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Statistical analysis of the relationship between mortality and nosocomial factors in patients with septicemia and the importance of *Pseudomonas aeruginosa*

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Abstract: The ongoing evolution of sepsis as a condition constitutes a global health concern and necessitates continuous monitoring and investigation of incidence rates, mortality factors, and disease patterns. This study sought to elucidate the frequency of bacterial cultures in patients with septicemia at our hospital and identify the factors influencing mortality. Zoonotic risk factors with reference to the literature were also taken into account.Independent variables of all patients diagnosed with sepsis were retrospectively screened to reveal factors affecting mortality. Incomplete or unclear data were not included. Continuous variables are represented as means and standard deviations, whereas binary variables are represented as percentages and frequency values. The distribution was evaluated using the Kolmogorov–Smirnov test. Separately, the Student's t-test or Mann–Whitney U test was used to compare differences for continuous variables between independent groups according to distribution status. Dichotomous variables were evaluated using the chi-squared or Fisher's exact test. Significant results found during univariate analysis were reevaluated using linear and binary logistic regression. Neither the length of hospital stay nor patient age was statistically significant, for mortality. Among dichotomous variables, sex also did not impact the mortality rate. Meanwhile, *Salmonella*, *Shigella*, and *Pseudomonas aeruginosa* infections were found to cause mortality. During the final statistical analysis using multiple logistic regression, only *P. aeruginosa* was a factor influencing the mortality rate. *P. aeruginosa* is an important pathogen that contributes to increased risks of mortality and zoonotic transmission among patients with sepsis.

Key words: Septicemia, *Pseudomonas aeruginosa*, zoonotic transmission, mortality, nosocomial infection

1. Introduction

The significance of the development of antibiotic resistance is well known and is related to the feasibility of transition zone creation for various diseases between humans/the environment, and animals. Although many studies have presented important evidence that antimicrobial drug-resistant bacteria may spread from animals to humans, some research also suggests that humans can also transmit resistant pathogens to animals in a reverse zoonotic event known as zooanthroponosis.

Separately, another significant issue of concern in the fight against infectious diseases is sepsis, which significantly increases the risk of mortality. Some clinical studies have estimated that sepsis occurring in 30%–50% of hospitalised patients' results in death [1,2]. Sepsis is a life-threatening condition that arises when the body's reaction to an infection damages its own structures and organs [3,4]. Currently, there is no gold-standard diagnosis for sepsis, and nonstandard descriptions limit the comparability of results among clinical and epidemiological studies [4]. It is thought that cornerstone risk factors for sepsis are those that are risk factors for zoonotic transition to humans. While animals in healthcare facilities have traditionally been limited to laboratories and research fields, their presence in patient care settings is now more common in both acute and long-term care settings. Unfortunately, this can lead to the potential transmission of zoonotic pathogens from animals to humans in these environments [5]. Although dogs and cats are common in health centre locations, other animals such as fish, birds, nonhuman primates, rabbits, and rodents are also used for research or as service animals. These animals can be a source of zoonotic pathogens and may pose a risk to both patients and health care personnel [5]. Despite advances in hospital treatment and care, current epidemiological studies show that sepsis remains a major burden worldwide [6]. Walkey et al. stated that the frequency of sepsis cases increased from 359/100,000 to 535/100,000 residents between 2003 and 2009, and this rate increased daily in the United States [7]. However, according to findings obtained by scanning

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ICD-9 codes, there are also studies in the United States stating that current hospital sepsis rates are higher than ICU, and it is stated that it ranges from about 25% to 30% [8]. Recently, the existing criteria for sepsis have been revised in an attempt to overcome the nondiscriminatory limitations between infections and sepsis [8]. In general, 50% of all sepsis cases are caused by Gram-negative rods, but half of them are correlated with a positive blood culture [9]. In septic shock, 50% to 60% of cases are the result of Gram-negative bacteria, while 5% to 10% of cases are caused by Gram-positive bacteria or circulatory fungal infections [10]. The prevalence of sepsis is expected to increase in the future due to the growth in antibiotic-resistant bacteria and an increase in the use of immunosuppressive therapy, invasive procedures, and device transplantations. At this time, sepsis is already the most common cause of death among patients hospitalised in noncoronary intensive care units [11]. This study sought to clarify the types of bacteria isolated from patients with sepsis at a single hospital in Turkey, determine whether there are any bacterial species that pose a particularly high zoonotic risk, and outline the risk factors capable of affecting patient mortality rates.

