Emerging Bacterial Pathogens Profile from Whole Blood Samples in Patients with Presumed Sepsis in the Intensive Care Unit in Kerbala/Iraq

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Abstract

the goal of the study is to evaluate the diagnostic utility of Presepsin& Calprotectin as biomarkers compared to the traditional used diagnostic methods in addition to investigate bacterial pathogens causes of septic patients. Methods: Before the collection of samples, all research groups' patients were informed, and verbal consent was acquired. The committee on publishing ethics of the college of medicine gave its approval to this work. University of Karbala, Iraq, and in compliance with the Human Rights and Biomedicine Convention. In this case- 40 persons (sepsis patients) were included. Demographic and clinical data and microbiological and laboratory findings, treatments, and outcomes such as ICU and hospital lengths of stay and mortality were all documented. Data were collected until the patient was discharged from the hospital or died. A total of forty blood samples were obtained from clinically diagnosed adult sepsis patients (sepsis group). The samples were collected from both sexes (20 males and 20 females ranging in age from (17 to 72) years' old who were at the ICU at Al-Hussein teaching hospital in Karbala and the Obstetrics hospital in Karbala city/Iraq between (November 2021 and April 2022). Result: Patients. Out of the 40 enrolled subjects, 23 septic patients had a confirmed bacterial etiology of sepsis (blood culture positive) and had confirmed either gram-positive or gram-negative etiology of sepsis, and 16 septic patients were negative for bacterial growth and the bacterial etiology not identified.We analyzed data from 24 patients suffering from bacterial sepsis 16(60%) of bacterial growth positive caused by gram-negative pathogens. The rest 7 (40%) of bacterial growth positive in septic patients were caused by gram-positive pathogens. The most frequently cultivated bacterial pathogens from positive blood cultured samples in this study were Salmonella typhi (16) isolates followed by Staphylococcus epidermidis (4), Staphylococcus aureus (3) and one fungus isolate candida barbican respectively. The study patient was divided into three groups according to the sepsis clinical course of the patients. Conclusion: The majority of sepsis-causing pathogens are bacteria. In the current investigation, adult sepsis was caused by both Gram positive and Gram-negative bacteria. Staphylococcus aureus, Staphylococcus epidermidis, and Salmonella typhi were among the most often found Gram positive and Gram-negative pathogens causing adult sepsis, respectively.

1. Introduction

Sepsis is a prevalent and potentially fatal infection in the intensive care unit that needs prompt and efficient antibiotic therapy. The most prevalent cause is bacterial infections, however viruses and fungi can also develop in people with comorbid illnesses and immunosuppression.[1]

Fever, tachycardia, and tachypnea are classic sepsis symptoms, a scattered inflammatory response produced by microbial infections. At least one organ malfunction has been associated with severe sepsis. When severe sepsis is combined with multiple organ system failures, the condition is known as septic shock.[2]

This life-threatening organ failure caused by a dysregulated host response to an infection is a medical emergency for which early detection, suitable, and prompt therapies are critical in reducing mortality and morbidity.[3]. Although there are numerous criteria for defining organ failure during sepsis, using 3 guidelines (the third iteration of the international consensus diagnostic definitions of sepsis) of Sepsis-related Organ Failure (SOFA) score to do so. The new concept gives us a better grasp of sepsis pathogenesis and more precise diagnostic criteria.[4, 5]

Septic shock is described as sepsis with circulatory collapse, which is the most severe kind of sepsis. "Adequate routine microbiologic cultures (including blood) should be obtained before beginning antimicrobial therapy in patients with suspected sepsis or septic shock when doing so outcomes in no significant delay in the start of antimicrobials," the recommendation for making a definitive diagnosis changed. [6]

Gram-positive bacteria were the most commonly found in sepsis patients in developed countries. S. aureus, Enterococcus species, and S. pneumoniae were the most prevalent Gram-positive organisms

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had been identified, whereas E.coli, K. pneumoniae, Acinetobacter baumannii, and Pseudomonas species were the most common Gram-negative organisms. [7]

The primary test for sepsis is still blood culture, albeit the findings are generally not available for 24 to 48 hours after the collection but still, the gold standard for diagnosis of bacterial sepsis, and the bacterial culture results can provide information for determining the microbiological etiology of an illness, assisting in the selection of suitable empiric antibiotic treatment.[8]

The objective of this study was to take close look at the bacterial makeup of adult sepsis in ICUs.

