Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i17.2659

Value of Neutrophil gelatinase-associated Lipocalin in serum and peritoneal fluids in the Diagnosis of Spontaneous Bacterial Peritonitis and the Prediction of in hospital mortality.

A Randomized controlled trial.

Alshymaa A. Ahmed [1], Maha Roushdy Abd El Wahed [1], Marwan Elgohary [2], Fatma Atef Ibrahim[1]* and Rehab Mohamed Ateya [1].

1. Department of clinical pathology, faculty of medicine, Zagazig University, Alsharquia.
2. Department of Internal medicine, faculty of medicine, Zagazig University, Alsharquia.

***Corresponding author**: Fatma Atef Ibrahim, Department of clinical pathology, faculty of medicine, Zagazig University, Alsharquia, Egypt.,Email: FatmaAtefIbrahim@gmail.com

Submitted: 20-08-2023; Accepted: 25-08-2023; Published: 02-09-2023

ABSTRACT

Background Spontaneous bacterial peritonitis (SBP) is one of the most critical complications in chronic liver disease patients with ascites. Lipocalin 2 (Lcn2), also known as neutrophil gelatinase associated lipocalin (NGAL), is a 25-kDa protein present in peroxidase-negative granules of neutrophils, and is released following neutrophil activation. Lipocalin-2 is a promising marker for diagnosis of infections, especially in chronic liver diseases (CLD).

Objective This study aims to evaluate role of serum and ascitic lipocalin-2 as a reliable biomarker in diagnosis of SBP and as a predictor of short term in-hospital mortality in patients with chronic liver disease -related SBP.

Methods This case-cohort study was conducted on 80 patients with CLD &Ascites, 40 patients with SBP infections and 40 patients without SBP . All patients were subjected to measurement of lipocalin-2 levels in both serum and ascitic fluids by enzyme-linked immune-sorbent assay (ELISA) kits . For SBP cases , Siderophores production was determined among bacterial isolates quantitatively by modified CAS assay method and analyzed genetically by multiplex polymerase chain reaction (PCR) ,then cases were followed up for 30 days in hospital mortality.

Results The results indicated that ascites Lipocalin-2 was significantly higher in patients with SBP compared to those without SBP. In ROC analysis, ascites Lipocalin-2 had an AUC of 0.845 as a marker for diagnosis of SBP cases . Sixteen (40%) patients died in the hospital. In the final multivariate model, MELD score, Creatinine , INR, Total and direct bilirubin remain significant predictors of in-hospital mortality ($P<0.05$).

Conclusion Ascitic fluid Lipocalin-2 level can be used as a diagnostic marker of SBP in hospitalized

patients with cirrhosis with high sensitivity and specificity, especially in chronic liver disease patients . MELD score remains an independent predictor of short-term survival in-hospital mortality.

Keywords: Neutrophil gelatinase-associated Lipocalin (NGAL) , Lipocalin 2 (Lcn2) , chronic liver disease (CLD), Spontaneous bacterial peritonitis (SBP), In hospital mortality.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as an infection of the previously sterile ascetic fluid that occurs without any clear intra-abdominal source of infection [1,2]. It is a serious complication of advanced liver disease and chronic severe hepatitis, with a prevalence of 10–30% among hospitalized patients [3, 4]. Different investigations reported varying hospital mortality rates in hospitalized patients with cirrhosis and SBP [5-9].

At present, clinical diagnosis of SBP is established on the presence of polymorphonuclear leukocyte (PMN) ≥ 250 cells/mm3 in ascites after exclusion of any finding suggestive of secondary peritonitis, regardless of ascitic fluid culture [10,12]. SBP often occurs during hospitalization and is regarded as a hospital-acquired infection or nosocomial (SBP diagnosis more than 48 hours after hospitalization) [11].It is considered one of the leading cause of death in this category of patients, with mortality rates of 20–40% [12,16,17].

Neutrophil gelatinase-associated lipocalin (NGAL) also known as lipocalin-2(Lcn2), a member of the lipocalin superfamily, is a low molecular weight secretion protein (25 kDa) , present in neutrophils and multiple other tissues[21,22,24]. Lipocalin 2 (Lcn2), acquired great attention as a bacteriostatic agent by depleting intracellular iron stores[18].It prevents iron acquisition by microorganisms via sequestering iron loaded bacterial siderophores [24].In addition, it represents an important component of the innate immunity to bacterial infections[19].

Lipocalin 2 has many functions including response to injury, as in the case of acute renal tubular damage, as well as involvement in the innate immune response to infection [26,27]. Lipocalin 2 is rapidly released in response to inflammation, ischemia, and metabolic disorders and has thus been investigated as a biomarker for a number of conditions [27–29]. Lipocalin 2 measurement may also be of significant utility in patients with advanced liver disease. Serum Lipocalin 2 is also elevated in the setting of liver injury. [20,30].

Elevated levels of lipocalin 2 have been detected in the blood of patients with bacterial urinary tract infection (UTI), community acquired pneumonia, sepsis, as well as in the cerebrospinal fluid and peritoneal fluid of patients with bacterial meningitis and peritonitis[23].Also, Increased Lipocalin 2 levels in plasma and ascitic fluid have showed significant correlation with SBP, but still requires an invasive sampling technique.[20]

Serum Lipocalin 2 is a promising marker for diagnosis of infections, especially in chronic liver disease [24, 25]. Also, ascitic fluid Lipocalin-2 may be a biomarker of peritonitis in patients with cirrhosis and an independent predictor of short term in hospital mortality, proper controlling for SBP and model for end-stage liver disease (MELD) score [20].

Gram-negative bacteria were the main causative agents of spontaneous bacterial peritonitis, with Escherichia coli and Klebsiella spp. being the most frequently isolated organisms [31]. Pathogenic E. coli and Klebsiella pneumoniae isolated from different clinical infections possesses a broad range of virulence-associated factors, including toxins, adhesions , lipopolysaccharides, and siderophores (enterobactin ,aerobactin, yersiniabactin and salmochelin),which are encoded by pathogenic islands and other mobile genetic elements [36, 37,40].

It is generally assumed that the acute-phase protein lipocalin 2 acts as an antimicrobial agent in human plasma and effectively prevents the systemic spreading of bacteria that depend on corresponding siderophores [103]. Lcn2 may tailor anti-infective response by inhibiting bacterial proliferation based on microbial iron metabolism [106].

Siderophores are small, high-affinity iron chelators produced by many microorganisms. Several siderophores have been previously detected in E .coli and klebsiella like Enterobactin (Ent), Aeribactin (Aer) , Yersiniabactin (Ybt) and Salmochelin (Sal), [39 ,40].They allow bacteria to take up protein-bound iron

from the host cells[38]. Siderophores constitute a key component of pathogenicity of gram negative bacterial infections.

Currently, there are five structural classifications for the iron chelators: catecholate, hydroxamate, carboxylate, phenolate and mixed siderophores [108]. The structural differences among siderophores portray the virulence of microbes, especially in regards to whether their siderophores could be sequestered, and thus subdued, by the host innate immune protein, lipocalin 2 (Lcn2) [107]. Lcn2 chelate specifically catecholate and some carboxylate siderophores and is unable to sequester hydroxamate and mixed siderophores [107].

The secretion of lipocalins are key in protecting the host from siderophore-mediated iron chelation; however, bacteria have further enhanced their countermoves for iron sequestering, including the generation of stealth siderophores [107]. Microbes employ various strategies to evade Lcn2 as bacteria continue to achieve their virulence through siderophore modifications, or utilization of alternate siderophores, can be explained by evasion of Siderocalin binding [109].

For Example , Enterobactin is produced by nearly all E. coli strains and produced by many other pathogenic enterobacteria, including Klebsiella spp. [100].The inefficacy of bacteria has been attributed to the absorption of this protein by Lipocalin 2. It has been demonstrated that modification of enterobactin through glycosylation by IroB results in the generation of salmochelins that can evade sequestration by Lipocalin 2 [101]. Enterobactin glycosylation is, therefore, an important virulence mechanism through which certain pathogenic strains can evade host immune defenses and obtain iron [102].

Therefore to resist Lcn2-mediated inhibition, pathogens such as Salmonella spp. And Klebsiella spp. have evolved to express stealth siderophores (e.g., salmochelin, aerobactin, and yersiniabactin), which augment siderophore-mediated iron acquisition and, therefore, microbial virulence and pathogenicity [107,108].

Salmochelins, for example, are derivatives of Ent that carry one or two C-linked glucose substituents on the catechol groups [104] and cannot form a complex with lipocalin 2 due to steric hindrance.[105] Thus, corresponding strains of Salmonella spp., Klebsiella pneumonia and E. coli evade the human innate immune system simply by making an ordinary siderophore more bulky[103].

Moreover, siderophores producing gram-negative bacteria acquire multidrug resistance (MDR) resulting in augmentation of infections. Patients with MDR Gram-negative bacterial infections are more likely to face worse outcomes, such as increased mortality and longer hospital stays [41].

Multidrug-resistant (MDR) organisms are predominantly found in nosocomial spontaneous bacterial peritonitis, being reported in about 20%–35% of the episodes [31]. The increasing prevalence of MDR bacterial infection has been associated with failure of empirical antibiotic therapy and poor prognosis [42] due to a higher mortality rate, an increased duration of in-hospital stays and higher healthcare related costs when compared to infections caused by susceptible strains [43].

Gram-negative bacteria have developed several mechanisms of resistance to currently used antimicrobials. One of the successful mechanisms for transmitting multiple-drug resistance among bacterial pathogens is horizontal transfer[112]. The spread of MDR isolates in the clinic has been attributed to commonly shared plasmids across bacteria such as K. pneumoniae, K. oxytoca, Escherichia coli, Enterobacter sp., and Salmonella sp[113,114].

Treatment of infections caused by pathogens such as E. coli and Klebsiella pneumoniae is threatened by the emergence and increase in antimicrobial resistance associated with genetic mobile elements such as plasmids that may also contain virulence genes [115]. These bacterial species are proficient pathogens because they are virulent, antibiotic resistant, and epidemic in nature [111].

In addition to the role of acquisition of virulence genes and its effect on the pathogenicity, the acquisition of resistance genes plays an important role in therapeutic failure and the increase of mortality rate. The inability to control the emergence of multi-drug (MDR), extensive drug (XDR) and pan drug resistance will increase the mortality rates to 10 million people by 2050[116,117]

Because of diversity in the mechanism of

acquisition of resistance and virulence factors of the bacterial strains, the correlation between resistance and virulence is a complex issue and is poorly understood [115].Knowledge and understanding of correlations between antibiotic resistance and virulence factors would therefore be helpful and enable antibiotic treatment of several infections [111].