2. Materials and methods

2.1. Patients and management

Prior to its implementation, the present study received institutional review board approval (decision no. 90057706-799, 27 June 2019). Eligible data for analysis were collected from 30 May 2015 to 30 May 2019 at Yirmidokuz Mayıs State Hospital. The records of all patients diagnosed with sepsis were reviewed retrospectively. Eighty-six infected patients with white blood cell counts of less than 4000/mm^3 or greater than 12,000/mm^3, which are laboratory markers for sepsis, were diagnosed by the culture method. Our target population included cases diagnosed as sepsis whose clinical and laboratory values could be confirmed. More specifically, we included white blood cell counts and data on other vitals in cases in which we could access official, clinical records clearly indicating sepsis. We also included patients whose culture results were positive per collected data of vitals such as respiratory rate, pulse rate, and body temperature per the general definition of sepsis; the condition involves a detected infectious pathogen together with systemic inflammatory reaction syndrome (SIRS) [12]. However, at least two of the following systemic inflammatory response criteria should be present when making a diagnosis: (a) a body temperature of below 36 °C or above 38 °C; (b) a heart rate of 90 beats/min, a respiratory rate of 20 breaths/min or more and a Pa CO_2 of less than 4.3 kPa; (c) a white blood cell count of less than 4000/mm^3; and (d) a count of greater than 12,000/mm^3 or immature cell 10% (band form) [12]. Patients with no clinical diagnosis of sepsis or those with a diagnosis code for sepsis but whose laboratory values were not compatible with sepsis were excluded. In other words, if there were no detected microbiologic pathogens or if the vitals data or white blood cell count were within normal limits, we did not consider these as sepsis cases. Among the study population, the characteristics we aimed to examine as secondary outcomes were C-reactive protein (CRP) level, urea, creatinine, hemoglobin, partial thromboplastin time, prothrombin time, international normalised ratio (INR), fever, respiratory rate, and heart rate to identify factors affecting mortality. Incomplete or unclear data were not included. Age, sex, length of hospital stay, clinical patient distribution, and microorganisms isolated from patients with sepsis were analysed separately by considering demographic patterns.

2.2. Statistical analysis

When evaluating continuous variables, the Student's t-test or the Mann–Whitney U test was used to compare differences between independent groups according to distribution status, which was determined by the Kolmogorov–Smirnov test. When evaluating binary variables, the chi-squared or Fisher's exact test was performed. For 95% safety limits, a P-value of less than 0.05 was adopted. According to univariate analysis results, factors affecting mortality were determined using multiple linear or binary logistic regression. Statistical analysis was conducted using the Statistical Package for the Social Sciences Version 22 software program (IBM Corp., Armonk, NY, USA).

3. Results

The average age of the patients was 75.25 ± 16.51 years old, and there was a significant difference in mean age between the sexes. Men were diagnosed with sepsis at a younger age (70.59 ± 18.9 years vs. 79.51 ± 12.68 years; P = 0.013). There was also no statistically significant difference regarding the length of hospital stay observed between men and women (28.90 ± 33.29 days vs. 26.31 ± 29.78 days; P = 0.747) (Table 1). While there was no statistically significant difference in mortality rate according to sex, patients under general intensive care more often died as a result of septicemia (Table 1).

When we used the chi-squared test as a univariate test to compare cases of white blood cell counts of less than 4000 × 10^9 cells/L to those with more than 12,000 × 10^9 cells/L, which are important whole blood criteria for the diagnosis of sepsis and bacteria detected by culture, a statistical significance between mortality and *Salmonella, Shigella, Staphylococcus aureus*, or *Pseudomonas aeruginosa* infections were found (Table 2).

