries where there is poor infection prevention practice (3-5). Blood stream infection (BSI), urinary tract infection (UTI), surgical site infection (SSI) and respiratory tract infection (RTI) are among the most frequently reported sites of NIs (2,3,6).

NIs are caused by a variety of organisms, including bacteria, fungi, viruses and parasites, but about 90% of NIs are due to bacteria(3).

The predominant bacteria commonly isolated in NIs include: *Staphylococcus aureus, Coagulase negative staphylococci* (CoNS), *Enterococcus spp., Acinetobacter spp., Pseudomonas aeruginosa* and members of the Enterobacteriaceae family like *Escherichia coli, Proteus mirabilis* and *Klebsiella pneumonia(*6, 7). Bacterial infections can arise from exogenous or endogenous sources and are transmitted by either direct or indirect contact between patients, healthcare workers, contaminated objects, visitors, or even various environmental sources.

Although NIs affect all age groups, children have enhanced susceptibility to many infections because of the immaturity of their immune system, increased use of medical devices and environmental contamination. The emergence of difficult to treat MDR strains in a hospital environment, further complicates NIs in this population (8).

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This may lead to increased mortality, length of stay in the hospital (LOS) and increased cost [5, 9]. Frequency and pattern of NIs also varies by country, region, type of hospital, whether wards or intensive care units (ICU) [10]. In Ethiopia, previous studies focused mainly on adults or the general population [2, 7, 11]. However, there is limited data about NIs in pediatric patients[6]. Hence, we determined the prevalence of NIs, antibiotic resistance pattern of bacterial isolates and associated factors among pediatric patients in Hawassa University Comprehensive Specialized Hospital. The hospital does not have a pediatric intensive care unit (PICU) facility.

PATIENTS AND METHODS

Study design, study period and study area: A cross sectional study was conducted from March to August, 2016 in Hawassa University Comprehensive Specialized Hospital (HUCSH). The hospital is located in Hawassa; the capital city of the Southern Nations, Nationalities Peoples' Regional State (SNNPR), which is located 275 km south of Addis Ababa. The hospital provides both inpatient and outpatient health services catering to over 6 million people.

Study population: Pediatric patients who had no evidence of infection on admission, but developed sign and symptoms of infection after at least 48 hours of hospitalization were included in the study.

Variables of the study: Socio-demographic characteristics i.e. age, sex, residence and clinical characteristics including length of stay in hospital, malnutrition, use of external indwelling device, nasal oxygen, intravenous cannula, urinary catheter, nasogastric tube, antibiotic use before having surgery, types of surgical procedures, taking prophylaxis before surgery were the independent variables assessed.

Data collection

Socio-demographic and clinical data were collected using structured questionnaires. Nutritional status, weight and height of children were determined by trained nurses, and clinical examination was carried out by the attending physician. One or more types of clinical specimens were collected from an individual based on the clinical decision of the attending physician. Accordingly, blood was collected when blood stream and respiratory infections were suspected; whereas urine and wound swabs were collected from UTI and SSI suspected patients, respectively.

***Sample collection and laboratory investigation*:** Two bottles of blood samples (1 ml for each bottle) from two different sites of peripheral vein were collected aseptically (disinfecting with 70% alcohol and 2% tincture of iodine) from children suspected of BSI and RTI (such as pneumonia) by experienced nurses. The collected blood sample was inoculated directly into Thioglycollate broth blood culture bottles (Abtek LTD). Bottles were incubated aerobically at 37°C for 7 days and observed for signs of bacterial growth (turbidity, hemolysis, air bubbles or gas production and clot formation) on daily basis for up to 7 days. Growth in either of the bottles was considered positive. Bottles which showed sign of growth were gram stained and sub-cultured on blood agar plate (Abtek LTD), chocolate agar plates (incubated at 5% CO_2 atmosphere), MacConkey (Abtek LTD), and mannitol salt agar plates (Abtek LTD). These plates were than aerobically incubated for 18–24 hours at 37°C. Blood cultures with no bacterial growth after 7 days were sub-cultured before being reported negative.