Although many studies have focused on the relationship between Lipocalin 2 and AKI, there are relatively few studies on Lipocalin 2, especially ascitic Lipocalin 2 and SBP, an important complication of cirrhosis .Therefore, The aim of this work is to investigate the role of serum and ascitic Lipocalin-2 to identify patients with SBP and to detect short term in hospital mortality predictors in these patients and to evaluate the correlation between Lipocalin 2 levels and antibiotic resistance as well as siderophores production. Also, we try to understand relation between Siderophores as a virulence factor and antimicrobial resistance .

METHODS

2.1. Study Design

Between September 2019 and January 2021, this case-cohort study was carried on eighty patients with chronic liver disease and ascites who were admitted to Internal medicine department , Faculty of Medicine , Zagazig University. They were classified into two groups: group 1 included 40 chronic hepatic patients with SBP and group 2 included 40 chronic hepatic patients without SBP ,then , SBP cases were followed up for 30 days during hospital admission (to detect short term in hospital mortality).

2.2. Ethical Approval

The current study was approved by the Institutional Review Board (IRB) committee of Zagazig University (no. ZU-IRB # 5523/25-8-2019).

2.3. Patients Diagnosis

In the presence of clinical symptoms and signs, diagnosis of chronic liver disease was based on the results of clinical and biological examinations and ultrasound and other imaging techniques [46], a patient was diagnosed with Ascites (clinical Complication of chronic liver disease with clinical examination and radiological ultrasound diagnosis of ascites volume ≥150 mL) [44] and cases of SBP were diagnosed on the basis of PMN ≥ 250 cells/mm3 and/or positive ascitic fluid culture for single organism with exclusion of acute renal impairment ,renal replacement therapy and secondary peritonitis as in ,intra- abdominal surgery or malignancy[45,46]. Blood and Peritoneal fluid samples were taken from every enrolled patient in our study on the day of paracentesis [20] . In-hospital mortality was defined as death in the hospital after 30 days [20].The site of SBP acquisition was classified as nosocomial as the diagnosis was made 48 h or longer after hospitalization [47] .

2.4. Data and Sample Collection

Eighty patients diagnosed with chronic liver disease and Ascites were included ,their data were collected from electronic medical health records including age and sex and investigated with laboratory findings that include Ascitic fluid analysis (all patients underwent paracentesis then ascitic fluid was subjected to cytology, culture and biochemical examination), Complete Blood Count (CBC), Coagulation profile: Prothrombin time (PT) and international normalized ratio (INR), Liver function tests (LFT), Kidney function tests (KFT) and Electrolytes . Severity of liver disease was assessed by Model for End-Stage Liver Disease (MELD) score for each patient . All patients were subjected to measurement of neutrophil gelatinase-associated lipocalin (NGAL) levels in both serum and ascitic fluids (Serum and Peritoneal fluid samples were collected in sterile syringes). For SBP cases , Phenotypic and Genotypic Detection of Siderophores were done ,then they were followed up for 30 days in hospital mortality.

2.5. Quantitative mesurement of Neutrophil Gelatinase Associated Lipocalin (NGAL)

Serum and ascitic fluid Levels of Lipocalin were detected quantitatively with enzyme-linked immunosorbent assay (ELISA) kits (SunRed biotechnology co., Shanghai), used according to the manufacturer's instructions. The limit of detection for this assay is in the range of 12–3000 ng/mL.

2.6. Microbiological Identification

Blood agar and MacConkey agar were used to cultivate clinical specimens (Oxoid Co., England). Standard microbiological procedures (colony morphology and Gram stain) and biochemical reactions characteristics using VITEK® 2 compact system and ID-GN card (BioMérieux, Marcy L'Etoile, France) [48].

2.7. Phenotypic Detection of Siderophores

Siderophores production by bacterial isolates grown in M9 medium [50,51] were determined quantitatively by using CAS liquid medium according to Schwyn and Neilands (1987) and by the method of Alexander and Zuberer (1991) in which 2-[Nmorpholino] ethanesulfonic acid (MES buffer) (FisherScientific, Belgium) was used as a buffer solution instead of solution 2 in the original method [50,51].

2.8. Molecular Detection of siderophores

DNA extraction was conducted according to the manufacturer guidelines employing the G-spinTM Genomic DNA Extraction Kit (iNtRON Biotechnology, Inc., Korea). Multiplex PCR was used to detect universal housekeeping gene for all bacteria , three significant siderophores genes in K. pneumonia (Ent B ,Iut A ,Ybt S) and three significant siderophores genes in E.coli (Ent B ,Iut A ,Irp 1) [49]. A total volume of 20 µl was adequately prepared for the PCR reaction mixture, including 2 µl (100 ng) of template DNA, 10 µl of PCR master mix, and 1 µl (5 pmol) of each primer . For the amplification, the thermal cycling conditions were employed: a two-minute initial denaturation step at 95 □C, followed by 35 cycles of DNA denaturation at 95 □C for 30 seconds, primer annealing at 60 □C for 60 seconds for K. pneumonia while primer annealing at 55 □C for 60 seconds for E.coli , and primer extension at 72 □C for one minute and a five-minute final extension step at 72 □C Then, gel electrophoresis and U.V. visualization was conducted for the PCR products [49].

Table 1: Primers used in Multiplex PCR in this study as regard klebseilla spp

Gene	sequence	ann	Bp
YBTS	F 5` GACGGAAACAGCACGGTAAA 3` R 5` GAGCATAATAAGGCGAAAGA 3`	60	242
entB	F 5` GTCAACTGGGCCTTTGAGCCGTC 3` R 5` TATGGGCGTAAACGCCGGTGAT 3`	60	400
iutA	F 5` GGGAAAGGCTTCTCTGCCAT 3` R 5` TTATTCGCCACCACGCTCTT 3`	60	920

Table 2: Primers used in Multiplex PCR in this study as regard E.coli spp

Gene	sequence	ann	Bp
Entb	F 5` GCGACTACTGCAAACAGCAC 3` R 5` TTCAGCGACATCAAATGCTC 3`	55	382
IRP1	F 5` AGAGCGGAAATAACCGAACA 3` R 5` GTAAACAGGCCGTGACGATT 3`	55	221
IUTA	F 5` CCAGCCTCAAACTCCATCAT 3` R 5` ACAGCCGACAACTGGACTCT 3`	55	157

2.9. Calculation of MELD Score: MELD = (0.957 x log (Serum Creatinine) + 0.378

x log (Total Bilirubin) + 1.120 x log (INR) + 0.643) x 10 (for dialysis, Creatinine = 4) (52).

STATISTICAL METHODS

The data were statistically analyzed using (Statistical Package for Social Science) SPSS software version 20.0. Normality of data was tested by Shapiro Wilk test of normality .The collected data were summarized in terms of mean± Standard Deviation (SD) (parametric data) and median and range (non-parametric data) for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Independent t-test (t) to detect difference in the mean between two parametric data, while the Mann-Whitney (MW) test was used to detect difference between two non- parametric data. The Kruskal Wallis test (KW) was used to detect difference between more than two group regarding non-parametric data. The Chi-square test (x2) and the Fisher Exact (FET) were used to detect difference between proportions as appropriate.

Receiver Operating Characteristics (ROC) analysis was carried out to evaluate the diagnostic performance of Ascitic Lipocalin-2 for SBP. The best cut-off point and the corresponding sensitivity and specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Area under the Curve (AUC) were estimated.Cox regression model (proportional-hazards) was used to control the effects of the confounding variables to determine the risk factors associated with mortality. The Spearman Correlation coefficient (rho) was used to assess the correlation between serum and ascitic Lipocalin-2 levels and estimated parameters.

All statistical comparisons were two tailed with statistical significance at P value <0.05 (S) , P value <0.001 indicate highly significant (HS) while a P value >0.05 was considered non-significant..

RESULTS

1- Spontaneous bacterial peritonitis (SBP) Group against Non Spontaneous bacterial peritonitis Group.

Table (1): Socio-demographic characteristics among both groups:

		Studied groups		X^2	P
		SBP n=40	Non SBP n=40		
Sex	Male	22 (55.0%)	24 (60.0%)	0.102	0.749 NS
	Female	18 (45.0%)	16 (40.0%)		
Age	Mean ± SD	60.6 ± 12.5	57.8 ± 10.2	t-test 0.76	0.451 NS

This table shows that SBP and Non SBP groups are properly matched regarding age and sex , there are statistically non-significant differences between studied groups regarding Socio-demographic characteristics (P>0.05).

Table (2): Comparison between serum and ascitic Lipocalin-2 levels between SBP and non-SBP groups:

Lipocalin-2	SBP			Non SBP			MW	P
	Mean	S.D	Median (range)	Mean	S.D	Median(range)		
Serum lipocalin-2 n=20	730.89	426.70	604.5 (285.1-1880.3)	606.45	214.0	642.5 (290.3-923.1)	0.414	0.678 NS
Ascitic lipocalin-2 n=20	628.9	427.5	564.8 (167.4-1694.1)	405.33	167.55	288.34 (46.3-820.5)	2.07	**0.047 S**

This table shows a statistically significant difference between studied groups regarding Ascitic Lipocalin-2 level (P<0.05), while there is statistically non-significant difference between studied groups regarding Serum Lipocalin-2 level (P>0.05).

Table (3): Receiver operator characteristic (ROC) analysis for ascitic Lipocalin-2 as a marker for the diagnosis of SBP:

AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	P
0.845	297.8	95.6%	92.5%	95%	95%	95%	.000

ROC analysis for ascitic Lipocalin-2 as marker for diagnosis of SBP reveals that at a cut-off value of: 297.8 (ng/dl), sensitivity is: 95.6%, specificity is: 92.5%, positive predictive value(PPV) is: 95%, negative predictive value(NPV) is: 95%, area under curve (AUC) at 0.845, it is statistically high significant (P>0.05).

2- Spontaneous bacterial peritonitis (SBP) Group : Klebseilla isolates against E. coli isolates

Table (4): Comparison between Siderophores production among KP and E.coli isolates:

	Bacterial isolates	**n**	**Mean**	**S.D**	**Median (Range)**	**P MW**
Siderophores production	KP	28	45.2	12.7	44.16 (29-77.21)	**0.011 S**
	E.coli	12	26.3	13.5	22.8 (7.5-46.7)	

This table shows a statistically significant difference between KP and E.coli isolates regarding Siderophores production (P<0.05).