The Student's t-test was used to compare fatal and nonfatal cases of sepsis. Here, we observed a statistically significant difference in neutrophil percentage, blood urea

Table 1. Demographic patterns of age and length of hospital stay and general demographic patterns of patients diagnosed with septicemia in terms of sex and clinical distribution according to mortality.

	Mean ± standard deviation	Sex (mean ± standard deviation) (n = 86)		P < 0.05
		Male (n = 41)	Female (n = 45)	
Age	75.25 ± 16.51	70.59 ± 18.97	79.51 ± 12.68	0.013
H-LOS	27.54 ± 16.5	28.90 ± 33.29	26.31 ± 29.78	0.704
	Mortality		Total number of cases	P < 0.05
	Mortality cases (n = 41)	Discharged cases (n = 45)	Total number of cases (n = 86)	
Male	29 (53.7%)	12 (29.3%)	41	0.153
Female	25 (46.3%)	20 (60.6%)	45	
GICU	52	24	86	0.018
Other clinics	1	9		

Length of hospital stay [(H-LOS) (days)], age (years); GICU: general intensive care unit. Other clinics include centres for palliative care, chest diseases, internal medicine, coroner intensive care, orthopedia, and traumatology.

nitrogen value (BUN), creatine, INR, and active partial thromboplastin time (APTT) between fatal and nonfatal cases (Table 3).

Using logistic regression analysis performed after advanced statistical analysis, which followed the univariate analysis, the presence of *P. aeruginosa* appeared statistically significant among the cases diagnosed with sepsis and was observed to be the most important factor contributing to mortality (Table 4).

Separately, the results of the univariate analysis showed that the percentage of neutrophils was different between the patients who died and those who survived, although the values were not located within the confidence limits (Table 4).

4. Discussion

The term 'zoonos' is used to discuss diseases that concern both humans and animals. Transition to humans can be from either domestic or wild animals. Along these lines, many animals and their products can serve as reservoirs for zoonotic pathogens [13].

On the other hand, a 'nosocomial infection' is when hospital-acquired or health-related infections occur in patients under or receiving medical care. These infections, which are common worldwide, constitute a major medical problem in both developed and developing countries. Nosocomial infections are reported at rates of 7% in developed countries and 10% in developing countries [14]. Bacterial, viral, and fungal pathogens can be responsible for nosocomial infections. Common routes of infection in this context include central catheterisation, urinary catheterisation, surgical area infections, and ventilator-related pneumonia [14]. As these infections occur in patients under hospitalisation, they may also cause long-term disability and economic burden [15].

Nosocomial infections affect a large number of patients worldwide. According to Jones et al., *P. aeruginosa*, *K. pneumoniae*, *Acinetobacter* spp., and *Escherichia coli* have developed as notable nosocomial pathogens due to their multidrug resistance. This may be related to the outcome of *P. aeruginosa* as the most impactful pathogen in our study. Moreover, among the isolates tested by Jones et al., *P. aeruginosa* appeared as the bacteria most resistant to antibiotics [16]. In 2019, Scott et al. reported that *P. aeruginosa* strains obtained in a veterinary department, which displayed high levels of resistance to polymyxin, had a significantly similar genetic status between some human and animal isolates. The authors also proposed that continuous surveillance is needed in both animals and humans [17]. Flammer reported that many enteric bacteria spread primarily through the fecal–oral route but that the transfer of enteric bacteria can be effectively reduced by proper hygiene, animal husbandry, and disinfection [18]. Pittet et al. also documented the effectiveness of simple precautions such as hand washing, which is very easy and important to do [19]. In research conducted by Hall et al. and Brand et al., it was stated that zoonotic bacteria affecting morbidity and mortality were observed among people, including *P. aeruginosa* [20,21]. Because *P. aeruginosa* transmission can occur from birds taken from pet shops whose ancestors were tropical sea birds, we believe that consideration of this point is important. *P. aeruginosa* is a very common pathogen that can be found in almost 20% of nosocomial infections in the blood circulation of all Gram-negative bacteria [22]. In addition to being widespread, this pathogen has been shown

Table 2. Results of the bacterial species isolated from cultures; white blood cell counts (low ≤ 4000 10^9 cells/L and leukocytosis ≥ 12,000 10^9 cells/L) were evaluated for chi-squared mortality.