For children suspected of UTI, clean catch midstream urine sample, after cleansing the urethral meatus, or catheter urine (in patients catheterized for management purpose) was collected in a wide mouthed sterile container. A loopful of urine sample (1µl) was inoculated on blood agar and MacConkey plates. Significant bacteriuria was considered for those with 10^3 CFU/ml of catheterized urine and 10^5CFU/ml of midstream urine(12). For children suspected of SSI, swabs were collected after cleansing the wound site with normal saline and inoculated on BAP, MAC and mannitol salt agar (MSA). Both the inoculated urine and swab were incubated aerobically at 37 °C for 18-24 hours.

The isolates obtained were identified by standard microbiological techniques, namely Gram staining, colony characteristics, and biochemical properties including catalase, coagulase (free and bound), PYRase, bile esculin, growth on mannitol salt agar, antibiotic disc (bacitracin and optochin) and hemolytic activity on blood agar plate for Gram positive isolates, and triple sugar iron, mannitol fermentation, motility, indole, citrate utilization, urease, lysine iron agar, oxidase, growth factors (x, v and xv) and H_2S production for Gram negative isolates(12). *Coagulase negative Staphylococci* (*CoNS*) were only considered to be causative pathogens if two blood samples drawn on separate occasions showed the growth of the same pathogen.

Antibiotic susceptibility testing: Antibiotic susceptibility test was performed with the Kirby-Bauer disk diffusion method (13). In brief, three to five pure colonies of test organism were transferred into a tube containing 5 ml nutrient broth and mixed gently until a homogenous suspension was formed. The turbidity of the suspension was compared with 0.5 McFarland standards to standardize inoculum density. A sterile cotton swab was dipped into the suspension and excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacterial suspension evenly over the entire surface of the Mueller-Hinton agar (Abtek LTD, UK) for non-fastidious organisms and Muller Hinton agar with 5% defibrinated sterile sheep blood for fastidious organisms. The following antimicrobial disks were used: Abtek LTD, UK, penicillin (P:10 unit), erythromycin (E:15 µg), ampicillin (AMP:10 µg), amoxicillin-clavulanic acid (AMC:20/10 µg), trimethoprim-sulphamethoxazole (SXT:1.25/23.75 µg), chloramphenicol (CAF:30 µg), gentamicin (CN:10 µg),ciprofloxacin (CIP:5 µg), norfloxacin (NOR:10µg), ceftazidime (CAZ:30 µg), ceftriaxone (CTR:30 µg) and cefoxitin (CXT:30 µg), cefuroxime (CRX:30µg), doxycycline (DOX:30µg), streptomycin (STR:10µg) and clathromycin (CLM :15µg). After 18–24 hours' incubation at 37° C, zone of growth inhibition was measured to the nearest whole number in millimeter using a caliper. The zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline [14] as susceptible (S), intermediate (I) or resistant (R).

Quality control: The validity and completeness of the data were verified by the principal investigator daily. Sterility of culture media and biochemical tests was checked by overnight incubation of uninoculated media from each batch of preparation. Standard strains of E. *coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were used for culture and antibiotic susceptibility testing internal quality assurance.

Data entry: Data entry and analysis was performed using SPSS version 20. Frequencies and cross tabulations were used to summarize descriptive statistics. Bivariate and multivariate logistic regression analyses were used to assess the possible risk factors of NI. All variables with $p < 0.25$ in the bivariate analysis were further entered into multivariate logistic regression model and variables which had $p < 0.05$ were retained as independent predictors of culture confirmed NI.

Descriptive statistics was also used to explain antimicrobial susceptibility patterns. Odds ratio and 95% confidence interval (CI) were computed to assess the presence and degree of association between dependent and independent variables.

Ethical consideration: Ethical approval was obtained from Hawassa University, College of Medicine and Health Sciences, Institutional Review Board (IRB). A permission letter was obtained from the hospital administration. Informed written consent and/or assent were obtained from children and/or guardians. Patient information was anonymized and de-identified prior to analysis. Culture results were reported to the responsible physician.