Table (5): Comparison of siderophores genes distribution among kp and E.coli isolates:

Siderophores genes	Bacterial isolates			
	KP (n=28)	E.coli (n=12)	X^2	P
Ent B n=22	18 (64.3%)	4 (33.3%)	2.82	
Ent B +Others n=12 (Ent B with Iut A or Ybts)	4 (14.3%)*	8 (66.7%)**		0.243 NS
Others n=6 (Iut A or Ybts)	6 (21.4%)***	0 (0%)		

* two EntB+IutA and two EntB+YbtS
** eight EntB+IutA
*** four cases IutA and two case YbtS

This table shows statistically non-significant differences between KP and E.coli isolates regarding Siderophores genes (P>0.05).

3-Relation between Lipocalin 2 level and different studied parameters among SBP group

Table (6): Correlation coefficient between ascitic and serum Lipocalin-2 levels with Siderophores production among SBP group :

	Ascitic Lipocalin-2		Serum Lipocalin-2	
	r-value	P-value	r-value	P-value
Siderophores production	0.336	0.148	-0.234	0.321

This table shows non-significant correlation between Lipocalin-2 levels in serum and ascitic fluid and Siderophores production (P>0.05).

Table (7): Relation between Lipocalin-2 level in serum and ascitic fluid and Siderophores genes among SBP group:

	Siderophores genes	**n**	**Mean**	**S.D**	**Median (Range)**	**H**	**P**
Serum Lipocalin-2	Ent B	22	556.11	165.65	583.04(285.1-769.1)	3.92	0.14 NS
	Ent B +Others	12	1112.03	580.2	1018.2 (568.7-1880.2)		
	Others	6	609.46	353.24	491.9(329.9-1006.5)		
Ascitic Lipocalin-2	Ent B	22	539.63	282.78	508.2 (167.4-979.9)	1.17	0.557 NS
	Ent B +Others	12	889.2	627.37	641.56 (284.4-1694.1)		
	Others	6	435	221.34	369.01(255.1-682.5)		

This table shows statistically non-significant differences between Siderophores genes and Lipocalin-2 levels in serum and ascitic fluid (P>0.05).

Table (8): Relation between Lipocalin-2 levels in serum and ascitic fluid and Antimicrobial resistance patterns among SBP group:

	Bacterial isolates			H	P
	XDR n=12	MDR n=16	Others n=12		
Serum Lipocalin-2 Mean ±SD Median(Range)	929.237±446.14 719.38(575.2-1366.9)	750.788±498.7 597.34(434.07-1880.24)	506.033±190.3 478.60(285.09 -741.7)	4.056	0.131 NS
Ascitic Lipocalin-2 Mean ±SD Median(Range)	777.11±508.9 647.45(284.44 - 1694.1)	545.06±490.6 341.04(167.34-1664.26)	592.47±243.23 633.77(305.9- 870.59	2.208	0.331 NS

This table shows statistically non-significant differences between Lipocalin-2 levels in serum and ascitic fluid and XDR ,MDR and other bacterial isolates (P>0.05).

4- Relation between Virulence and Resistance :

Table (9): Relation between Siderophores genes and XDR, MDR and Other isolates:

Siderophores genes	Bacterial isolates			X^2	P
	XDR n=12	MDR n=16	Others n=12		
Ent B (n=22)	4(18.1%)	6(27.2%)	12(54.5%)	6.157	0.187 NS
Ent B +Others (n=12) (Ent B with Iut A or Ybts)	8(66.6%)	4(33.3%)	0(0.0%)		
Others (n=6) (Iut A or Ybts)	0(0.0%)	6(100%)	0(0.0%)		

Others : remaining resistant and sensitive isolates.

This table shows statistically non-significant differences between Siderophores genes and XDR ,MDR and other isolates among SBP group (P>0.05).

Table (10): Relation between Siderophores production and Antimicrobial resistance:

	Bacterial isolates			H	P
	XDR n=12	MDR n=16	Others n=12		
Siderophores production Mean ±SD Median(Range)	30.345±15.17 30.88(7.5-53.67)	35.45±12.36 35.02(21.66-57.82)	52.71±12.62 49.15(41.43-77.21)	6.72	**0.034 S**

Others :remaining resistant and sensitive isolates.

This table shows statistically significant differences between Siderophores production and XDR ,MDR and other isolates (P<0.05).

5-Analysis of Mortality Risk factors :

Table (11): Univariate and Multivariate regression analysis of factors predicting 30 days mortality among SBP cases:

parameter	Uni-variate analysis		Multi-variate analysis	
	RR (95%CI)	P	RR (95% CI)	P
Age	0.72(0.621-1.52)	0.511		
TLC in fluid	0.93(0.71-3.11)	0.374		
LDH in fluid	1.03(0.762-2.09)	0.208		
Glucose in fluid	1.23(0.85-4.21)	0.129		
Total protein in fluid	0.54(0.426-3.19)	0.850		
WBCs	1.87 (0.97-3.23)	**0.04**	1.12 (0.943-3.21)	0.07
Hb	2.45 (1.15-5.22)	**0.01**	0.987(0.841-3.76)	0.987
PLT	2.11 (1.56-4.22)	**0.03**	0.998 (0.889-1.65)	0.121
PT	1.25 (1.02-2.33)	0.911		
PTT	1.12 (0.932-2.43)	0.212		
INR	1.77 (1.14-3.12)	**0.001**	3.23 (2.34-7.17)	**0.002**
Total bilirubin	2.43 (1.17-5.22)	**0.01**	2.66 (1.65-3.16)	**0.02**
Direct bilirubin	1.11 (0.993-2.22)	**0.02**	1.22 (1.03-2.78)	**0.03**
Total protein	0.99 (0.732-1.01)	0.414		
Albumin	1.07 (0.653-2.11)	0.221		
ALT	1.33 (0.612-2.34)	0.211		
AST	0.996 (0.858-1.22)	0.267		
Creatinine	2.13 (1.22-4.11)	**0.02**	1.87 (1.12-6.33)	**0.002**
BUN	0.929 (0.899-1.55)	0.332		
MELD	2.25 (1.16-4.66)	**0.02**	2.67 (1.78-6.33)	**0.001**
Siderophores production	0.85(0.668-3.54)	0.463		
Serum Lipocalin-2	1.52(0.991-4.12)	**0.03**	0.87(0.684-1.72)	0.453
Ascitic Lipocalin-2	2.63(1.25-6.34)	**0.02**	0.982(0.821-2.1)	0.178

This table shows that in uni-variate analysis, WBCs , Hb, PLT , INR, Total bilirubin, Direct bilirubin, Creatinine, MELD, Serum and Ascitic Lipocalin-2 are all associated with in-hospital mortality (P<0.05), while in final multi-variate model, MELD , Creatinine , INR, Total and direct bilirubin remain significant predictors of in-hospital mortality(P<0.05).

Table (12): Relation between different studied parameters and mortality among SBP cases:

Parameter	MORTALITY		P
	Died N=16	Survived N=24	
SEX Male (n=22) Female (n=18)	 12 (75%) 4(25%)	 10(41.6%) 14(58.3%)	 0.312 NS
Bacterial isolates Kp (n=28) E. coli(n=12)	 10(62.5%) 6(37.5%)	 18(75%) 6(25%)	 0.92 NS
ESBL Positive (n=24) Negative (n=16)	 12(75%) 4(25%)	 12(50%) 12(50%)	 0.514 NS
Carbapenemases Positive (n=12) Negative (n=28)	 8(50%) 8(50%)	 4 (16.6%) 20(83.3%)	 0.273 NS
XDR XDR (n=12) Non XDR (n=28)	 8(50%) 8(50%)	 4 (16.6%) 20(83.3%)	 0.273 NS
MDR MDR (n=16) Non MDR(n=24)	 4(25%) 12(75%)	 12(50%) 12(50%)	 0.514 NS
Siderophores genes -Ent B (n=22) -Ent B +Others (n=12) (Ent B with Iut A or Ybts) -Others (n=6) (Iut A or Ybts)	10(62.5%) 6(37.5%) 0(0.0%)	12(50%) 6(25%) 6(25%)	0.707 NS

This table shows statistically non-significant differences between Mortality and different studied parameters such as Sex ,different bacterial isolates , ESBL ,Carbapenemases ,XDR , MDR and Siderophores genes (P>0.05).

DISCUSSION

Nowadays, more and more clinicians pay close attention to co-infection in decompensated liver cirrhosis because of its prognostic effect; in particular, SBP has attracted much attention because it can cause AKI and increase the mortality rate [46,53,54].

SBP is a common cause of hepatic decompensation, with an estimated prevalence of 12% in hospitalized patients with cirrhosis and ascites [55]. Siderophores constitute a key component of pathogenicity of gram negative bacterial infections causing SBP [51,78,79].

Many research groups are attempting to find markers and/or methods for the accurate diagnosis of SBP [76]. Therefore, there is an increasing demand for a marker that can assist in screening for SBP and predict the prognosis of SBP patients [46].

In this work, SBP patients include 22(55%) male and 18(45%) female, Mean age ± SD are 60.6±12.5 years, which is similar to Abd-Elsalam et al. [67] who found majority of SBP patients were males (62.2%), with mean age (60 ±6 years), also another study by Hafez et al., [68] which agrees with our result that majority of SBP patients were males (63%) and mean age of patients was 63.06 ± 9.67.These findings may be explained by

higher proportion of SBP patients were diagnosed with viral Hepatitis C cirrhosis which is more common in males at similar ages[121].

On the contrary , Hamed et al.,[75] involved 92 SBP patients, most of them were females 52 (56.5%) versus 40 males (43.5%). Their mean age was (57.1 ± 12) years which disagree with our results.

This variation may be due to differences in study population , sample size , type of studied patients (whether inpatients or outpatients, symptomatic or asymptomatic), patient and environmental conditions ,different therapeutic regimens and different geographical areas implying difficulties with recommending antibiotic treatment and prophylaxis in international guidelines.

