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Gender and Age Dependent Bacteriological Etiology of Community-Acquired Blood Stream Infection

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Abstract

Background: Bloodstream infection constitutes one of the most serious situations in infectious disease. **Objective:** The purpose of the presents study was to determine the gender and age dependent etiology of community- acquired urinary blood stream infection. **Methodology:** This was a retrospective analysis of blood samples from clinically suspected cases of blood stream infection. Samples were collected at Ad-din Women's Medical College Hospital, Dhaka & Rushmono Specialized Hospital, Dhaka from January 2018 to September 2018. All the samples were collected from inpatient's and outpatient's department of our hospital during the study period and processed in Microbiology laboratory. The BD BACTEC FX40 automated blood culture method was used to isolate bacterial pathogens and antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method. **Results:** A total of 483 (16.1%) pathogens were isolated from 3018 bacteremia suspect patient blood specimens. Gram-negative cocci (58.4%) were predominant organisms recovered followed by Gram-positive bacilli (41.6%). Majority of BSI were caused by Gram negative bacteria predominantly *Salmonella Typhi* (31.1%). *Salmonella species* was found less prevalent in the children (55%) and more frequent in the age groups 16 to 30 years (85.0%) and more than 60 years (100.0%). Acinetobacter species was found less prevalent in age group 16 to 30 years (7.5%). *Escherichia coli* was found only few prevalent in the 1 to 15 (0.5%) and higher frequent in the age groups 16 to 30 years (2.5%). *Salmonella species* isolates appeared to be sensitive to ceftriaxone (91.3%), meropenem (90.7%) and cotrimoxazole (76%). Sensitivity rates of ciprofloxacin, gentamycin and levofloxacin were 87.3% for coagulase-negative *Staphylococcus species* (CoNS). **Conclusion:** Both patients' age and gender are significant factors in determining bloodstream infection.

Keywords: Gender; age group; drug sensitivity; blood stream infection

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Introduction

Blood stream infections caused by bacteria range from self-limiting to life-threatening. Microbial invasion of the bloodstream can have very serious immediate consequences such as shock, multiple organ failures, and disseminated intravascular coagulopathies. Changing patterns of epidemiology, lack of proper antimicrobial guidelines in the locality, the emergence of antimicrobial resistance, and paucity of good diagnostic facilities are the major factors connected to the surge in BSI associated morbidity and mortality[1].

Previous studies revealed that the number of cases of BSI are increasing worldwide[2,3] .Blood stream infection caused by bacteria are among the main causes of mortality and morbidity across the globe,1

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particularly, the mortality range from 4 to 41.5% depending on the severity, age and gender[4]. Besides, high incidence rates of BSI were seen during advanced age due to weak immuno-competency, most often clubbed with some co-morbid conditions[1].

Many bacteria such as *Staphylococcus aureus*, *alpha-hemolytic Streptococci*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella Typhi* and *Acinetobacter species* have been reported as a cause of bacteremia with variation in distribution from place to place. As an example, *Salmonella enterica* is a frequently isolated pathogen from blood samples in both African and Asian regions, however their serotypes differ substantially. *Salmonella Paratyphi* is the predominant organism in the *Salmonella* group in Africa whereas *Salmonella Typhi* is the most frequently isolated organism in Asia. Besides their isolation rate and their antibiotic susceptibility pattern varies substantially[1].

Depending on the age, the severity of infection, other risk factors and the mortality rate for BSI varies between 4.0 and 41.5%. Globally, bloodstream infection affects about 30 million people leading to 6 million deaths[5] with 3 million newborns and 1.2 million children suffering from sepsis annually[6]. Changing patterns of epidemiology, lack of proper antimicrobial guidelines in the locality, the emergence of antimicrobial resistance and paucity of good diagnostic facilities are connected to the surge in BSI associated morbidity and mortality[7].

Increasing antimicrobial resistance is a worldwide concern. It is a serious challenge for health care professionals in prescribing suitable antimicrobial therapy as many bacterial pathogens have developed resistance to most of the antibiotics. Early diagnosis plays a crucial role in managing BSI, and hence, prompt detection of such infections is a critical function of clinical microbiology laboratories. Blood culture is a vital tool for the detection of BSI and remains the gold standard for bacteremia detection. Empiric antimicrobial therapy is based on knowledge of the microbial profile and their antimicrobial sensitivity patterns, clinical and epidemiological data. Irrational use of drugs has led to an increase of multidrug-resistant bugs and thus worsened the condition[8].