	Mortality (+)	Mortality (–)	P < 0.05
Age (years)	73.17 ± 17.97	78.77 ± 13.23	0.128
Length of hospital stay (days)	28.59± 31.39	25.78 ± 31.66	0.69
P. aeruginosa (+)	22	5	0.012*
WBC ≤ 4000 or WBC ≥ 12,000 10^9 cells/L	64 (81%)	15 (19%)	0.396
Klebsiella spp.	3	4	0.275
Klebsiella pneumoniae	14	4	0.139
Acinetobacter spp.	8	3	0.436
Micrococus luteus	1	0	0.432
Burkholderia cepacia	0	1	0.198
Acinetobacter baumanii	4	4	0.460
Difteroid bacil	3	2	0.922
Salmonella	9	0	0.013*
Shigella	8	0	0.022*
Serratia marcescens	0	1	0.198
Escherichia coli	12	5	0.420
Proteus mirabilis	2	1	0.867
Meticillin-sensitive *Staphylococcus aereus*	0	1	0.198
Coagulase (–) *Staphylococcus aereus*	5	1	0.401
Staphylococcus haemolyticus	1	0	0.379
Coagulase (+) *Staphylococcus aereus*	0	3	0.05*
Staphylococcus hominis	1	2	0.554
Staphylococcus epidermis	3	0	0.291
Streptococcus spp.	0	1	1.000
Enterococcus spp.	0	1	1.000
Pseudomonas spp.	4	1	0.646
Candida albicans	7	2	0.473
Candida spp.	11	4	0.323
Enterobacter cloaca	0	1	1.000
Proteus vulgaris	1	0	1.000
Proteus spp.	5	3	1.000
Klebsiella oxytoca	0	1	0.379

to promote adverse outcomes with high mortality and morbidity rates in patients. Therefore, significant efforts by researchers to identify survival-related factors and treatment options are ongoing. In this clinical study, we found that *P. aeruginosa* was a contributing factor in 40.7% of deaths among patients with sepsis, which is consistent with the literature. In the literature, patient mortality following *P. aeruginosa* infection has been associated with both microbial and host-related factors [23]. Bacterial infections that cause mortality have especially high internal virulence characteristics. Balibar et al. showed that Gram-negative bacteria have multiple mechanisms of resistance against antibacterial drugs, and this is attributed to their cell envelope structure and to the fact that molecules that pass through this envelope structure are often ejected by multiple drug-flow pumps. For these reasons, Balibar et al. preferred using *P. aeruginosa* in their in vitro studies [24]. In contrast, Wu et al. [25] reported that *P. aeruginosa* caused cytotoxicity in many cell types and through production of 1 of 2 type III secreted exotoxins: ExoS or

Table 3: Univariate analysis results of continuous independent variables by Student's t-test.

Total number of patients (n = 86)	Mortality (+) (n = 54)	Mortality (–) (n = 32)	P-value
Age (years)	73.17 ± 17.97	78.77 ± 13.23	0.128
Length of hospital stay (days)	28.59 ± 31.39	25.78 ± 31.66	0.692
Fever	38.15 ± 0.69	38.06 ± 0.78	0.597
Pulse	96.72 ± 8.26	98.39 ± 6.67	0.304
Respiratory rate	23.96 ± 19.78	20.48 ± 2.74	0.319
Leukocyte count	16647 ± 15368	17889 ± 11207	0.801
Percentage of neutrophils	84.4 ± 7.64	74.4 ± 10.81	0.015*
Hgb	9.69 ±2.07	9.81 ± 1.70	0.809
CRP	53.4 ± 98.5	48.5 ± 64.9	0.826
Ca	7.85 ± 0.79	8.12 ± 0.91	0.227
Albumin	2.29 ± 0.71	2.46 ± 0.50	0.349
APTT	48.35 ± 22.35	39.9 ± 8.35	0.037*
PT	23.06 ± 15.68	22.51 ± 29.4	0.926
INR	2.03 ± 1.69	1.26 ± 0.199	0.007*
BUN	56.8 ± 40.35	38.8 ± 29.82	0.044*
Creatinine	1.56 ± 0.89	1.10 ± 0.91	0.041*

CRP: C-reactive protein; Ca: calcium; APTT: active partial thromboplastin time; PT: prothrombin time; INR: international normalised ratio; Hgb: hemoglobin.