In the current study , Hepatic patients with SBP had significantly higher ascitic lipocalin-2 than those without SBP with substantial statistically significant difference between them. While, there was no significant difference among studied groups as regard serum levels of lipocalin-2. In accordance with our results , Cullaro et al.,[20] reported that Ascites Lipocalin 2 levels were significantly elevated in SBP patients than non SBP .While, as regard Serum Lipocalin 2 levels, there was no significant difference between them .Also, another study is consistent with our results as regard baseline ascitic Lipocalin 2 levels in SBP group , which were significantly more than those in non SBP among decompensated liver cirrhosis patients [46].Infection and inflammation cause a rise in Lipocalin-2 levels, which explains this.

Furthermore, Our results agree with , Biomy et al., [73] who reported that ascitic fluid Lipocalin 2 results was significantly higher in SBP group (171.55+89.13) ng/dl than in non SBP group (45.59+18.95) ng/dl, and are also similar to Liu et al., [46] who stated that ascitic fluid Lipocalin 2 presented in (83.9%) of patients with SBP in comparison to (35.4%) of patients without SBP with significant statistical difference between two studied groups, this may be explained by Lipocalin 2 is marker which elevated during infection and inflammation, thus it is elevated in SBP which is bacterial infection [20].

These findings confirm the fact that lipocalin 2 is increased during bacterial infection as it is an essential component of the antimicrobial innate immune system. It has bacteriostatic function by binding and sequestration of bacterial siderophores, thus depriving bacteria of iron [24,77] .

Based on ROC curve analysis:

at a cutoff value 297.8 ng/ml , Lipocalin-2 had a sensitivity of 95.6% , specificity of 92.5%, positive predictive value (PPV) of 95%, negative predictive value (NPV) of 95%, accuracy of 95% and AUC was 0.845 for the diagnosis of SBP (it was statistically high significant P-value 0.000). Biomy et al.,[73]found that regarding ROC analysis for ascitic Lipocalin 2 as marker for diagnosis of SBP revealed that, At a cut-off value of: 100.8 (ng/dl), sensitivity was: 97.62%, specificity was 97.67%, positive predictive value was: 97.62%, negative predictive value was: 97.67%, under curve (AUC) at :0.974 which is indicative of an excellent predictive biomarker of SBP [71].

Among SBP studied patients , 28 (70%) klebseilla isolates ,While E.coli isolates were 12 (30%). This is inconsistent with previous studies [56,68,95] which reported that E.coli was the most prevelant organism causing SBP, this could be explained by most of SBP patients were decompensated liver cirrhosis and admitted to ICU unit which prone to klebseilla hospital acquired infections.

Concerning Siderophores production, klebseilla isolates were found to be the most potent siderophores producers among SBP patients . However , the lowest percent siderophores unit is produced by E.coli isolates . This difference in Siderophores production among studied isolates could be because the growth and amount of siderophores produced by an organism under different culture conditions are different as regard different types of organisms and different forms of infections .Up to our Knowledge , this is the first study to identify Siderophores production by KP and E.coli isolates among SBP patients.

Both E.coli and Klebseilla isolates involved in this study were evaluated for the existence of the most often documented Siderophores genes. According to our

research, Enterobactin Biosynthesis (Ent B) is the most predominant gene among studied isolates (55%), followed by Aerobactin receptor (Iut A) (10%) and then Yersiniabactin Biosynthesis (Ybt S) (5%). Irp 1 gene used for Ecoli strains is not detected in any of the isolates. As regard bacterial isolates in this study ,some of them (30%) demonstrate co-expression of more than one Siderophores . Five isolates (25 %) express these two genes (Ent B and Iut A), while one isolate (5 %) expresses both genes (Ent B, Ybt S).

Up to our Knowledge , No previous literature concerning Siderophores genes of Klebseilla and E.coli isolates among SBP patients. However, Several literatures studied siderophores genes in other different clinical diseases.

Regarding Klebseilla isolates, Enterobactin (Ent B) gene was more predominant than other siderophore genes in our study which agree with Rastegar et al., [97] that found a majority of both hvKP and cKP isolates were demonstrated to carry Ent B gene, a finding in line with most previous reports [3, 10, 27]. In our study , Total Ent B gene (alone and combined) was identified in (78.6%) of all KP isolates. Similar to our results in EGYPT, Wasfi et al., [81] showed that enterobactin gene (entB) was detected in 78.5 % of MDR K. pneumonia isolates, and Naga et al., [40] found that Ent B gene was detected in 68% of kp isolates using PCR. This result showed that this iron chelator gene is identified in almost K. pneumoniae clinical isolates in many types of infection . Also , Kumar et al., [82] reported that entB gene was found among 65% of MDR K. pneumonia. Many results were highlighted by several studies like Kuş et al [86], Shakib, et al [83], Aljanaby et al [87]who demonstrated that entB gene was detected in 96.2%,81.4% and 100% klebseilla spp. respectively. In the current study, entB combined with others (Iut A, Ybts) genes were detected in (14.2%) klebseilla isolates which differ from another study that showed existence of the genes encoding entB in combination with others iutA and Kfu which was found in 66% and 68% respectively [40].

Total IutA gene (combined and alone) was detected in 21.4% among all Klebseilla isolates while another study in Egypt , showed that iutA gene was identified in 11.4% of kp isolates [83] . Also , in another study by Naga et al.,[40] in EGYPT , Iut A gene was detected in 34.5% of studied klebseilla isolates. In Fertas-Aissani et al., [85] study , Iut A gene was detected in 5.5% of klebseilla spp [84,85].

Total Ybts gene (combined and alone) was detected in 14.3% of all Klebseilla isolates. Shakib et al.,[83] found that ybtS gene was positive for 60% of studied isolates. Another study Rastegar et al., [97] reported that more than half of their isolates possessed ybtS. These variations may be due to difference in study population , sample size, patient and environmental conditions and type of infection.

As regard E.coli isolates , Enterobactin Biosynthesis (Ent B) gene was detected in 33.3% of isolates, while , entB combined with others (Iut A) genes was detected in 66.7%, therefore Total entB (alone and combined) was detected among all E.coli isolates. while , Yersiniabactin Biosynthesis (Irp1) gene was not detected in any of the E.coli isolates . In agreement with our result, Daoud et al.,[88] found that entB gene was detected in (92%) of all isolates, and Sarowska et al., [89] who found ent B in most E. coli strains, both commensal and pathogenic. Also , as regard IutA , Matin et al,.[90] reported that IutA gene frequency among uropathogenic E.coli strains was 58.3%. And Daoud et al.,[88] reported that the iutA gene was present in 100% of isolates. While Candan & Aksöz,[91] reported that iutA was 92% among tested isolates by multiplex PCR .This may be due to small number of tested E.coli isolates in our study. In contrast to our study , Daoud et al.,[88] reported that irp1 gene was found in 60% of isolates, thus indicating that these strains carries a high-pathogenicity island (HPI) which irp1 gene is encoded on ,which is not found in our studied isolates as irp1 gene was not present in E.coli strains .

Up to our Knowledge ,this is the first study to report the distribution of Siderophores genes in K. pneumoniae and E.coli isolates among SBP patients in either EGYPT or worldwide.

As regard relation between Lipocalin-2 levels in serum and ascitic fluid and different studied parameters among SBP group , non-significant relation were found between them and Siderophores production ,

Siderophores genes and Antimicrobial resistance patterns (XDR , MDR and other bacterial isolates) (P>0.05).

Concerning relationship between virulence and antimicrobial resistance among bacterial isolates ,We found statistically significant differences between Siderophores production and XDR , MDR and other isolates among SBP group (P<0.05), While non-significant differences between Siderophores genes and XDR , MDR and other isolates among SBP group (P>0.05) were found in this study . Several studies have reported the presence of a relationship (both positive and negative, and both direct and indirect) between antimicrobial resistance and virulence among bacterial pathogens. The relationship between resistance and virulence among bacteria depends on the bacterial species, the specific mechanisms of resistance and virulence, the ecological niche, and the host [118].As regard relationship between antimicrobial resistance and virulence among bacterial pathogens three scenarios have been reported: (i) an increase of resistance accompanied by an increase of virulence; (ii) an increase of resistance accompanied by a decrease of virulence; and (iii) an increase of resistance that does not cause effects on virulence. Hence, positive and negative relationships between antibiotic resistance and virulence exist, and depend on the antibiotic studied, the mechanism of resistance and the type of bacteria[118]. In addition to the effects that the acquisition of antibiotic resistance has on virulence, a co-selection of both characteristic can occur through mobile genetic elements such as plasmids and integrative and conjugative elements (integrative conjugative elements, ICEs: transposons, PAIs, integrons, etc.)[118]. It is possible that simultaneous presence of virulence factors and resistance genes on resistance transferable elements such as integrons and conjugative plasmids increases the possibility of spreading both virulence traits and antibiotic resistance via horizontal gene transfer [119] .

As regard relationship between AMR and virulence in K. pneumonia, Sundaresan et al.,[120] reported that ESBL and carbapenemase harbouring isolates were mapped with major virulence genes (rmpA , magA , ybtS, alls, iutA),they found that 98% of the ESBL and carbapenemase Klebseilla isolates harboured ybtS and iutA[120].

Although most previous studies have focused separately on virulence or resistance, our report is the first study to assess /focus on the potential link / interplay /correlation /relationship between the antibiotic resistance profiles and distribution of Siderophores production as a virulence factor in the K. pneumoniae and E.coli isolates among SBP patients .

However, because little is known about the type and extent of this relationship, future studies are required to fully investigate and understand this point and find solutions for the long standing AMR problem , Perhaps a new treatment can be found to overcome these pathogens by targeting common biochemical pathways for the synthesis of proteins related to the virulence and resistance genes. Also , Further Studies on other virulence associated genes and resistance genes could be resulted in the discovery of new aspects of this association.

SBP is one of the most prevalent and deadly cirrhosis consequences [92].Determining the prognosis of SBP would be essential for formulating a proper management plan, as well as selection of robust antimicrobial coverage, and setting up appropriate follow-up schedules[93]. Lipocalin-2 has been found to be elevated in infections, inflammation, ischemia, and metabolic disorders, many of the etiologies of hepatic decompensation [20,94]. Thus , we hypothesized that Lipocalin-2 would be a marker of not only SBP, but also mortality. We , also set out to research SBP patients' short-time mortality rate and mortality determinants.

The 30-day in-hospital mortality of SBP is variable but has been reported to be anywhere between 18% [122] and up to 31.9% [54,93].

Alexopoulou et al.,[45] and Wong et al., [95] reported overall 30-d-mortality 37.7% and 30.8% respectively which are nearly consistent with our results as regard overall mortality which was 40%.