2. Material and Methods

Before the collection of samples, all research groups' patients were informed, and verbal consent was acquired. The committee on publishing ethics of the college of medicine gave its approval to this work. University of Karbala, Iraq, and in compliance with the Human Rights and Biomedicine Convention. In this case- 40 persons (sepsis patients) were included. Demographic and clinical data and microbiological and laboratory findings, treatments, and outcomes such as ICU and hospital lengths of stay and mortality were all documented. Data were collected until the patient was discharged from the hospital or died.

A total of forty blood samples were obtained from clinically diagnosed adult sepsis patients (sepsis group). The samples were collected from both sexes (20 males and 20 females ranging in age from (17 to 72) years' old who were at the ICU at Al-Hussein teaching hospital in Karbala and the Obstetrics hospital in Karbala city/Iraq between (November 2021 and April 2022). For each patient, case information sheets comprising age, gender, and other factors were completed.

Patients were ruled out if they had a different diagnosis, such as pulmonary thromboembolism, burns, severe pancreatitis, anaphylaxis, adrenal insufficiency, thyrotoxicosis, or alcohol withdrawal.

To assess organ dysfunction in the sepsis group, the Sequential Organ Failure Assessment (SOFA) score was generated daily for four days. The presence of pathogenic bacteria in blood cultures was used to make the etiologic diagnosis.

Blood collection

A 10 ml blood sample was taken from each patient after cleaning the area above the vein and using a new sterile needle for each vein puncture Within an hour of blood draining, the 10 ml blood samples were transported to the laboratory 5 mL of blood sample used for blood culture and cultured in a bottle for bacterial identification using the BACT/ALERT PLUS Culture bottle (BIOMERIEUX, France)culture system and incubated until the BacT/Alert instrument (BACTEC, Becton Dickinson) which will signal it as positive or negative.

The other 5 mL of blood was set in a gel tube to get the serum from patients, then centrifuged 1000 x g (or 3000 rpm) for 15 minutes to obtain serum, which was then frozen at -20 °C for subsequent immunological and blood biochemistry tests.

Isolation and Identification of bacteria

Bacterial isolates were detected by inoculating 5ml of blood into a culture bottle, which was then incubated at 37°C for up to 7 days until the final report.

Subcultures were made from all bottles either negative or positive (both bottles which showed signal or not), Brain Heart Infusion broth was utilized for bacterial resuscitation, and blood agar and MacConkey agar were also employed for bacterial identification. They were incubated at 37°C overnight. Each bacterial isolate in the study was subjected to phenotypic bacterial identification, which included colony characterizations, and Gram-stain morphology to distinguish between gram-positive and gram-negative bacteria and check the isolate's purity, and bacterial biotyping and identification at the species level using the Vitek-2 system (BioMerieux).

Using a sterile swab, 1-2 colonies from each isolate's pure culture were collected an+d suspended in a specific transparent plastic polystyrene test tube (a 12 x 75 mm) given by the manufacturer company containing 3 ml sterile saline (aqueous 0.45 percent to 0.50 percent NaCl, pH 4.5 to 7.0) and vortexed.

The turbidity of the bacterial suspension was adjusted using a turbidity meter (DensiChek TM) supplied by the company, and the final concentration of the suspension should be between 0.5-0.63, as needed for the GN and GP Identification cards. The VITEK® 2 Identification cards were warmed to room temperature and utilized in accordance with the product information manuals (BIOMERIEUX).[9]

3. Result

Patients. Out of the 40 enrolled subjects, 23 septic patients had a confirmed bacterial etiology of sepsis (blood culture positive) and had confirmed either gram-positive or gram-negative etiology of sepsis, and 16 septic patients were negative for bacterial growth and the bacterial etiology not identified.

We analyzed data from 24 patients suffering from bacterial sepsis 16(60%) of bacterial growth positive caused by gram-negative pathogens. The rest 7 (40%) of bacterial growth positive in septic patients were caused by gram-positive pathogens. The most frequently cultivated bacterial pathogens from positive blood cultured samples in this study were Salmonella typhi (16) isolates followed by Staphylococcus epidermidis (4), Staphylococcus aureus (3) and one fungi isolate candida barbican respectively. The study patient was divided into three groups according to the sepsis clinical course of the patients. The baseline characteristics of the study subjects are explained in table 3.1.