In most of the cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Selection of right antibiotic for empiric therapy is of utmost importance. Continuous monitoring trends in the microbiology of BSI pathogens and their antibiotic susceptibility patterns are therefore important to guide empiric antibiotic treatment strategies and infection control programs. Therefore, the purpose of the presents study was to determine the gender and age dependent etiology of community acquired blood stream infection.

Methodology

Study Settings & Population: This was a retrospective observational analysis a total of 3018 samples from clinically suspected cases of blood stream infection were collected at Ad din Medical College and Hospital, Dhaka from January 2018 to September 2018. All the samples were collected from inpatient's and outpatient's department of our hospital during the study period and processed in Microbiology laboratory.

Blood Culture Procedure: About 10 ml of venous blood for adults and 2-3 ml for children was collected aseptically using 70.0% alcohol and 2.0% tincture iodine and transferred in to automated blood culture bottles. The BD BACTEC FX40 automated blood culture method was used. In case of a positive growth, the BD BACTEC FX40 automatically gives an alert. Blood culture bottles were with no alert signal of bacterial growth after recommended days of incubation is considered culture negative. The positive bottles were sub cultured on MacConkey's agar, blood agar and chocolate agar media. The chocolate agar plates were incubated inside a candle jar to provide 5.0 to 10.0% CO2, whereas the other two agar plates (blood agar and MacConkey's agar) were incubated aerobically for 18 to 24 h at 37ºC.

Isolation and Identification of Bacteria: Isolates were further processed according to standard operating procedure (SOP) of the laboratory for its complete identification. Pure cultures of bacterial isolates were subsequently subjected to species identification and confirmation. Gram positive isolates were identified using catalase and coagulase tests. Isolates of members of *Enterobacteriaceae* family were identified biochemically by means of a series of tests: catalase, indole, citrate, urease, H_2S production and triple-sugar iron. Non lactose fermenting Gram negative bacteria were identified by indole, triple-sugar iron, urease, oxidase and catalase tests.

Procedure of Antimicrobial susceptibility tests: Antimicrobial susceptibility tests were performed by using the Kirby-Bauer disc diffusion method and susceptibility patterns were determined following CLSI guidelines[9]. Diameters of the zone of inhibition were measured to the nearest millimeter and categorized as sensitive, intermediate and resistant according to CLSI guidelines[9]. Isolates were classified as either susceptible or resistant to an antibiotic and all the isolates with inter-mediate resistance were classified as resistant. Culture media and antibiotic discs used in the study were obtained from Oxoid Ltd., UK. Quality control for media was done by randomly taking the prepared culture media and incubating overnight to see for any growth. In this study multi-drug resistance (MDR) was defined as simultaneous resistance to more than two antimicrobial agents. Isolates of *Staphylococcus aureus* were further tested for methicillin resistance according to the CLSI guidelines by using cefoxitin disc.

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Chi-square test was used for comparison of categorical variables and Student t test was applied for continuous variables. Every efforts were made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance. Differences between case and control were tested.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local IRB. As this was a retrospective study, no consent form is needed to perform the study.

Results

This study shows 150 (31.1%) were males and 333 (68.9%) were females. From 483 isolates recovered from patients, the spectrum of microbes included 201 (41.6%) Gram-positive cocci (GPC) and 282 (58.4%) Gram-negative bacilli (GNB). Salmonella was the most frequently Gram-negative isolated blood borne bacterial pathogen in this study accounting for 31.1% of the total isolates. CoNS was the most Gram-positive isolated blood borne bacterial pathogen according to 39.1% (Table 1).

Gram negative bacteria were isolated from male and female which was 92 (61.3%) and 189 (56.7%) respectively. The difference between the male and female was statistically significant (p=0.001). Salmonella species were the most commonly isolated from the specimens which was 77 (51.3%) and 73 (21.9%) isolates in male and female respectively. Gram positive bacteria were isolated from male and female which was 51(34.0%) and 144(43.2%) respectively. The difference between the male and female was statistically significant (p=0.001) (Table 2).

Table 1: Distribution of bacteria isolated from blood sample (n=483)

Type of growth	Frequency	Percent
Gram negative	282	58.4
• *Salmonella*	150	31.1
• *Acinetobacter*	87	18.0
• *Enterobactar*	33	6.8
• *E. coli*	9	1.9
• *Klebsiella*	2	0.4
Gram positive	201	41.6
• *CoNS*	189	39.1
• *Staph. Aureus*	12	2.5

Table 2: Distribution of Bacteria Isolated from Blood Sample according to Gender (n=483)