On the other hand, in Hamed et al., [75] and Cullaro et al., [20] studies, in-hospital mortality rate among SBP patients was 15.2%, 11.0% respectively.

This can be attributed to local antibiotic prescribing habits and difference in effectiveness of infection control

program indifferent health institutes. Lack of antibiotic surveillance, antibiotics misuse, and weak infection control measures may also contribute to the high mortality rate .As regard our data , use of prophylactic antibiotics was low in our group of SBP patients, which probably explains this condition [123] .

The high mortality rate among our Nosocomial SBP cases could be also related to gut dysbiosis among patients with advanced cirrhosis. Nevertheless, the question of whether the alteration of gut microbiome observed in N-SBP was the consequence of the disease itself or could potentially lead to more advanced disease remains un answered .But researchers also think that the high mortality rate for patients with SBP reflects both the presence of infectious diseases and the underlying illness itself [95].

A number of studies have sought to identify prognostic factors in patients with SBP [124, 125, 126, 127, 128, 129]. In uni-variate cox analysis, WBCs , Hb, PLT , INR, Total bilirubin, Direct bilirubin, Creatinine, MELD, Serum and Ascitic Lipocalin-2 were all associated with 30 day in-hospital mortality , while in final multi-variate model, MELD , Creatinine , INR, Total and direct bilirubin remain significant predictors of short term in-hospital mortality in our study.

These results emphasized the importance of the timely treatment in complications. More importantly, reducing days of hospital stay before SBP, which meant early diagnosis and treatment, was also closely related to 28-day mortality [44]. Also ,these findings have been reciprocated in most similar studies and would be expected as all of these characterize more advanced liver disease [131].

As a comparative study , Cullaro et al., [20] showed in univariate analysis, ascites Lipocalin-2 (OR 1.04 per 10 ng/mL, p\0.01), serum Lipocalin-2 (OR 1.01 per 10 ng/ mL, p\0.01), MELD at sample collection (OR 1.15, p\0.01), SBP at sample collection (OR 7.07, p\0.01), Absolute neutrophilic count of the ascitic fluid (OR 1.03 per 100 neutrophils, p = 0.049), and intrinsic AKI (iAKI) or hepatorenal syndrome (HRS) at sample collection (OR 7.83, p\0.01) were all associated with in-hospital mortality. while , In the final multivariate model, ascites Lipocalin-2 (OR 1.02 per 10 ng/mL, p\0.01), presence of SBP at sample collection (OR 9.76, p\0.01), and MELD at sample collection (OR 1.11, p = 0.01) remained significant predictors of in-hospital mortality.

Elzouki et al., [93] study , multivariate Cox proportional hazards regression model analysis showed that MELD score (hazard ratio [HR]: 1.29; 95% confidence interval [CI]: 1.10 to 1.92; p=0.023); CTP (B/C) score (HR=1.23; 95% CI: 1.05 to 1.82; p=0.027), and AKI (HR=2.09; 95% CI: 1.41 to 3.47; p=0.01) were significantly predictive of in-hospital mortality which agree with our results .

In another study , Liu et al., [44] revealed that as regard predictors of 28-day mortality in patients with acute decompensated cirrhosis with culture-positive spontaneous ascitic infections (SAI), In univariate analysis, days of hospital stay before culture-positive SAI, prevalence of XDR isolates, severe hepatic encephalopathy (HE) (grade 2-4), upper gastrointestinal bleeding (UGB), WBC count, neutrophil count, aspartate transaminase (AST), alanine aminotransferase (ALT), serum creatinine, total bilirubin, INR, and Model for End-Stage Liver Disease score (MELD) and MELD-Na scores were significantly correlated with outcomes of patients with acute decompensated cirrhosis who developed culture-positive SAI ,while Multivariate analysis demonstrated that mortality variables were independently associated with days of hospital stay before culture-positive SAI, UGB, WBC count, INR, ALT, serum creatinine, and total bilirubin which is nearly consistent with our study.

These Literatures are consistent with the findings of our study, which showed a significant increase in excess mortality in patients with AKI compared to patients with normal kidney function [93]. Moreover, this suggests that patients with poorer liver reserve are either more likely to develop SBP or to decompensate following the occurrence of SBP [95].

Alexopoulou et al., [45] study reported Cox univariate analysis, variables that had at least a trend (P < 0.10) for association with 30-d survival included age (P = 0.089), INR (P = 0.001), creatinine (P = 0.036), total bilirubin (P = 0.001) and XDR infection (P = 0.007) while, In multivariate Cox regression analysis, factors

adversely affecting outcome were XDR infection (HR = 2.263, 95%CI: 1.005-5.095, P = 0.049), creatinine (HR = 1.125, 95%CI: 1.024-1.236, P = 0.015) and INR (HR = 1.553, 95%CI: 1.106-2.180, P = 0.011) .

As regard correlation of isolate type and drug resistance with mortality, Ning et al., [96],In Multivariate analysis showed that only patients infected with Klebsiella spp. had higher hazard ratio of 30-day mortality compared to those with Escherichia coli .Also, in the same study , carbapenem-resistance is associated with significantly lower 30-day survival probability ($p < 0.01$). Thus, this can be a life-threatening factor for cirrhotic patients with ascitic fluid infection.

In Liu et al., [46] , univariate and multivariate Cox regression analyses were used to further explore the prognostic indicators for the SBP group. In brief, ascitic Lipocalin-2 was the most significant independent risk factors, which could relevantly predict the prognosis of SBP in decompensated liver cirrhosis patients.

Also , In Elzouki et al., [93] study showed a significantly higher mortality rate in patient cohort with multi-drug resistant (MDR) bacteria than in those with other bacterial isolates.

Similar to our study , Hamed et al., [75] found there was no significant relationship between age or sex and mortality .

However , no previous study has evaluated correlation between Siderophores genes and short term in hospital mortality among SBP cases. This hypothesis could be given with the identification of relation between pathogen virulence and in hospital mortality for which there is evidence that Controlling virulence factors of Bacteria associated with infection in hepatic patients can improve the regression of liver cirrhosis and this improvement is associated with a better prognosis.

All identified independent risk factors could be utilized to develop a multiple clinical risk evaluation system to help clinicians identify the highest-risk subsets of patients [44]. Moreover , MELD score appears to be the most accurate predictor than others which mainly affect short term outcome of SBP cases among decompensated liver diseases. A higher MELD score could be used as the threshold for initiating more aggressive treatment or closer monitoring among SBP patients.

Though this is a global problem the spectrum of infecting organisms and the pattern of sensitivity to antibiotics will necessarily show significant variability based on geographical location, prevalent antibiotic policies and pattern of antibiotic use. The importance of prompt initiation of appropriate antibiotic therapy has been shown to significantly improve out-comes and so more local guidelines based on more local data will need to be formulated to ensure improvement of outcomes [131].

In conclusion, Lipocalin-2 proved to be a reliable biomarker of SBP Diagnosis in hospitalized patients with cirrhosis ,it can be used as a diagnostic marker with high sensitivity and specificity in SBP infection, especially in chronic liver disease patients. MELD score appears to be the most accurate predictor of 30 day mortality.

SUMMARY

To summarize, our present study found that, in decompensated liver cirrhosis patients, high levels of ascitic Lipocalin-2 (NGAL) can play an important role in screening for the occurrence of SBP. We found that an ascitic Lipocalin 2 of 297.8 ng/mL was the optimum cutoff value for the diagnosis of SBP in CLD patients. In addition, Gram-negative bacteria remain the most prevalent cause of SBP. Multiplex PCR allows rapid, reproducible, and sensitive detection of virulence genes carried by bacterial isolates. In addition, the method is less time consuming than MLST determination and is suitable for screening virulent clones .As regard short term in hospital mortality , MELD score , Creatinine , INR, Total and direct bilirubin levels still predict the cirrhosis SBP patients' short-term prognosis(poor outcome).

CONCLUSION

Ascitic Lipocalin-2 (NGAL) level is a biomarker of SBP in hospitalized patients with cirrhosis ,it can be used as a diagnostic marker with high sensitivity and specificity in SBP infection, especially in chronic liver disease patients .Gram-negative bacteria were the major pathogens involved in SBP in the cirrhotic patients .Regarding siderophores production by quantitative method among bacterial isolates, Klebsiella strains were found to be more potent siderophores producers than E.coli strains ,While siderophores production by

molecular method using multiplex PCR to detect siderophores genes shows Enterobactin gene (EntB) is more predominant than other siderophore genes among isolated bacteria with higher frequency in Klebsiella strains. Nosocomial SBP often had a poorer outcome than other forms. MELD score remains an independent predictor of short-term in-hospital mortality .

RECOMMENDATIONS

Ascitic Lipocalin-2 (NGAL) may not only be a biomarker for monitoring SBP but also may be a predictor for more severe outcomes in decompensated cirrhosis-related SBP. These findings need to be validated in another prospective studies and may in future be incorporated in prognostic models.

Another recommendation , patients with culture-negative SBP (ascitic PMN ≥ 250 cells/µl and a negative culture result) should be included in further studies.

Furthermore, it is well advised to study the expression of the Siderophores and other virulence genes using real time PCR.

We also recommend following up patients after discharge to study long-term mortality.

LIMITATIONS

Our study had some limitations. First, our study aimed to analyze the microbiological characteristics of patients with culture- positive SBP, thus culture-negative patients were excluded from our study, Thus, selection bias may have occurred . Second, we included Klebsiella and E.coli strains because these strains remain most prevelant cause of SBP. Third ,we studied only short-term mortality (in-hospital mortality). Forth , patients with culture-negative SBP (ascitic PMN ≥ 250 cells/µl and a negative culture result) were not included in this study. Thus, our findings, such as independent risk factors of 30-day mortality in patients with SBP, could not be applied to those patients.

Lastly, our sample size is small ,this may have compromised its statistical power.A future prospective multicentre study would be ideal to verify our findings

Funding: No Fund Received

Institutional Review Board Statement: The experimental procedures of the current study were approved by the Ethics Committee at the Faculty of Medicine, Zagazig University, Alsharquia, Egypt (no. ZU-IRB # 5523/25-8-2019).