Table 3.1:Bacterial sepsis group characteristics N =40			
Age (mean ± SD)	(17-72)		40.67±14.24
Gender	Male		20(50%)
	female		20(50%)
Etiology	Gram-positive	7	Staphylococcus epidermidis (4) Staphylococcus aureus (3)
	Gram-negative	16	*Salmonella typhi* (16)
	Fungi	1	Candida (1)
Patients with blood culture tested	40		
The positive blood culture result	24(60%)		
The negative blood culture result	16(40%)		
Sepsis clinical course patients	Sepsis (s)	Severe sepsis(ss)	Septic shock(sx)
	20	10	10

Of the thirty -five positive bacterial growth there was sixteen (60%) isolates of the bacterial pathogens were gram-negative and the rest 7 (40%) bacterial pathogens were gram-positive as explained in table 3.2.

Table 3.2 Bacterial isolates and their frequency from positive blood culture patients with sepsis.		
Gram reaction	Number of Bacterial isolates	Percentage %
Gram-positive	7	40
Gram-negative	16	60
Total	23	100

According to the severity of the disease of septicemia. The patients were classified into the levels (total=20 sepsis) , (total=10 sever sepsis) and (total=10 septic shock).In Sepsis(s) clinical course level the blood culture result was positive growth in 12 (60%) of twenty patients with sepsis(s) and 8(40%)was bacterial growth negative while In the sever sepsis course level the blood culture result was positive growth in 3(30%) of ten patients with severe sepsis(ss) and 7 (60%) was bacterial growth negative .The blood culture result of the septic shock level was positive in 9 (90%) and only one patient (10%) was growth negative. the results of bacterial growth between the clinical course of the patient's study subgroups according to Sepsis clinical course showed statistical significance (P=0.04). the significance is between sever sepsis and septic shock group where significant most of patients had positive bacterial growth.as illustrated in table 3.3.

Table 3.3 Blood culture results according to the sepsis clinical course of patients of the study				
Sepsis clinical course	Total no. of patients N-=40	Blood culture result		P-value
		Growth positive N= 24	Growth negative N=16	
Sepsis(s)	20	12(60%)	8(40%)	
Severe sepsis(ss)	10	3(30%)	7(60%)	
Septic shock(sx)	10	9(90%)	1(10%)	
Total	40	24	16	
0.040*-Sepsis Vs sever septic =0.121 -Sepsis Vs septic shock=0.90 -Sever sepsis Vs septic shock= 0.006* * Chi-square test, Significant difference at P<0.05				

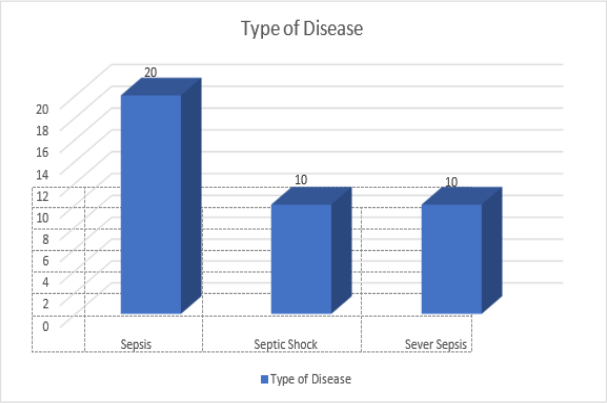

Figure (3.1): Frequencies of patients based on severity of sepsis

As shown in table 3.4 etiological pathogen distribution for patient group after blood culturing were as follows: Staph aureus 3(7.5%), Staphylococcus epidermidis 4(10%), Salmonella typhi 16(40%) & Candida albicans 1(2.5%).