Operational definition: Methicillin resistant *Staphylococcus aureus* (MRSA): *S. aureus* resistance for cefoxitin disk (≤21mm) (15).

***Multi-drug resistant bacteria (MDR*)**: A bacterial isolate which is resistant to one or more antibiotics in three or more classes of antimicrobials agents(16).

Malnutrition*:* We used the World Health Organization (WHO) definition which refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients (17).

RESULTS

Socio-demographic characteristics and uses of devices: Out of 939 children admitted in neonatology and pediatric wards in HUCSH during the study period, 384 (41.0%) were clinically suspected of NIs. among these, 147 (38.3%) were neonates (age ≤28days) admitted to the neonatology ward and the rest 237(61.7%) were older and were admitted to the pediatric ward. The majority of the children, 220 (57.3%) were male. The median and mean (standard deviation (SD)) age of children during admission was 0.42 days and 1.8 (SD=3.3) years, ranging from 1day to 14 years. Almost two-thirds (227/384, 59.2%) of the children were urban residents, 135(35.2%) had some degree of malnutrition, 183(47.7%) had admission greater than 5 days and 209(54.4%) had taken antibiotics at the time of sample collection. The greatest proportion of patients 304(79.2%) used at least one medical device either for therapeutic feeding or diagnosis during their stay in the hospital and 25(6.5%) children were clinically suspected for SSI. Of these, 22(88%) had taken antibiotic prophylaxis before surgery **(Table 1**).

Magnitude and associated factors: The overall magnitude of culture confirmed NI was 21.4% (82/384; 95%CI, 17.2 - 25.8 %). Children aged 6–10 years (32.3%) and rural residents (35%) had the highest rate of NIs. The overall rate of device-associated NI was 65 (21.3%) while the rate of nasopharyngeal (NG) tube associated infection was the highest with 54(45.7%). In this study, factors associated with culture confirmed NI were analyzed.

A multivariate analysis indicated that patients with a length of stay of more than 5 days in the hospital were almost three times more likely to develop NI (aOR=2.76, 95%CI 1.58-4.84) than those with less than 5 days of hospital stay. Moreover, malnourished children were almost 2 times more likely to develop NI (aOR=1.99, 95%CI 1.083-3.653) compared to their well-nourished counterparts **(Table 1).**

Table 1. Culture confirmed nosocomial infection in relation to socio-demography and associated risk factors among pediatric patients at Hawassa University Comprehensive Specialized Hospital, from March-August, 2016

Variables		Suspected NIs N(%)	Culture confirmed NIs N (%)	COR (95%CI)	aOR(95%CI)
Age	≤28days	147(38.3)	32(21.8)	0.78(0.26-2.33)	
	29days-2yrs	151(39.3)	25(16.6)	0.56(0.18-1.68)	
	3-5yrs	36(9.4)	10(27.8)	1.08(0.31-3.78)	
	6-10yrs	31(8.1)	10(32.3)	1.33(0.38-4.74)	
	11-14yrs	19(4.9)	5(26.3)	1.00	
Sex	Male	220(57.3)	48(21.7)	1.07(0.65-1.75)	
	Female	164(42.7)	34(20.7)	1.00	
Residence	Rural	157(40.9)	55(35.0)	1.00	1.00
	Urban	227(59.2)	27(11.8)	1.44(0.86-2.40)	1.05(0.58-1.89)
LOS	≤5days	201(52.3)	24(11.9)	1.00	1.00
	>5days	183(47.7)	58(31.7)	3.42(2.02-5.80)***	2.76(1.58-4.84) ***
Malnutrition	Yes	135(35.2)	39(28.9)	1.95(1.19-3.20)	2.10(1.16-3.78)*
	No	249(64.8)	43(17.3)	1.00	1.00
Device use	Yes	304(79.2)	65(21.3)	1.01(0.55-1.84)	
	No	80(20.8)	17(21.2)	1.00	
Nasal oxygen	Yes	78(20.3)	12(15.3)	0.54(0.27-1.08)	
	No	306(79.7)	70(22.9)	1.00	
IV cannula	Yes	285(74.2)	61(21.4)	1.01(0.58-1.77)	
	No	99(25.8)	21(21.2)	1.00	
Urinary catheter	Yes	36(9.4)	4(11.1)	0.43(0.15-1.26)	0.33(0.10-1.08)
	No	348(90.6)	78(22.3)	1.00	1.00
NG tube	Yes	118(30.6)	54(45.7)	1.16(0.69-1.95)	
	No	267(69.4)	28(10.5)	1.00	
Antibiotics used	Yes	209(54.4)	51(24.4)	1.50(0.91-2.47)	1.01(0.58-1.76)
	No	175(45.6)	31(17.7)	1.00	1.00
Surgery	Yes	25(6.5)	16(60.0)	6.54(2.81-15.19)	3.13(0.25-39.48)
	No	359(93.5)	67(18.7)	1.00	1.00
Surgical procedure	Elective	15(60.0)	10(53.3)	0.49(0.09-2.66)	
	Emergency	10(40.0)	6(70.0)	1.00	
Prophylaxis	Yes	22(88.0)	14(63.6)	7.57(3.05-18.76)	2.77(0.19-39.68)
	No	3(12.0)	2(66.7)	1.00	1.00