Type of growth	Male	Female	P value
Gram negative	92 (61.3%)	189 (56.7%)	0.001
• *Salmonella*	77 (51.3%)	73 (21.9%)	0.001
• *Acinetobacter*	14 (9.3%)	73 (21.9%)	0.016
• *Enterobactar*	1 (0.7%)	32 (9.6%)	0.001
• *E. coli*	0 (0.0%)	9 (2.7%)	0.001
• *Klebsiella*	0(0.0%)	2 (0.6%)	0.031
Gram positive	58 (38.7%)	144 (43.2%)	0.001
• *CoNS*	51 (34.0%)	138 (41.4%)	0.001
• *Staph. Aureus*	7(4.7%)	6 (1.8%)	0.019

Salmonella was found less prevalent in the children (55%) and more frequent in the age groups 16-30 years (85%) and >60 years (100%). *Acinetobacter* was found less prevalent in age group 16-30 years (7.5%) and more frequent in the age groups <1 years (22.7%) and 1-15 years (15.7). *Enterobacter* was found less prevalent in the 1-15 years (1%) and more frequent in the age groups 31-59 years (100%) and <1 years (9.7%). *E. coli* were found only few prevalent in the 1-15 (.5%) and higher frequent in the age groups 16-30 years (2.5%). *Klebsiella* was found in less than 1 year (0.8%). *Staphylococcus aureus* was found only 1-15 years (6.3%). CoNS was found less prevalent in the 16-30 (2.5%) and more frequent in the age groups less

Table 3: Distribution of bacteria isolated from blood sample according to age and gender (n=483)

Organism	Gender	<1 yrs	1 -15 yrs	16 -30 yrs	31 -59 yrs	≥60 yrs
	All	238	191	40	7	7
	Male	35	96	19	0	0
	Female	203	95	21	7	7
Salmonella	All	4(1.4%)	105(55.0%)	34(85.0%)	0(0.0%)	7(100.0%)
	Male	0(0.0%)	58(60.4%)	19(100.0%)	0(0.0%)	0(0.0%)
	Female	4(2.0%)	47(49.5%)	15(71.4%)	0(0.0%)	7(100.0%)
Acinetobacter	All	54(22.7%)	30(15.7%)	3(7.5%)	0(0.0%)	0(0.0%)
	Male	0(0.0%)	17(14.6%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	54(26.6%)	16(16.8%)	3(14.3%)	0(0.0%)	0(0.0%)
Enterobacter	All	23(9.7%)	2(1.0%)	1(2.5%)	7(100.0)	0(0.0%)
	Male	0(0.0%)	1(1.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	23(11.3%	1(1.1%)	1(4.8%)	7(100%)	0(0.0%)
E. coli	All	7(2.9%)	1(0.5%)	1(2.5%)	0(0.0%)	0(0.0%)
	Male	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	7(3.4%)	(1.1%)	1(4.8%)	0(0.0%)	0(0.0%)
Klebsiella	All	2(0.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	Male	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	2(1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Staph. aureus	All	0(0.0%)	12(6.3%)	0(0.0%)	0(0.0%)	0(0.0%)
	Male	0(0.0%)	6(6.3%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	0(0.0%)	6(6.3%)	0(0.0%)	0(0.0%)	0(0.0%)
CoNS	All	147(61.8%	41(21.5%)	1(2.5%)	0(0.0%)	0(0.0%)
	Male	34(97.1%	17(17.7%)	0(0.0%)	0(0.0%)	0(0.0%)
	Female	113(55.7%)	24(25.3%)	1(4.8%)	0(0.0%)	0(0.0%)

than 1 year (61.0%) and 1 to 15 years (21.5%) (Table 3).

Salmonella isolates appeared to be sensitive to ceftriaxone (91.3%), meropenem (90.7%) and cotrimoxazole (76%). *Acinetobacter* were found to be sensitive (74.4% and 75.9%) to levofloxacin and vancomycin. Sensitivity of *E. coli* was 88.9% to cirprofloxacin, 77.7% to cotrimoxazole. Sensitivity rates of vancomycin was 93.6% and ciprofloxacin, gentamycin and levofloxacin were 87.3% for coagulase-negative *Staphylococcus spp.* (CoNS). *Staphylococcus spp.* were most sensitive (91.7%) to the action of vancomycin and (83.3%) to amikacin (Table 4).