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Biomy, H., Ramadan, N., Ameen, S., Kandil, A. E. din, & Galal, Z. (2021). Evaluation of Ascitic Fluid Neutrophil Gelatinase Associated Lipocalin in patients with Spontaneous Bacterial Peritonitis. Benha Medical Journal, 0(0), 0–0. https://doi.org/10.21608/bmfj.2021.73302.1410
2. Elsherif, A. A., Eldahshan, M. A., Hussein, M. S., & Mohamed, A. M. (2016). Asymptomatic Spontaneous Bacterial Peritonitis in Adult Egyptian Patients with Decompensated Liver Cirrhosis: A Prospective Cohort Study. International Journal of Advanced Biomedicine, 1(1): 5–9.
3. Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. J Hepatol. (2014) 61:1385–96. doi: 10.1016/j.jhep.2014.08.010
4. Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. World J Hepatol. (2016) 8:307–21. doi: 10.4254/wjh.v8.i6.307
5. Alexandre dA. Akin criteria as a predictor of mortality in cirrhotic patients after spontaneous bacterial peritonitis. Ann Hepatol. (2014) 13:390−5. doi: 10.1016S1665-2681(19)30870-1
6. Iliaz R, Ozpolat T, Baran B, Demir K, Kaymakoglu S, Besisik F, et al. Predicting mortality in patients with spontaneous bacterial peritonitis using routine inflammatory and biochemical markers. Eur JGastroen Hepat. (2018) 30:786– 91. doi: 10.1097/MEG.0000000000001111
7. Schwabl P, Bucsics T, Soucek K, Mandorfer M, Bota S. Blacky A. Reiberger T Risk factors for development of spontaneous bacterial peritonitis and subsequent mortality in cirrhotic patients with ascites. Liver Int. (2015) 35:2121–8. doi: 10.1111/liv.12795
8. Terg R, Gadano A, Cartier M, Casciato P, Lucero R. Munoz A. Abecasis R Serum creatinine and bilirubin predict renal failure and mortality in patients with spontaneous bacterial peritonitis: a retrospective study. Liver Int. (2009) 29:415–9. doi: 10.1111/j.1478-3231.2008.01877.x
9. Xiang, Shoushu, Juntao Tan, Chao Tan, Qian Xu, Yuanjiu Wen, Tiantian Wang, Chen Yang, and Wenlong Zhao. 2022. “Establishment and Validation of a Non-Invasive Diagnostic Nomogram to Identify Spontaneous Bacterial Peritonitis in Patients With Decompensated Cirrhosis.” Frontiers in Medicine 8(January):1–10. doi: 10.3389/fmed.2021.797363.
10. Abdel-Razik, Ahmed, Waleed Eldars, Rania Elhelaly, Ahmed A. Eldeeb, Mostafa Abdelsalam, Niveen El-Wakeel, and Alsaid Aboulmagd. 2018. “Homocysteine: A New Diagnostic Marker in

Spontaneous Bacterial Peritonitis." European Journal of Gastroenterology and Hepatology 30(7):779–85. doi: 10.1097/MEG.0000000000001109.

11. Shi, Lei, Dan Wu, Lei Wei, Suxia Liu, Peng Zhao, Bo Tu, Yangxin Xie, Yanan Liu, Xinhua Wang, Liying Liu, Xin Zhang, Zhe Xu, Fusheng Wang, and Enqiang Qin. 2017. "Nosocomial and Community-Acquired Spontaneous Bacterial Peritonitis in Patients with Liver Cirrhosis in China: Comparative Microbiology and Therapeutic Implications." Scientific Reports 7(April):1–7. doi: 10.1038/srep46025.
12. Fouad, T. R., Abdelsameea, E., & Elsabaawy, M. (2019). Urinary neutrophil gelatinase-associated lipocalin for diagnosis of spontaneous bacterial peritonitis. 49(3), 189–192. https://doi.org/10.1177/0049475519830265
13. Singal AK, Salameh H and Kamath PS. Prevalence and in-hospital mortality trends of infections among patients with cirrhosis: a nationwide study of hospitalised patients in the United States. Aliment Pharmacol Ther 2014; 40: 105–112.
14. Barreales M and Ferna´ ndez I. Spontaneous bacterial peritonitis. Rev Esp Enferm Dig 2011; 103: 255–263.
15. Kaymakoglu S, Eraksoy H, Okten A, et al. Spontaneous ascitic infection in different cirrhotic groups: prevalence, risk factors and the efficacy of cefotaxime therapy. Eur J Gastroenterol Hepatol 1997; 9: 71–76.
16. Cheong HS, Kang CI, Lee JA, et al. Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. Clin Infect Dis Off Publ Infect Dis Soc Am 2009; 48: 1230–1236.
17. Tandon P and Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Semin Liver Dis 2008; 28: 26–42.
18. Goetz DH, Holmes MA, Borregaard N, et al. The neutrophil mlipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 2002; 10: 1033–1043.
19. Berger T, Togawa A, Duncan GS, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to E. coli infection but not to ischemia reperfusion injury. Proc Natl Acad Sci USA 2006; 103: 1834–1839.
20. Cullaro G, Kim G, Pereira MR, et al. ascites neutrophil gelatinase-associated lipocalin identifies spontaneous bacterial peritonitis and predicts mortality in
21. hospitalized patients with cirrhosis. Dig Dis Sci 2017; 62: 3487–3494.
22. Kjeldsen L, Johnsen AH, Sengeløv H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem. 1993;268(14):10425-10432.
23. Bagshaw SM, Bennett M, Haase M, Haase Fielitz A, Egi M, Morimatsu H, et al.(2010):Plasma and urine neutrophil gelatinase associated lipocalin in septic versus non septic acute kidney injury in critical illness. Intensive Care Med 2010; 36:452–461.
24. Nasioudis D and Witkin SS (2015): Neutrophil gelatinase associated lipocalin and Innate immune responses to bacterial infections. Med Microbiol Immunol 2015; 204:471–479.
25. Ghoniema EM, Abd El Aziza AM, Abd El Motelba TM, Salama AA, and El Gazzara HM (2017): The value of lipocalin 2 as a predictive biomarker of bacterial infection in hepatic patients.Menoufia Medical Journal, Volume 30 | Number 4 | October-December 2017[Downloaded free from http://www.mmj.eg.net on Friday, April 12, 2019, IP: 197.56.184.94
26. Behairy BE, Salama EI, Allam AA, Ali MA and Abd Elaziz AM (2011):Lipocalin-2 as a Marker of Bacterial Infections in Chronic Liver Disease: A Study in Egyptian Children THE EGYPTIAN JOURNAL OF IMMUNOLOGY Vol. 18 (2), 2011 Page: 31-36.
27. Flo TH, Smith KD, Sato S, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004;432(7019):917–921.
28. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. BBA Rev Cancer. 2012;1826:129–169.
29. Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatr Nephrol. 2009;24:2387–2392.
30. Verna EC, Brown RS, Farrand E, et al. Urinary neutrophil gelatinase-associated lipocalin predicts mortality and identifies acute kidney injury in cirrhosis. Dig Dis Sci. 2012;57:2362–2370. 11. Roth GA, Nickl S, Lebherz-Eichinger D, et al. Lipocalin-2 serum levels are increased in acute hepatic failure. TPS. 2013;45:241–244.
31. Roth GA, Nickl S, Lebherz-Eichinger D, et al. Lipocalin-2 serum levels are increased in acute hepatic failure. TPS. 2013;45:241–244.
32. Piano S, Brocca A, Mareso S, Angeli P. Infections complicating cirrhosis. Liver international : official journal of the International Association for the Study of the Liver. 2018;38 (Suppl 1):126-33.
33. Fiore M, Maraolo AE, Gentile I, Borgia,G. Leone,S. and Sansone,p. et al. (2017).Current concepts and future strategies in the antimicrobial therapy of emerging gram-positive spontaneous bacterial peritonitis. World J Hepatol. 9(30):1166–1175
34. Acevedo J. Multiresistant bacterial infections in liver cirrhosis: clinical impact and new empirical antibiotic treatment policies. World J Hepatol.2015;7(7):916–921.