IV-intravenous, NG-nasogastric, LOS: length of hospital stay, COR- crude odd ratio, a OR- adjusted odd ratio, *-p<0.05; **-p<0.01; ***- P<0.0001

Multi-drug resistant bacteria: The overall magnitude of MDR was 78(88.6%). Multidrug resistance (MDR) among Gram negative and positive bacteria was 46 (97.9%) and 32 (78%), respectively.

Among MDR strains, 8% of the isolates were resistant to three classes of antibiotics, the rest 71(91%) were resistant to more. None of the isolated pathogens was sensitive or resistant to all of the antibiotic classes tested **(Table 2).**

Table 2: Multi-drug resistance pattern of bacterial isolates from admitted pediatric patients at Hawassa University Comprehensive Specialized Hospital, from March-August, 2016.

Bacterial isolates	Antibiogram pattern N (%)								
	R1	R2	R3	R4	R5	R6	R7	R8	MDR
S. aureus (n=16)		2 (12.5)	2 (12.5)	1(6.25)	1 (6.25)	1 (6.25)	7(43.8)	2 (12.5)	14 (87.5)
CoNS (n=13)		3 (23.1)		2(15.4)	1(7.7)	2 (15.4)	2(15.4)	3 (23.1)	10 (76.9)
Enterococcus spp (n=6)			1 (16.7)	1(16.7)			3(50)	1 (16.7)	6(100)
S. pyogenes (n=3)	1 (33.3)	1 (33.3)	1 (33.3)						1(33.3)
S. pneumoniae (n=3)	1 (33.3)	1 (33.3)	1 (33.3)						1(33.3)
Klebsiella spp (n=21)				1(4.8)	2(9.5)	3 (14.3)	15 (71.4)		21 (100)
E. coli(n=14)		1(7.1)		3(21.4)	2 (14.3)	1(7.1)	7(50)		13 (92.9)
Pseudomonas spp (n=4)				1(25.0)	1 (25.0)		2(50)		4(100)
Citrobacter spp(=3)			1 (33.3)	1(33.3)			1(33.3)		3(100)
Acinetobacter spp (n=2)							2(100)		2(100)
H. influenzae(n=2)			1(50)		1(50)				2(100)
E. cloacae (n=1)						1(100)			1(100)
Total (=88)	2(2.3)	8(9.0)	7(8.0)	10 (11.4)	8(9.0)	8(9.0)	39 (44.3)	6(6.8)	78 (88.6)

CoNS: coagulase negative staphylococci, R_0: sensitive to all classes of antibiotics; R_1: resistance for 1 class of antibiotics; R_2: resistance for two classes of antibiotics; R_3: resistance for three classes of antibiotics; R_4: resistance for 4 classes of antibiotics; R_5: resistance for 5 classes; R_6: resistance for 6 classes of antibiotics; R_7 resistance for 7 classes of antibiotics; R_8: resistance for 8 classes of antibiotics; MDR: Total multi-drug resistance.