Table 3.4 Etiological pathogens distribution for patients' group	
Pathogen name	N (%)
Staph aureus	3(7.5)
Staphylococcus epidermidis	4(10)
Salmonella typhi	16(40)
Candida albicans	1(2.5)
No growth	16(40)

The frequency and distribution of bacterial species according to stages of infection in patients 'investigated subgroups of sepsis (S) (total=20), severe sepsis(SS) (total=10), and septic shock(Sx) (total=10) for each Clinical course of sepsis was as follows: for Staphylococcus epidermidis (n = 4), isolated from one (20%) blood samples in a patient with severe sepsis course and three (37.5%) isolates from blood samples in patients with septic shock course, Staphylococcus aureus(n = 3), three (30%) isolates of Staphylococcus aureus isolated from blood samples in a patient with sepsis course and no isolates from the two other subgroups recorded. Salmonella typhi (n = 16), isolated in 7

blood Samples (70%) isolates from sepsis,4(80%) isolates in severe sepsis and 5 (62.5%) isolates from septic shock patients as illustrated in table (3.5). There is no significant variation between the percentages of isolates between the sepsis clinical course subgroups.

Table3.5: Type of bacterial isolates from patients according to the sepsis clinical course of the patients of the study					
Type of bacterial isolates	Total	sepsis	Severe sepsis(ss) No. (%)	Septic shock(sx) No. (%)	P-value
Staphylococcus epidermidis	4	0(0%)	1(20%)	3(37.5%)	0.105
Staphylococcus aureus	3	3(30%)	0(0%)	0(0%)	
Salmonella typhi	16	7(70%)	4(80%)	5(62.5%)	

The present study's findings showed that both sexes (men and females) were receptive to bacterial infection with males being more responsive to infection having the higher percent of infection (66.7%). The bacterial growth results were compared in the 40 screened adult patients, between both male(total=20) and female (total=20) according to (gender) who were included in the study there was significant variation between male and female in bacterial growth results in which 16 (66.7%) of 20 males with sepsis were positive for bacterial growth while of 20 females with sepsis only 8(33.3%) was positive for bacterial the rest were negative and no bacterial growth recorded.

The mean age of the patients was (40.67±14.24). When the data of bacterial growth analyzed compared to the age groups there was no significant variation between the age intervals of study patients, but the age range of (28 to 53) years were the most associated with infection as recorded in table (3.6).

Table (3.6) distribution of bacterial growth results according to the gender and age interval				
Variable	Category	Bacterial growth		P-value
		Positive Growth N= 24	Negative Growth N=16	
Gender	Male	16(66.7%)	4(25%)	0.022*
	Female	8(33.3%)	12(75%)	
	Total	24	16	
Age	15 - 27 Years	5(20.9%)	4(25%)	0.956
	28 - 40 Years	8(33.3%)	5(31.25%)	
	41 - 53 Years	8(33.3%)	4(25%)	
	More Than 54 Years	3(12.5%)	3(18.75%)	
	Total	24	16	
Chi-square test, Significant difference at P<0.05				

The table 3.7 explain that: Out of the 40 enrolled subjects, 23 patients had a confirmed bacterial etiology of sepsis, gram-positive 7(40%) or gram-negative 16 (60%) etiology of sepsis. We analyzed data from 7 patients suffering from sepsis caused by gram-positive pathogens and 16 patients suffering from sepsis caused by gram-negative pathogens The comparison of the baseline characteristics of study patients who diagnosed with gram-positive and gram-negative between males and female sepsis patients and between the patients age groups intervals which did not reach statistical significance.

Table (3.7): Distribution of gender and age groups based on results of bacterial growth type (Gram Positive & Gram Negative)				
Variable	Category	Bacterial growth type		P-Value
		Gram positive N=7	Gram negative N=16	
Gender	Male	3(42.9%)	10(62.5%)	0.38
	Female	4(57.1%)	6(37.5%)	
Age	15 - 27 Years	2(28.6%)	2(12.5%)	0.08
	28 - 40 Years	1(14.3%)	7(43.8%)	
	41 - 53 Years	1(14.3%)	6(37.5%)	
	More Than 54 Years	3(42.9%)	1(6.3%)	

4. Discussion

Sepsis is a fatal illness that raises healthcare issues on a worldwide scale and is linked to unfavorable clinical and financial results. Even in today's cutting-edge healthcare systems, sepsis mortality remains too high. Numerous studies have shown that timely provision of effective antibiotic therapy is essential for the patient's life.[10]

Therefore, it is essential for patient care that the laboratory provides early and useful information on the identification and antibiotic susceptibility of the bacterium causing sepsis.