Table 4. Evaluation of the significant results in univariate analysis resubjected to further statistical analysis by multiple linear and binary logistic regression.

Total number of patients (n = 86)	Mortality (+) (n = 54)	Mortality (–) (n = 32)	P	Adjusted odds ratio (95% CI)/ adjusted mean difference (95% CI), 95% CI for EXP (B)	P-value
P. aeruginosa (+) vs. (–)	22 (40.7%) vs. 32 (59.3%)	5 (11.2%) vs. 28 (84.8%)	0.012	3.54* (1.09–11.454)	0.03*
White blood cell number (WBC) < 4000 vs. WBC > 12,000 (+/–) 10^9 cells/L	38 (74.5%) vs. 13 (25.5%)	24 (82.8%) vs. 5 (17.2%)	0.396	0.365 (0.11–1.23)	0.103
Percentage of neutrophils	84.4 ± 7.64	74.4 ± 10.81	0.015	0.404 (0.08–0.04)	0.002*

ExoU [26,27]. When we examined the multiple resistance mechanisms of this bacterium, the low permeability of the outer membrane was found to result from the structural presence of β-lactamases, the efficacy of outflow pumps, and the ability to express resistance genes (e.g., following genetic gain for β-lactamases). Notably, these mechanisms are often present simultaneously, which lead to the highly resistant phenotypes found in both humans and animals [26,27]. Therefore, susceptibility testing is especially important for *P. aeruginosa* in clinical practice. We believe that detection of resistance to antibacterial agents in this study is very important in terms of the isolates concomitant to both human and animal origin. As we emphasise in Tables 3 and 4, when we look at what affected mortality in our study, some markers that initially were hypothesised as having predictive properties were not found to be significant in other studies, univariate analysis, or the final logistic regression analysis. These results maybe dependent on the limited number of cases and may also depend on the limited number of studies available in this area. Among the variables that were mentioned above, urea, creatinine, CRP, hemoglobin, partial thromboplastin time, prothrombin time, INR, fever, respiratory rate, and heart rate were not found to be significant variables. Instead, the most

significant factor influencing mortality was *P. aeruginosa* infection. We believe that the presence of *P. aeruginosa* appeared as a significant risk factor for mortality [3.53 (1087–11.54); P < 0.036)] (Table 4) in this study probably because of the organism's multiple resistance mechanisms and high virulence characteristics. Host factors correlated with poor outcomes include neutropenia, prolonged bacteremia duration, pneumonic source, shock, renal failure, and metastatic foci of infection [28,29]. On the other hand, host factors associated with higher rates of mortality and morbidity include other important medical problems and neutropenia, long-term exposure to bacteremia, pneumonic infection focus, renal failure shock, and metastatic foci of infection [28,29]. In this study, the percentage of neutrophils was found to be significant following a univariate analysis, which was not in agreement with outcomes reported in the literature. Similarly, the percentage of neutrophils was found to be a significant risk factor for *P. aeruginosa*-induced mortality, although it was not statistically within the confidence limits using multiple linear regression (84.4% ± 7.64% and 74.4% ± 10.81%; P = 0.015) [AOR: 0.404 (0.08–0.04); P = 0.002)] (Table 4).