Sensitivity rates of vancomycin was 93.6% and ciprofloxacin, gentamycin and levofloxacin were 87.3% for coagulase-negative *Staphylococcus spp.*

Table 4: Drug-sensitivity Profiles of Gram-negative Isolates

Antibiotic	*Salmonella* (n=150)	*Acinetobacter* (n=87)	*Enterobacter* (n=33)	*E. coli* (n=9)	*Klebsiella* (n=2)
Amikacin	89(59.3%)	13(14.9%)	15(45.5%)	1(11.1%)	0(00)
Azithromycin	82(54.7%)	6(6.9%)	21(63.6%)	1(11.1%)	2(100)
Ceftriaxone	137(91.3%)	57(65.5%)	23(69.7%)	1(11.1%)	2(100%)
Ciprofloxacin	97(64.7%)	50(57.5%)	17(51.5%)	8(88.9%)	0(00)
Cotrimoxazole	114(76%)	45(51.7%)	9(27.3%)	7(77.7%)	2(100%)
Gentamycin	77(51.3%)	37(42.5%)	10(30.3%)	1(11.1%)	2(100%)
Imipenem	14(9.3%)	13(14.9%)	1(3%)	0(00)	0(00)
Levofloxacin	25(16.7%)	65(74.4%)	15(45.5%)	0(00)	0(00)
Meropenem	136(90.7%)	10(11.5%)	21(63.6%)	6(66.7%)	0(00)
Ampicillin	76(50.7%)	35(40.2%)	10(30.3%)	2(22.2%)	0(00)
Doxycycline	85(56.7%)	0(00)	8(24.2%)	0(00)	0(00)
Vancomycin	0(00)	66(75.9%)	0(00%)	5(55.6%)	0(00)
Chloramphenicol	77(51.3%)	28(32.3%)	16(48.5%)	5(55.6%)	1(50%)
Tetracycline	81(51.4%)	0(00)	27(81.8%)	4(44.4%)	0(00)

(CoNS). *Staphylococcus spp.* were most sensitive (91.7%) to the action of vancomycin and (83.3%) to amikacin (Table 5).

Table 5: Drug-sensitivity profile of Gram-positive isolates

Antibiotic	CoNS (n=189)	*Staph. aureus* (n=12)
Amikacin	164(86.7%)	10(83.3%)
Azithromycin	49(25.9%)	0(0.0%)
Ceftriaxone	105(55.6%)	0(0.0%)
Ciprofloxacin	165(87.3%)	9(75%)
Cotrimoxazole	119(63%)	0(0.0%)
Gentamycin	165(87.3%)	8(66.7%)
Imipenem	48(25.4%)	7(58.3%)
Levofloxacin	165(87.3%)	0(0.0%)
Meropenem	37(19.6%)	0(0.0%)
Ampicillin	110(58.2%)	6(50.0%)
Doxycycline	96(50.8%)	5(41.7%)
Vancomycin	171(93.6%)	11(91.7%)
Chloramphenicol	92(48.7%)	0(0.0%)
Tetracycline	97(51.3%)	0(0.0%)

Discussion

Bacterial or fungal bloodstream infections are common among adults and children. This study reveals the importance of bloodstream infections in the different age and gender of patients.

In this study, *Salmonella* (31.1%) was the predominant gram-negative bacteria followed by *Acinetobacter* (18%), *Enterobacter* (6.8%), *E. coli* (1.9%) and *Klebsiella spp.* (0.4%) and *S. aureus.* CoNS (39.1%) (Table-1) was predominant gram-positive bacteria. Several studies from Bangladesh have identified S. Typhi as a common cause of bloodstream infection in this region and reported *Salmonella species* to be responsible for almost half of the disease burden associated with BSI in Dhaka[10]. More or less similar observations have been seen in cases of bacteremia in different countries, though the proportion and prevalence of the bacterial agents varied[11,12]. As the only source of Salmonella infection is the infected human and fecal contamination of drinking water and food supplies, the highest percentage of Salmonella isolates in this study indicate the necessity of proper waste management and infection control practices. Over the past two decades, CoNS, the usual skin commensals are increasingly being considered bloodstream pathogens in select settings. Improper methods of blood collection and the presence of long standing intravascular catheters are recognized as possible modes of spread of BSI by CoNS. In fact, two studies[11,13] reported CoNS as the most common isolate causing BSIs in ICU patients.

This study showed that prevalence of bloodstream infection following data stratification was not consistent across all age groups further divided by gender. *Salmonella* was found less prevalent in the children (55%) and more frequent in the age groups 16-30 years (85%) and >60 years (100%). *Acinetobacter* was found less prevalent in age group 16-30 years (7.5%) and more frequent in the age groups 10-<1 years (22.7%) and 1-15 years (15.7%). Enterobacter was found less prevalent in the 1-15 years (1%) and more frequent in the age groups 31-59 years (100%) and <1 years years (9.7%). E. coli was found only few prevalent in the 1-15 (0.5%) and higher frequent in the age groups 16-30 years (2.5%). *Klebsiella* was found in <1 years (0.8%). *Staph aureus* was found only 1-15 years (6.3%). CoNS was found less prevalent in the 16-30 (2.5%) and more frequent in the age groups <1 years years (61%) and 1-15 years (21.5%). This finding are consented with previous studies Magliano et al[14].