Several studies have shown that the clinical implementation of rapid microbiological diagnostics of sepsis results in decrease in mortality, length of stay and health care costs and shorter time to appropriate antimicrobial therapy [11]

Blood cultures are now the most reliable way to

diagnose sepsis. Blood cultures allow for the identification of bacterial pathogens and are sensitive and simple to perform. [12],Until recently the identification of microorganisms from sub-cultures of the positive blood culture usually took between 24-72 hours.

Numerous underlying disorders and diseases, such as chronic illnesses or those using chemotherapy or immunosuppressive medications, have been shown to contribute to the development of sepsis.[10], One may argue that a large variety of microorganisms are capable of causing sepsis. Protozoa, fungi, and bacteria are some of them. However, bacteria and yeast are the most frequent causes of sepsis.

According to the study's most general findings, the frequency of microbiological etiology from positive blood culture patients with sepsis was both gram negative and gram positive and candida. Other researchers throughout the world have made similar findings.

The most conclusive findings from this study showed that more Gram-negative bacteria than Gram-positive bacteria were present in the majority of sepsis patients. others have found a predominance of Gram-positive bacterial pathogens [13],[14]

The most frequent pathogens responsible for sepsis, according to several research, are either Gram-positive or Gram-negative bacteria. Sepsis in the ICU is mostly caused by gram-negative bacteria as reported by several researchers.[15], [16], [17] and this is concurred with the study results.

Gram-negative bacteria, for instance, are responsible for around 50–70% of nosocomial infections in Brazil, Nepal, the United States, and other parts of the world; comparable data has also been reported.[18], [19], [20]

Causative bacterial pathogens were not found in 40% of the current study patients. The Gram-negative bacteria play the leading role in patient's groups of our study and such microorganism causing the initial infection naturally dominates as Blood stream infection pathogen. In general, the proportions of Gram-negative and Gram-positive bacteria causing sepsis did not reach statistical significance.

Other researchers across the globe have made comparable observations. For instance, a key indicator of the outcome is the kind of bacterium causing sepsis. The most recent European Prevalence of Infection in Intensive Care (EPIC II) research discovered that gram-negative organisms were more frequent, despite the fact that most earlier studies have shown an increase in the prevalence of gram-negative organisms (62.2 percent). S. aureus (20.5%), Pseudomonas species (19.9%%), Enterobacteriacae (mainly E. coli, 16.0%, salmonella 57%) and fungus were the most prevalent infecting organism patterns, which were similar to those observed in past study (19 %). With significant infection rates in various regions of the world, acinetobacter was discovered in 9% of all illnesses.[21], [18]

Bacteria's cell wall constituents and extracellular metabolites strongly stimulate the immune system. Endotoxin, a component of the Gram-negative cell wall, plays a key role in the immuno-activation of the host response in Gram-negative bacteria. Gram-positive bacteria have pro-inflammatory cell wall components such peptidoglycan and lipoteichoic acid but lack endotoxin. Both Gram-negative and Gram-positive bacteria are capable of producing virulent exotoxins. [22], [23]

Initially it was thought that the major organisms that caused bacterial sepsis were gram-negative bacteria. However, over the past 25 years it has been shown that gram-positive bacteria are the most common cause of sepsis and this in contrast with our study.[22]

An important role in the etiology of sepsis in adults in our study was played by Salmonella typhi n=16 which was the prominence bacterial pathogen followed by and Staphylococcus epidermidis, Staphylococcus aureus (n=3) n=4 and candida albican (n=1)

Ramachandran reported that Some of the most frequently isolated bacteria in sepsis are Staphylococcus aureus ,Streptococcus pyogenes, Klebsiella spp., Escherichia coli ,salmonella and Pseudomonas aeruginosa.[22] . also several bacterial etiological surveys studies for sepsis revealed that Various species of Klebsiella, Enterobacter, Acinetobacter, and Pseudomonas, E. coli, salmonella and S. aureus are major sepsis etiologies with regional variation [24] among all causative pathogens which are similar to the rates in previous reports in developing and under developing countries.[3], [5], [24]