On the subject of BUN, Al-Aloul et al. reported 8 cases of acute renal failure (ARF) following the administration of intravenous aminoglycosides for the treatment of pulmonary exacerbations with an epidemic highly resistant *P. aeruginosa* strain in adult patients with cystic fibrosis [30]. In our present study, BUN and creatinine levels were not recorded as statistically significant. Bilgili et al. noted that although the most common cause of ARF in critically ill patients is sepsis, limited knowledge exists about sepsis-related ARF. The authors stated that medications that have long been successful in animals for the treatment of ARF have unfortunately not been similarly effective in most people experiencing sepsis and sepsis shock in intensive care units. They further suggested that sepsis is a common cause of death and that this necessitated new investigations into the diagnosis and treatment of sepsis-related ARF [31]. On the other hand, Souza et al. [32] stated that exposure to *P. aeruginosa* may cause septic shock, especially in patients with chronic renal failure. Therefore, we believe that the creatinine level, which is important in renal function, is higher in cases that experience mortality and that abnormal renal function may have the potential to progress to failure more rapidly in cases where this pathogen is present. Pradhan et al. emphasised that CRP is useful in identifying patients with sepsis diagnosed with SIRS. In addition, they stated that CRP could be a very useful marker in resource-restricted areas where sepsis specialists are not available and especially in places where data on procalcitonin or interleukins are not accessible [33]. In our study, although there was no difference between mortal and nonmortal cases diagnosed with sepsis, this does not minimise the fact that CRP may be beneficial in the diagnosis of sepsis, although it is not predictive for all aspects of the condition. According to the results of a study on calves published in 2006 considering the use of APTT and prothrombin time, FDPs, and PLT for diagnosing sepsis, the animals with questionable septic shock demonstrated a spectrum of hemostatic dysfunction [34]. In our study, these markers were not statistically and significantly effective in making a diagnosis of sepsis. According to the results of Quinten et al. in 2015, sepsis lacks a reliable and easily obtainable way to measure disease activity. Along these lines, to discern the treatment effect of sepsis patients, the authors stated that trends showing an increase in oxygen saturation and blood pressure and a decrease in heart rate, respiratory rate, and body temperature could be considered positive responses. Based on the results of that study, it is underlined that the use of vitals data should be further considered. Additionally, the authors stressed that it is an advantage that these data can be collected easily, inexpensively, and noninvasively, alongside being able to indicate significant changes where they appear [35]. In our study, there was no statistical significance between mortal and nonmortal sepsis cases in terms of body temperature, heart rate, and respiratory rate. For this reason, we believe that vitals data may be more suitable for conducting follow-up rather than predicting mortality from sepsis. In 2001, Dennesen et al. found that tracheal colonisation by resistant pathogens such as *P. aeruginosa* emerged in the second week of treatment [36].

Additionally, Bisbe et al. reported that following univariate and multivariate analyses of the factors affecting prognosis [37] among 133 consecutive sequences in *P. aeruginosa* bacteremia, *P. aeruginosa* was the most common cause of negative nosocomial bacteremia (25.6%). In the present study, when we examined the average hospitalisation period of the patients who died, we noted that they stayed in the hospital for nearly 1 month (28.59 ± 31.39 days). In addition, Bisbe et al. [37], in their consecutive analysis of 133 cases, reported direct (45%) or indirect (5%) mortality rates in association with infection and noted that approximately half of the recorded deaths occurred during the first 2 days following the detection of bacteremia [37]. Approximately half of these cases were patients hospitalised in intensive care units or in renal or bone marrow transplantation units [37]. As emphasised in the literature, we believe that this pathogen should be proactively addressed where possible, especially in intensive care units because of the potential for rapid onset and the high risk of mortality. In our study, we found a 3.5-fold increase in mortality and observed that 98.2% of sepsis-related deaths were

caused by *P. aeruginosa*. However, other bacterial or viral pathogens may also cause a similar risk in the future. Thus, the possibility of zoonotic transmission must always be considered, especially when facing epidemic tragedies.

In conclusion, the risk of passing zoonotic diseases to people is increasing, and this can lead to very tragic consequences worldwide. *P. aeruginosa* with zoonotic colonisation and transition features can lead to an infection that can also cause sepsis and mortality, especially in patients in intensive care. Due to the potential of zoonotic transmission, it should always be kept in mind as it is still a very important zoonotic risk factor affecting patient care outcomes.

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