This study shows *Salmonella* were more frequent in male than female which was 51.3% versus 21.9%. All others organism were found more frequent in female than male. The deference was statistically significant ($P<0.05$). Kiffer et al[15] conducted a study comparable to ours, in terms of patient's population (both males and females of any age), number of isolates (35 782), and selected age groups. They also found lower percentage of E. coli isolation in patients younger than 13 years or older than 60 years (69.0% and 68.8%) as compared to the age group 13–60 years (79.7%)[16] ; higher difference in E. coli rates of isolation, between males and females, in the youngest (27.2%) and the oldest (25.8%) age groups with respect to the 13–60 years age group (8.9%);[17] a higher prevalence of *E. faecalis* (16.4%) and *P. aeruginosa* (14.7%) in males older than 60 years, approximately three and six times higher, respectively, as compared to females of the same age group[18].

In this study found a high percentage of *Salmonella* isolates appeared to be sensitive to ceftriaxone (91.3%), meropenem (90.7%) and cotrimoxazole (76%) which is consistent with studies carried out in Nepal, Pakistan and Bangladesh[5,19,20].This might give us some hope that in future we can again start using these antimicrobials for treatment to *Salmonella*. This finding shows 64% and 54.7% Salmonella Typhi were susceptible to amikacin and azithromycin. A study from Nepal also reported a low rate of azithromycin resistance among tested antibiotics[5].

In this study *Acinetobacter* were found to be sensitive (75.9% and 74.4%) to levofloxacin and vancomycin which were consistent with other studies[9,11,12,21]. *Enterobacter* were susceptible to tetracycline and ceftriaxone (78.9%) and (81.8%) which is consistent with studies carried out in Nepal, India and Pakistan[9,19,20].This might give us some hope that in future we can again start using these antimicrobials for treatment to *Enterobacter*.

This study observed sensitivity of *E. coli* was 88.9% to cirprofloxacin, 77.7% to cotrimoxazole and 66.7%% to meropenem. Similar findings have been observed across Saudi Arabia and China[22,23]. *Klebsiella* isolates showed sensitivity rate of 100% to azithromycin, ceftriaxone, cotrimoxazole and gentamycin. However other studies from India, Nepal and Ethiopia showed all the isolates of Gram-negative bacteria were susceptible to cotrimoxazole and meropenem[8,14,24].

In this study, CONS was mostly sensitive to vancomycin (93.6%). The sensitivity rates of ciprofloxacin, gentamycin and levofloxacin were 87.3% for Coagulase-negative *Staphylococcus spp.* (CoNS) which were consistent with other studies[9,21]. They reported CoNS infections were amenable to levofloxacin, gentamicin, and chloramphenicol (90% sensitivity). *Enterococcus spp.* had mixed sensitivities toward gentamicin, chloramphenicol, and tetracycline. Ampicillin was however totally effective for *Enterococcus spp.* Alpha and beta hemolytic *Streptococcus spp.* were uniformly sensitive to penicillin and other beta lactam antibiotics.

Staphylococcus spp. were most sensitive (91.7%) to the action of vancomycin and (83.3%) to amikacin. Ciprofloxacin and gentamycin where other alternatives *Staphylococcus spp.* isolates were highly responsive (75% and 66.7%). Similar study Banik et al.8 *staphylococcus spp.* were most responsive (100%) to the action of teicoplanin, vancomycin, and chloramphenicol. Gentamicin and levofloxacin were other alternatives responsive (>90%) to gentamicin, clindamycin, quinolones, and chloramphenicol besides erythromycin and tetracycline.

Conclusion

In a nutshell, this study reveals that, *Salmonella species* significantly cause blood stream infection in males of age group of 16- 30 years whereas all other microorganisms are more prevalent among females. *E.coli species* are less frequent among patients younger than 13 years and older than 60 years. *Salmonella species* shows highest sensitivity towards meropenem and ceftriaxone. Gram positive organisms are most sensitive to vancomycin and amikacin.

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Conflict Of Interest
The authors have no conflicts of interest to disclose.

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Authors' contributions
Dr. Nafisa Rashid conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Dr. Ritu Saha & Dr. Tarana Jahan contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Dr. Md. Mahbubur Rahman, Dr.Nasrin Akhter Maya and Dr. Asma Ul Hosna involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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