35. Abdel Salam, F., El-Toukhy, N., El- kharbotly, E., Mohamed, M., & Ameen, S. (2022). Serum Endocan Levels In Patients with Spontaneous Bacterial Peritonitis. Benha Medical Journal, 0(0), 0–0. https://doi.org/10.21608/bmfj.2022.118948.1538
36. ALMEIDA, P. R. L. de, LEÃO, G. S., GONÇALVES, C. D. G., PICON, R. V., & TOVO, C. V. (2018). Impact of Microbiological Changes on Spontaneous Bacterial Peritonitis in Three Different Periods Over 17 Years. Arquivos de Gastroenterologia, 55(1): 23–27.
37. Wang Y., Zhao S., Han L., Guo X., Chen M., Ni Y., Zhang Y., Cui Z., He P. (2014) Drug resistance and virulence of uro- pathogenic Escherichia coli from Shanghai, China. J. Antibiot. (Tokyo). 67(12): 799–805. https://doi.org/ 10.1038/ja.2014.72; http://dx.doi.org/10.1038/ja.2014.72
38. Fuursted K SL, Hansen F, Dam K, Bojer MS, Hammerum A et al. Virulence of a Klebsiella pneumoniae strain carrying the New Delhi metallo-beta-lactamase-1 (NDM-1). Microbes and Infection. 2012;14(2):155-158.
39. Wu CC, Wang CK, Chen YC, Lin TH, Jinn TR, Lin CT. IscR regulation of capsular polysaccharide biosynthesis and iron-acquisition systems in Klebsiella pneumoniae CG43. PLoS One. 2014;9(9):e107812.
40. Holden VI, Bachman MA. Diverging roles of bacterial siderophores during infection. Metallomics. 2015;7(6):986-995.
41. S. Naga, I., Ghazal, A., S. Alomari, S., & Gaballah, A. (2021). Detection of Biofilm and Siderophore Encoding Genes Implicated in the Pathogenesis of Klebsiella pneumoniae Isolated from Different Clinical Specimens. Egyptian Journal of Medical Microbiology, 30(1), 101–108. https://doi.org/10.51429/ejmm30113
42. Wilson BR, Bogdan AR ,Miyazawa M, Hashimoto K, and Tsuji Y (2016): Siderophores in Iron Metabolism: From Mechanism to Therapy Potential . Trends Mol Med. 2016 December ; 22(12): 1077–1090. doi:10.1016/j.molmed.2016.10.005
43. Chon YE, Kim SU, Lee CK, Park JY, Kim DY, Han KH, et al. Community-acquired vs. nosocomial spontaneous bacterial peritonitis in patients with liver cirrhosis.Hepato-gastroenterology. 2014;61(136):2283-90.
44. Piano S, Singh V, Caraceni P, Maiwall R, Alessandria C, Fernandez J, et al. Epidemiology, predictors and outcomes of multi drug resistant (MDR) bacterial infections in patients with cirrhosis across the world. Final results of the "Global study". Digestive and Liver Disease. 2018;50(1):2-3.
45. Liu, J., Gao, Y., Wang, X., Qian, Z., Chen, J., Huang, Y., & Meng, Z. (2020). Culture-Positive Spontaneous Ascitic Infection in Patients with Acute Decompensated Cirrhosis : Multidrug-Resistant Pathogens and Antibiotic Strategies. 61(2), 145–153.
46. Alexopoulou, A., Vasilieva, L., Agiasotelli, D., Siranidi, K., Pouriki, S., Tsiriga, A., Toutouza, M., Dourakis, S. P., Alexopoulou, A., Vasilieva, L., & Agiasotelli, D. (2016). Extensively drug-resistant bacteria are an independent predictive factor of mortality in 130 patients with spontaneous bacterial peritonitis or spontaneous bacteremia. 22(15), 4049–4056. https://doi.org/10.3748/wjg.v22.i15.4049
47. Liu H, Zhu P, Nie C, Ye Q, Gao Y, Liu H, Pang G, Han T. The value of ascitic neutrophil gelatinase-associated lipocalin in decompensated liver cirrhosis with spontaneous bacterial peritonitis. J Clin Lab Anal. 2020 Jun;34(6):e23247. doi: 10.1002/jcla.23247. Epub 2020 Feb 26. PMID: 32100329; PMCID: PMC7307354.
48. pimentel R, Leitão J, Gregório C, Santos L, Carvalho A, Figueiredo P: Spontaneous Bacterial Peritonitis in Cirrhotic Patients: A Shift in the Microbial Pattern? A Retrospective Analysis. GE Port J Gastroenterol 2022;29:256-266. doi: 10.1159/000518585
49. Hackman, Henry Kwadwo, George Osei-adjei, Andrew Gordon, Emmanuel Laryea, and Solomon Quaye. 2013. "The Reliability of Using Vitek 2 Compact System to Detect Extended-Spectrum Beta-Lactamase-Producing Isolates in Escherichia Coli and Klebsiella The Reliability of Using Vitek 2 Compact System to Detect Extended-Spectrum Beta-Lactamase-Producing Isolates ." (July 2014).
50. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global Dissemination of Carbapenemase-Producing 384 Klebsiella pneumoniae: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. Front 385 Microbiol. 2016;7:895. Published 2016 Jun 13. doi:10.3389/fmicb.2016.00895.
51. Maniatis T., Fritsch E.F., Sambrook J. (1982) Molecular clo- ning: a laboratory manual. Cold Spring Harbor Lab. Press, New York.
52. Khazaal, M. T., El-Hendawy, H. H., Mabrouk, M. I., Faraag, A. H. I., & Bakkar, M. R. (2022). Antibiotic resistance and siderophores production by clinical Escherichia coli strains. Biotechnologia, 103(2), 169–184. https://doi.org/10.5114/bta.2022.116211
53. Kamath P, Kim W (2007): Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). Hepatology,45:797-805.
54. Ascione T, Di Flumeri G, Boccia G, De Caro F. Infections in patients affected by liver cirrhosis: an update. Infez Med. 2017;25(2):91-99.
55. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK: Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010, 139:1246-56,

1256.e1-5. 10.1053/j.gastro.2010.06.019
56. Borzio M, Salerno F, Piantoni L, et al. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. Dig Liver Dis. 2001;33:41–48.
57. Yousef MM, Amer AI, M. Zidan A, A. Amer F, M. ElsaidTash R. Spontaneous Bacterial Peritonitis in the Medical Intensive Care Unit of a University Hospital in Egypt: Frequency, Bacteriological Profile, Risk Factors and Outcomes. The International Arabic Journal of Antimicrobial Agents. 2016;6(2):5-14.
58. Salem AM, Eliwa HM, El-saeed NH. Prevalence and characteristics of spontaneous bacterial peritonitis in hospitalized patients with ascites due to liver cirrhosis [Master thesis]. Faculty of Medicine: Cairo University; 2008.
59. Zaki MES, El Shabrawy WO, El-Eshmawy MM, Eletreby SA. The high prevalence of Listeria monocytogenes peritonitis in cirrhotic patients of an Egyptian Medical Center. Journal of Infection and Public Health. 2011;4(4):211-6.
60. El-Amin H, Sabry AMM, Ahmed RE, Makhlouf NA. Types and microbiological spectrum of infections in patients with cirrhosis: A single-centre experience in Upper Egypt. Arab journal of gastroenterology : the official publication of the Pan-Arab Association of Gastroenterology. 2017;18(3):159-64.
61. Muhammad T, Shereen AA, Mabrouk R, Abdallah A. Frequency of Spontaneous Bacterial Peritonitis in Patients of Liver Cirrhosis with Ascites at Tanta University Hospitals. The Medical Journal of Cairo University. 2019;87(March):147-52.
62. Makhlouf NA, Ghaliony MA, El-Dakhli SA, Elfatth AMA, Mahmoud AA. Spontaneous Bacterial Peritonitis among Cirrhotic Patients in Upper Egypt: Clinical and Bacterial Profiles. Journal of Gastroenterology and Hepatology Research. 2019;8(6):3014-9.
63. Kamal SM, Abdelhakam SM, Massoud YM, El Hafeez A, El Aziz KA, Kamal HA. Clinical Profile of patients with Ascitic Fluid Infection at Ain Shams University Hospitals. The Egyptian Journal of Hospital Medicine. 2018;72(9):5241-50.
64. Metwally K, Fouad T, Assem M, Abdelsameea E, Yousery M. Predictors of Spontaneous Bacterial Peritonitis in Patients with Cirrhotic Ascites. Journal of clinical and translational hepatology. 2018;6(4):372-6.
65. Mir M, Rather M, Kadla S, Wani Z, Shah N, editors. Study of etiological profile and resistance pattern of spontaneous bacterial peritonitis in chronicBenha medical journal, vol. 39, issue 2, 2022 664 liver disease. Journal of gastroenterology and hepatology; 2019: WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA.
66. Goel S, Pandey PT, Kumar A, Kumar N, Arora DK, Singh A. Clinico-bacteriological profile of spontaneous bacterial peritonitis in cirrhosis of liver with ascites. International Journal of Research in Medical Sciences. 2019;7(11):4256-60.
67. Oladimeji A, Temi A, Adekunle A et al. (2013): Prevalence of spontaneous bacterial peritonitis in liver cirrhosis with ascites. The Pan African Medical Journal, 15: 128-32.
68. Abd-Elsalam, F., Zeinelabedin, M., Abdelrahman, S., & Gabal, H. (2022). Bacteriological Profile and Antimicrobial Resistance in Ascitic Fluid of Patients with Community Acquired and Nosocomial Spontaneous Bacterial Peritonitis. Benha Medical Journal, 0(0), 0–0. https://doi.org/10.21608/bmfj.2022.97727.1490
69. Hafez, M. Z., Abdallah, H., Abdallah, M., Khairy, K., & Abdellatif, K. (2020). Prevalence of Spontaneous Bacterial Peritonitis in Cirrhotic Patients with Ascites and Its Pattern in Aswan University Hospital. 81(October), 1444–1448.
70. Pimentel, R., & Santos, L. (2022). Spontaneous Bacterial Peritonitis in Cirrhotic Patients : A Shift in the Microbial Pattern ? A Retrospective Analysis. 256–266. https://doi.org/10.1159/000518585
71. Taherifard, E., Hosseini-asl, M. K., & Mosayebi, M. A. (2021). Distribution and antimicrobial resistance pattern of pathogens causing spontaneous bacterial peritonitis in patients with cirrhosis at Namazi hospital , southern Iran. 13(3), 425–426.
72. Mohammed A. R.(2018). Plasma Neutrophil Gelatinase-Associated Lipocalin as a biomarker for the diagnosis of hepatorenal syndrome and spontaneous bacterial peritonitis in cirrhotic patients
73. Ximenes RO, Farias AQ, Helou CM. Early predictors of acute kidney injury in patients with cirrhosis and bacterial infection: urinary neutrophil gelatinase-associated lipocalin and cardiac output as reliable tools. Kidney Res Clin Pract 2015; 34:140–145.
74. Biomy, H., Ramadan, N., Ameen, S., Kandil, A. E. din, & Galal, Z. (2021). Evaluation of Ascitic Fluid Neutrophil Gelatinase Associated Lipocalin in patients with Spontaneous Bacterial Peritonitis. Benha Medical Journal, 0(0), 0–0. https://doi.org/10.21608/bmfj.2021.73302.1410
75. Metwally, K., Fouad, T., Assem, M., Abdelsameea, E., and Yousery, M. (2018). Predictors of spontaneous bacterial peritonitis in patients with cirrhotic ascites. Journal of Clinical and Translational Hepatology; 6(4): 1–5.
76. Hamed, A. A., Morsy, K. H., & Mohammad, A. N. (2022). Prognostic Factors of Short-Term Mortality in Spontaneous Bacterial Peritonitis Patients. 88(July), 4117–4120.
77. Lee SS, Min HJ, Choi JY, et al. Usefulness of ascitic

fluid lactoferrin levels in patients with liver cirrhosis. BMC Gastroenterol.2016;16(1):132.