Distribution of bacterial isolates by site of nosocomial infections

Three hundred eighty-four children (40.9%) had a total of 462 NIs, 310 children (80.7%) had only a single site of NI, and 74 children (19.3%) had two or more NI sites. The most common site of NIs was BSI 296 (64.1%) followed by UTI 88 (19%), SSI 25 (5.4%) and pneumonia 53 (11.5%).

Out of the 384 patients, 82 (21.4%) had culture confirmed Nis and 88 bacteria were isolated from them. Polymicrobial growth was observed in 5(6.1%) of the patients, while the 77(93.1%) showed growth of single strains.

Among the 88 bacteria isolated, 46.6% (41/88) were isolated from patients clinically suspected of BSI followed by UTI 22.7% (20/88), SSI 18.2% (16/88) and pneumonia 12.5 % (11/88).

Gram negative bacterial species, 47 (53.4%), were more frequently isolated than Gram positive bacteria, 41(46.6%). The predominant bacterial species isolated was *Klebsiella spp.* 21(23.9%) followed by *S. aureus* 16(18.2%), *E. coli* 14(15.9%) and *CoNS* 13 (14.8%). *CoNS* was the commonest bacteria in BSI 13(31.7%) followed by *S. aureus* (19.5%). *E. coli* was the most common bacteria for UTI 8(45%) **(Table3).**

Table 3: Distribution of 88 bacterial isolates by site of nosocomial infection among pediatric patients at Hawassa University Comprehensive Specialized Hospital, from March-August, 2016.

Bacterial isolate	**Site of nosocomial infections**				
	BSI N (%)	UTI N (%)	SSI N (%)	Pneumonia N (%)	Total
S. aureus	8(19.5)	2(10.0)	4(25.0)	2(13)	16(18.2)
CoNS	13(31.7)	-	-	-	13(14.8)
Enterococcus spp	5(12.2)	-	1(6.25)	-	6(6.8)
S. pyogenes	3(7.3)	-	-	-	3(3.4)
S. pneumoniae	1(2.4)	-	2(12.5)	-	3(3.4)
Subtotal Gram positive	30(73.1)	2(10.0)	7(43.75)	2(18.2)	41(46.6)
Klebsiella spp	5(12.2)	8(40.0)	4(25.0)	4(36.3)	21(23.9)
E.coli	2(4.9)	9(45.0)	3(18.75)	-	14(15.9)
Pseudomonas spp	-	-	1(6.25)	3(27.3)	4(4.5)
Citrobacter spp	1(2.4)	1(5.00)	1(6.25)	-	3(3.4)
Acinetobacter spp	2(4.9)	-	-	-	2(2.3)
H. influenza	1(2.4)	-	-	1(9.1)	2(2.3)
Enterobacter cloacae	-	-	-	1(9.1)	1(1.1)
Subtotal Gram negative	11(26.9)	18(90)	9(56.25)	9(81.8)	47(53.4)
Total	41(46.6)	20(22.7)	16(18.2)	11(12.5)	88(100)

BSI: blood stream infection, UTI: urinary tract infection, SSI: surgical site infection, RTI: Respiratory tract infection. Antibiotics resistance pattern of gram positive bacteria.

The antibiotic resistance pattern of 41 Gram positive bacterial isolates was determined for 15 drugs. High frequency of resistance (>80%) occurred against penicillin (84.2%) and trimethoprim-sulphamethoxazole (81.3%). Intermediate levels of resistance (60-80 %) were seen for ampicillin (61%), gentamicin (62%), erythromycin (61%) and ceftazidime (76%).

Low frequency of resistance (<60%) was seen against fluoroquinolones (ciprofloxacin and norfloxacin) (45.7%), amoxicillin/clavulanic acid (55.2%), ceftriaxone (50%), doxycycline (39.7%), chloramphenicol (51%), cefuroxime (38%) and clarithromycin (45.7%). Among the predominant Gram positive bacteria, 62.5% of *S. aureus* isolates were methicillin resistant (MRSA) **(Table 4).**

Table 4: Antibiotic resistance pattern of Gram positive bacterial species among pediatric patients at Hawassa University Comprehensive Specialized Hospital, from March-August, 2016.