In research published in 2016, Almuhayawi reported that the proportions of gram-positive and gram-negative bacteria that cause sepsis are often comparable. The most prevalent Gram-negative bacteria are Escherichia coli, Klebsiella species, Pseudomonas aeruginosa, Enterobacter species, and Serratia species, whereas the most prevalent Gram-positive bacteria are Staphylococcus aureus, CoNS, Enterococcus species, viridans streptococci, and Streptococcus pneumoniae.[10]

In our study salmonellae was the highest bacterial pathogen that recorded in positive patients blood culture samples and this was in accordance with several researchers showed that salmonella was the main bacterial pathogen between the bacterial etiology of sepsis patients as recorded data in their studies.[25],[26], [27],[28].

The study found one case of candidemia. which differs markedly from other reports of fungal infections ranging from 5·9–28·3% .[14], [29], [30]

Previous research has found that Candida fungus have a considerably larger role in the development of BSI in infants and children in their first year of life than in adults [5, 25, 26]. Sepsis caused by yeast is becoming more common. Currently, candidemia is thought to be the fourth most prevalent BSI in ICUs. The main pathogen causing candidemia has been identified as Candida albicans. However, throughout

time, the epidemiology of candidemia has altered quickly, increasing the likelihood of finding different Candida species.[10], [31].

The present study's findings showed that the age range of 39 to 49 years was the most impacted, and that both sexes (males and females) were receptive to bacterial infection, with males being more responsive to infection. and this is supported by a number of researches. [18], [11].

In this investigation, salmonella typhi isolates, Staphylococcus aureus, and Staphylococcus epidermidis were all correctly identified using the automated system VITEK2 compact system. The ability to recognize multiple Staphylococcus species that the traditional approach is unable to distinguish is another benefit of the VITEK 2 technology. Numerous commercially available automated systems have been looked at for routine laboratory usage; VITEK 2 displays a range of coagulase-negative Staphylococci genera and species. Several peer-reviewed studies have demonstrated that automated VITEK 2 technology and VITEK 2 ID cards provide reliable and accurate findings for Gram-positive cocci and Gram-negative bacilli that are clinically significant [18], [32], [33].

Variations in microbiological patterns of sepsis, severe sepsis and septic shock may be due to patient demographic differences, comorbidities, less widespread use of broad-spectrum antibiotics, or less use of invasive therapies or procedures. Nonetheless, in our investigation, microorganisms, in sepsis, sever sepsis and septic shock were not revealed significant difference.

Different findings may be caused by a variety of variables, such as the degree of antibiotic resistance, the patient's profile, treatment guidelines, and the clinical staff members who use the information from the microbiology laboratory in patient care.

The parameters in the clinical samples might be different from those in the simulated cultures. These may include the varied blood cell composition that is often seen in clinical blood cultures, antimicrobial drugs, and the transport time of blood culture bottles.

However, it would be extremely challenging to conduct the necessary research for the detection of uncommon microorganisms like anaerobic bacteria and uncommon clinical situations like polymicrobial sepsis in prospective clinical investigations. The results from the simulated samples may likely represent how well the techniques function analytically in everyday clinical practice.

Our research has certain drawbacks. First, because there was just one center, there could have been bias in regard to ICU admission, the caliber of treatment, and administration. Second, there was no attempt to assess ICU acquired infections, and third, we did not assess the treatment packages for Surviving Sepsis. Iraq should host a multicenter trial on the incidence and prognosis of severe sepsis treated with the sepsis bundle.

5. Conclusions

The majority of sepsis-causing pathogens are bacteria. In the current investigation, adult sepsis was caused by both Gram positive and Gram-negative bacteria. Staphylococcus aureus, Staphylococcus epidermidis, and Salmonella typhi were among the most often found Gram positive and Gram-negative pathogens causing adult sepsis, respectively. The most frequent bacterial cause of bacterial sepsis infection is Salmonella typhi. The present study's findings showed that the age range of (39 to 49) years was the most impacted, and that both sexes (men and females) were receptive to bacterial infection, with males being more responsive to infection. Identification of the sepsis pathogen involves a wide spectrum of both current and developing technology. As cutting-edge technology innovations develop and move from research to clinical practice, this sector is anticipated to continue to advance.

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Conflict of Interest

The authors declare that there is no conflict of interest

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