Antibiotics	**Bacterial isolates**					
	S. aureus (16)	**CoN S(13)**	**Enterococcus Spp. (6)**	**S. pyogenes (3)**	**S. pneumoniae (3)**	**Total (41)**
Ampicillin	10	7	6	0	1	24(58.5)
AUG	11	5	ND	ND	ND	16(55.1)
Cefoxitin	10		ND	ND	ND	10(62.5)
Penicillin	15	13	4	0	ND	32(84.2)
Ciprofloxacin	8	5	3	ND	ND	16(45.7)
Norfloxacin	7	6	3	ND	ND	16(45.7)
Gentamicin	9	9	ND	ND	ND	18(62.1)
Doxycycline	6	8	4	ND	ND	18(51.4)
Chloramphenicol	10	4	3	2	2	21(51.2)
SXT	11	13	ND	ND	2	26(81.3)
Ceftriaxone	8	7	ND	1	ND	16(50)
Ceftazidime	13	9	ND	ND	ND	22(75.8)
Cefuroxime	5	6	ND	ND	ND	11(37.9)
Erythromycin	10	7	6	1	1	25(60.9)
Clarithromycin	7	7	ND	2	0	16(45.7)

AUG amoxicillin/clavulanic acid, CONS – coagulase negative staphylococci, SXT- trimethoprim-sulphamethoxazole, ND-not done.

Antibiotic resistance pattern of Gram negative bacteria: Antibiotic resistance pattern of 47 Gram negative bacterial isolates was determined against 12 drugs. High frequency of resistance (>80%) was observed against ampicillin (88.4%), trimethoprim-sulphamethoxazole (88.4%), ceftriaxone (85.1%), gentamicin (84.3%), amoxicillin/clavulanic acid (83.7%), cefuroxime (83.7%) and streptomycin (80.5%). Intermediate level of resistance (60-80%) was seen in ceftazidime (76.5%). Low frequency of resistance (< 60%) was seen against ciprofloxacin (36%), norfloxacin (37.8%), chloramphenicol (53.5%) and doxycycline (56.1%) (**Table 5**).

Table 5: Antibiotic resistance pattern of Gram negative bacterial species among pediatric patients at Hawassa University Comprehensive Specialized Hospital, from March-August, 2016.

Bacterial isolates (No)		Antibiotics											
		AMP	AUG	DOX	CAF	NOR	SXT	CN	CIP	STR	CTR	CRX	CAZ
Klebsiella spp. (21)	R	19	19	17	13	6	20	20	6	19	20	20	18
E. coli (14)	R	12	10	4	7	7	12	11	6	11	10	11	11
P. aeruginosa (4)	R	ND	ND	ND	ND	2	ND	3	2	ND	4	ND	2
Citrobacter Spp (3)	R	3	3	1	1	1	2	1	1	1	1	1	1
Acinetobacter spp(2)	R	2	2	0	2	2	2	2	2	1	2	2	2
H. influenzae(2)	R	1	1	ND	0	ND	2	ND	0	ND	2	1	1
Enterobacter cloacae (1)	R	1	1	1	0	0	0	1	0	1	1	1	1
Total (=47)		38	36	23	23	17	38	38	17	33	40	36	36
		88.4	83.7	56.1	53.5	37.8	88.4	84.4	36	80.5	85.1	83.7	76.5

AMP-ampicillin, CIP- ciprofloxacin, CN- gentamicin, AUG- amoxicillin- clavulanic acid, CTR- ceftriaxone, DOX - doxycycline, CAF- chloramphenicol, NOR- norfloxacin, SXT- trimethoprim-sulphamethoxazole, CAZ- ceftazidime, CRX- cefuroxime, STR- streptomycin, ND-not done.

DISCUSSION

In our study, the overall magnitude of culture confirmed NI was 21.4% (82/384; 95%CI, 17.2 - 25.8 %). Children aged 6–10 years (32.3%) and rural residents (35%) had the highest rate of NIs. Length of hospital stay and malnutrition were the only significantly associated risk factors for NIs ($p< 0.05$). The overall magnitude of MDR was 88.6%. MDR frequency among Gram negative and positive bacteria was 97.9% and 78% respectively. Gram negative bacterial species (53.4%) were more commonly isolated than Gram positive bacterial species, 46.6%).

The predominant bacterial species isolated was *Klebsiella spp*. 21(23.9%) followed by *S. aureus* 16 (18.2%), *E. coli* 14(15.9%) and *CoNS* 13(14.8%).

High frequency of resistance (>80%) occurred against penicillin (84.2%) and trimethoprim-sulphamethoxazole (81.3%) among gram positive bacteria. Methicillin resistant *S. aureus* (MRSA) was 62.5%. High frequency of resistance (>80%) was observed against ampicillin (88.4%), trimethoprim-sulphamethoxazole (88.4%), ceftriaxone (85.1%), gentamicin (84.3%), amoxicillin/ clavulanic acid (83.7%), cefuroxime (83.7%) and streptomycin (80.5%) in gram negative bacteria.

According to our finding, the overall magnitude of NI in pediatric patients was 21.4% consistent with a study conducted in Peru (20%) (5) and Egypt (20%) (18). The magnitude of patients with NIs among pediatric patients reported from developing countries ranged widely from 3 % to 68.3% (1, 4, 6, 19).

The wide range in magnitude for the acquisition of NI in pediatric patients might be explained by differences in the severity of the underlying disease, the frequency of invasive device use, standards of infection prevention practice between different hospitals and the type of hospital setting (pediatric ward or ICU), length of hospitalization and use of antibiotics (19-23). Long hospital stay and malnutrition were found to be associated with NI in this study. The long hospital stay with higher rate of invasive device utilization for therapeutic feeding or diagnosis may reflect the severity of the underlying disease or malnutrition, both requiring greater care (6, 21) and strongly associated with the development of BSI, UTI and pneumonia.

The distribution of infections by site may vary with various factors, such as age, properties of hospitals and presence of an invasive device (22). As observed in most pediatric studies (4, 19, 20), we found BSI to be the most frequent NI, followed by UTI, SSI and pneumonia. We think that the higher frequency of BSI might be related to a higher rate of IV cannula utilization (74.2%), but also to other factors such as differences in device insertion techniques or further care, or both (5). On the contrary, RTI was the leading cause of NI among pediatric patients in Addis Ababa, Ethiopia (6) and elsewhere outside Ethiopia among pediatric ICU patients (24, 25).

As observed in most studies in developing countries (6, 25), Gram negative bacteria were more prevalent than Gram positive bacteria in hospitalized pediatric patients. In our study, *Klebsiella spp.* followed by *S. aureus*, *E. coli* and *CoNS* were the most commonly isolated nosocomial pathogens consistent with other studies (2, 6, 25). *E. coli* and *Klebsiella spp.* were the most frequently isolated pathogens in UTI. *Klebsiella spp.* was also a common pathogen in pneumonia and SSI. Species of the genus *Klebsiella* are the bacterial pathogens most often found associated with infections in health care settings, and infections may be endogenous or acquired through direct contact with an infected host (26, 27). *Klebsiella spp* has recently been reported to be a major cause of hospital acquired infections in two teaching hospitals of Ethiopia(2). It is likely that the higher frequency of *Klebsiella spp* in this study might be due to prolonged hospitalization, and use of devices like ventilators (breathing machines) or intravenous (vein) catheters (27). Likewise, *E. coli* is a member of the gastro-intestinal flora frequently responsible for UTI through ascending infection. This could be due to the presence of unique structures such as adhesin, pili, fimbriae and P1-blood group phenotype receptor in *Enterobacteriaceae* which help in their attachment to the uro-epithelium (28).

Coagulase-negative staphylococci and *S. aureus* were the most common pathogens in blood stream infections. *S. aureus* was also a common pathogen in SSI. Similarly, previous studies have also reported that these species were also the major pathogens of the afore mentioned clinical features(11). In fact, these organisms are ubiquitous in nature, frequently found on the skin, and invade the vascular endothelium at the insertion site of medical devices. Particularly, CoNS are readily able to form biofilms, and for this reason, they most frequently cause BSIs associated with indwelling foreign devices(29). In addition, because of survival ability on the environmental surface and ability to resist disinfection(8), *S. aureus* could also have an association as the main cause of post-surgical site infection where almost two-thirds (64%) of wounds were infected in our study (data not shown).

In this study, Gram negative bacteria showed high frequency (≥80%) of resistance to ampicillin, amoxicillin/clavulanic acid, and trimethoprim-sulphamethoxazole, which is in agreement with a study done in Addis Ababa(11). Likewise, a high frequency of antibiotic resistance to Gram positive bacteria was reported for penicillin and trimethoprim-sulphamethoxazole. This finding is also in agreement with studies in Addis Ababa (6, 11). However, resistance rate of both Gram positive and negative bacterial species to fluoroquinolones were comparatively lower (≤45.7%), consistent with a previous study in Addis Ababa (6). Among the most frequently isolated bacteria, *S. aureus* resistance, particularly of MRSA observed in this study was comparably higher (62.5%) than the estimate from a meta-analysis done in Ethiopia (32.5%)(30), Benin (52.5%)(31) and Hungary (52.2%)(32) but lower than in studies conducted in Mexico (oncology ICU, 90.6%)(33) and Taiwan (respiratory care centers ICUs, 100%)(34). The variation in the prevalence may be due to differences in time, place, method of MRSA detection in a laboratory and population involved in the study.

Surprisingly, the overall prevalence of MDR was extremely high (89.8%) in this study than that reported in studies conducted in some specific wards in Mexico (ICU, 39.5%)(33) and Thailand (52%) (35). The high prevalence of MDR in our study might be due to a high rate of invasive devices utilization, longer stay and use of broad spectrum antibiotics (54.4%), which possibly lead to selective pressure (35). Studies show that MDR Gram-negative organisms are significantly on the rise (9, 32).

In this study, Gram-negative bacteria showed extremely high frequency of MDR (97.9%) concordant with a study in Nepal(36). As multidrug resistant bacterial infections are associated with higher odds of infection-related mortality, extended duration of hospitalization and higher health care costs(9, 33, these high resistance rates are alarming scenarios for our pediatric wards.

This study has some limitations which need to be noted while interpreting the findings. Multi-drug resistant nosocomial pathogens of high epidemiological interest (i.e., vancomycin-resistant *S. aureus and* ESBL- producing Enterobacteriaceae) were also not assessed.

Conclusion

The prevalence of NI with MDR bacteria was high in the study area, particularly among patients with prolonged hospital stay and malnutrition. Moreover, there was a high rate of invasive device (IV cannula, urinary catheter, nasogastric tube) utilization and use of antibiotics. Blood stream infection was the most common site of NIs. *Klebsiella spp.* followed by *S. aureus* (62.5% of these were MRSA), *E. coli* and *CoNS*. Among all bacterial pathogens, 88.9% were MDR. An extremely high proportion of MDR (97.9%) was observed among Gram negative bacteria with all isolates except *E. coli* being 100% MDR. Therefore, continuous surveillance and strict implementation of infection prevention and control measures against NIs is urgently needed in our setting.

Authors' contributions: TA conceived the idea and developed the proposal. TA, ET, BN and AA reviewed and approved the proposal**.** TA, ET, BY, SA BN and AA: Contributed from inception of the research question to the design, proposal development, analysis, and preparation of the manuscript. TA and AA analyzed the data, were involved in the interpretation of results and critically reviewed the manuscript. All authors read and approved the final manuscript for publication.

Ethics approval and consent to participate: This study was approved by Institutional Review Boards (IRB) of Hawassa University, College of Medicine and Health Sciences and the Armauer Hansen Research Institute (AHRI). Informed written consent was obtained from each parent/guardian. Patient information was anonymized and de-identified prior to analysis.

Conflict of interest: The authors declare that they have no conflict of interest.

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