78. Nasioudis D, Witkin SS. Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. Med Microbiol Immunol 2015; 204:471–479.
79. Saha, M., Sarkar, S., Sarkar, B., & Sharma, B. K. (2016). Microbial siderophores and their potential applications : a review. 3984–3999. https://doi.org/10.1007/s11356-015-4294-0
80. Calhau, V. (2014). Antimicrobial resistance determinants among Escherichia coli and Klebsiella spp. Tesis, Universira, 151.
81. Ellermann M, Arthur JC. Siderophore-mediated iron acquisition and modulation of host-bacterial interactions. Free Radic Biol Med. 2017;105:68-78.
82. Wasfi, R., Elkhatib, W. F., & Ashour, H. M. (2016). Molecular typing and virulence analysis of multidrug resistant Klebsiella pneumoniae clinical isolates recovered from Egyptian hospitals. Scientific Reports, 6(December), 1–11. https://doi.org/10.1038/srep38929
83. Kumar, R., Das, A., Gaur, M., & Pattanayak, A. (2019). Genotypic validation of extended-spectrum β -lactamase and virulence factors in multidrug resistance Klebsiella pneumoniae in an Indian hospital. Pathogens and Global Health, 113(7), 315–321. https://doi.org/10.1080/20477724.2019.1705020
84. Shakib, P., Kalani, M. T., Ramazanzadeh, R., & Rouhi, S. (2018). Molecular detection of virulence genes in Klebsiella Pneumoniae clinical isolates from Kurdistan Province , Iran Biomed Press. 5(8), 2581–2589.
85. Abduljabbar, A., Aljanaby, J., Hassan, A., & Alhasani, A. (2016). Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. 10(22), 829–843. https://doi.org/10.5897/AJMR2016.8051
86. Fertas-Aissani R, Messai Y, Alouache S, Bakour R (2013). Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumonia strains isolated from different clinical specimens. Pathol. Biol. 61(5):209-216.
87. KUŞ H, ARSLAN, U., DAĞI, H. T., & FINDIK, D. Hastane Enfeksiyonu Etkeni Klebsiella pneumoniae İzolatlarında Çeşitli Virülans Faktörlerinin Araştırılması. Mikrobiyol Bul. 2017;51(4):329-339.
88. Aljanaby AAJ, & Alhasani, A. H. A. . Virulence factors and antibiotic susceptibility patterns of multidrug resistance Klebsiella pneumoniae isolated from different clinical infections. African Journal of Microbiology Research. 2016;10(22):829-843.
89. Daoud N., Abou Sulaiman A.and Al Kwatli K.H. VIRULENCE DETERMINANTS OF ESCHERICHIA COLI STRAINS HOSPITALS IN SYRIA. (2020). Bulletin of Pharmaceutical Sciences .Assiut University .43(2), 255–264.
90. J. Sarowska, B. Futoma-Koloch, A. Jama- Kmiecik, M. Frej-Madrzak, M. Ksiazczyk, G. Bugla-Ploskonska, et al., "Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports", Gut Pathogens, 11
91. Matin, Z., Clin, A., Antimicrob, M., Matin, F. Z., Rezatofighi, S. E., & Ardakani, M. R. (2021). Virulence characterization and clonal analysis of uropathogenic Escherichia coli metallo beta lactamase producing isolates. Annals of Clinical Microbiology and Antimicrobials, 4, 1–13. https://doi.org/10.1186/s12941-021-00457-4
92. Candan, E. D., & Aksöz, N. (2017). Escherichia Coli : Characteristics of Carbapenem Resistance and Virulence Factors. 60(December), 1–12.
93. Cholongitas E, Burroughs A (2012): The evolution in the prioritization for liver transplantation. Ann Gastroenterol., 25(1):6-13.
94. Elzouki, A., Hamad, A., Almasri, H., Ata, M., & Ashour, A. (2021). Predictors of Short-Term Mortality Following First Episode of Spontaneous Bacterial Peritonitis in Hospitalized Cirrhotic Patients Patient characteristics and outcomes. 13(10), 8–13. https://doi.org/10.7759/cureus.18999
95. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflam- mation and cancer. BBA Rev Cancer. 2012;1826:129–169.
96. Wong, Y. J., Kalki, R. C., Lin, K. W., Kumar, R., Tan, J., Teo, E. K., & Li, J. W. (2020). Short- and long-term predictors of spontaneous bacterial peritonitis in Singapore. 61(8), 419–425.
97. Ning, N., Li, T., Zhang, J., Qu, F., Huang, J., Liu, X., Li, Z., Geng, W., & Fu, J. (2018). Clinical and bacteriological features and prognosis of ascitic fluid infection in Chinese patients with cirrhosis. 1–11.
98. Rastegar, S., Moradi, M., Kalantar-Neyestanaki, D., Dehdasht, A. G., & Hosseini-Nave, H. (2021). Virulence factors, capsular serotypes and antimicrobial resistance of hypervirulent klebsiella pneumoniae and classical klebsiella pneumoniae in Southeast Iran. Infection and Chemotherapy, 53(1), 345–352. https://doi.org/10.3947/IC.2019.0027
99. Allred BE, Correnti C, Clifton MC, Strong RK, Raymond KN.2013 Siderocalin outwits the coordination chemistry of vibriobactin, a siderophore of Vibrio cholerae. ACS Chem Biol 2013; 8:1882–7.
100. Hider RC, Kong X.2010 Chemistry and biology of siderophores. Nat Prod Rep 2010;27:637–57.
101. Crosa, J.H.;Walsh, C.T.2002 Genetics and Assembly Line Enzymology of Siderophore Biosynthesis in Bacteria. Microbiol. Mol. Biol. Rev. 2002, 66, 223–

249.

102. Fischbach, M.A.; Lin, H.; Zhou, L.; Yu, Y.; Abergel, R.J.; Liu, D.R.; Raymond, K.N.;Wanner, B.L.; Strong, R.K.;Walsh, C.T.; et al.2006The Pathogen-Associated IroA Gene Cluster Mediates Bacterial Evasion of Lipocalin 2. Proc. Natl. Acad. Sci. USA 2006, 103,16502–16507.
103. Garénaux, A.; Caza, M.; Dozois, C.M.2011 The Ins and Outs of Siderophore Mediated Iron Uptake by Extra-Intestinal PathogenicEscherichia coli. Vet. Microbiol. 2011, 153, 89–98.
104. Dauner M, Skerra A 2020. Scavenging Bacterial Siderophores with Engineered Lipocalin Proteins as an Alternative Antimicrobial Strategy. Chembiochem. 2020 Mar 2;21(5):601-606. doi: 10.1002/cbic.201900564. Epub 2019 Dec 13. PMID: 31613035; PMCID: PMC7079049.
105. 1a) K. Hantke, G. Nicholson, W. Rabsch, G. Winkelmann, Proc. Natl. Acad. Sci. USA 2003, 100,3677–3682;b)B.Bister,D.Bischoff, G. J. Nicholson, M. Valdebenito, K. Schneider,G.Winkelmann,K.Hantke,R.D.Süssmuth, BioMetals 2004, 17,471–481
106. a) M. A. Fischbach, H. Lin, L. Zhou, Y. Yu,R.J.Abergel, D. R. Liu, K. N. Raymond, B. L. Wanner,R.K.Strong, C. T. Walsh, A. Aderem, K. D. Smith, Proc. Natl. Acad. Sci. USA 2006, 103,16502–16507;b)M.A.Fischbach, H. Lin, D. R. Liu, C. T. Walsh, Nat. Chem. Biol. 2006, 2,132–138.ChemBioChem
107. Guo, B., Wang, Q., Li, J., Gan, Z., Zhang, X., Wang, Y., & Du, H. (2017). Lipocalin 2 regulates intestine bacterial survival by interplaying with siderophore in a weaned piglet model of Escherichia coli infection. 8(39), 65386–65396.
108. Golonka R, Yeoh BS, Vijay-Kumar M. The Iron Tug-of-War between Bacterial Siderophores and Innate Immunity. J Innate Immun. 2019;11(3):249-262. doi: 10.1159/000494627. Epub 2019 Jan 3. PMID: 30605903; PMCID: PMC6487204.
109. Hider RC, Kong X. Chemistry and biology of siderophores. Nat Prod Rep. 2010 May; 27(5): 637–57.
110. Clifton, M. C., Rupert, P. B., Hoette, T. M., Raymond, K. N., Abergel, R. J., & Strong, R. K. (2019). Journal of Structural Biology : X Parsing the functional specificity of Siderocalin / Lipocalin 2 / NGAL for siderophores and related small-molecule ligands. Journal of Structural Biology: X, 2(May), 100008. https://doi.org/10.1016/j.yjsbx.2019.100008
111. Holden, V. I., Lenio, S., Kuick, R., Ramakrishnan, S. K., Shah, Y. M., & Bachman, A. (2014). Bacterial Siderophores That Evade or Overwhelm Lipocalin 2 Induce Hypoxia Inducible Factor 1 ⎵ and Proinflammatory Cytokine Secretion in Cultured Respiratory Epithelial Cells. 82(9), 3826–3836. https://doi.org/10.1128/IAI.01849-14
112. Wang Y., Zhao S., Han L., Guo X., Chen M., Ni Y., Zhang Y., Cui Z., He P. (2014) Drug resistance and virulence of uro- pathogenic Escherichia coli from Shanghai, China. J. Antibiot. (Tokyo). 67(12): 799–805. https://doi.org/ 10.1038/ja.2014.72; http://dx.doi.org/10.1038/ja.2014.72
113. Munoz-Price, L. S. & Quinn, J. P. The Spread of Klebsiella pneumoniae Carbapenemases: A Tale of Strains, Plasmids, and Transposons. Clinical Infectious Diseases 49, 1739–1741, doi: 10.1086/648078 (2009).
114. Falagas, M. E. & Karageorgopoulos, D. E. Extended-spectrum beta-lactamase-producing organisms. J Hosp Infect 73, 345–354, doi: 10.1016/j.jhin.2009.02.021 (2009).
115. Miro, E. et al. Spread of plasmids containing the bla(VIM-1) and bla(CTX-M) genes and the qnr determinant in Enterobacter cloacae, Klebsiella pneumoniae and Klebsiella oxytoca isolates. J Antimicrob Chemother 65, 661–665, doi: 10.1093/jac/dkp504 (2010)
116. Hennequin C., Robin F. (2016) Correlation between antimi- crobial resistance and virulence in Klebsiella pneumoniae. Eur. J. Clin. Microbiol. Infect. Dis. 35(3): 333–341. https://doi.org/10.1007/s10096-015-2559-7; https://www. ncbi.nlm.nih.gov/pubmed/26718943
117. Kallau NHG, Wibawan IWT, Lukman DW, Sudarwanto MB. Detection of multi-drug resistant (MDR) Escherichia coli and tet gene prevalence at a pig farm in Kupang, Indonesia. J Adv Vet Anim Res. 2018;5(4):388–396. doi:10.5455/javar.2018.e289
118. Ibrahim, R. A. (2020). Prevalence of Virulence Genes and Their Association with Antimicrobial Resistance Among Pathogenic E . coli Isolated from Egyptian Patients with Different Clinical Infections. 1221–1236.
119. Cepas, V., & Soto, S. M. (2020). Relationship between Virulence and Resistance among Gram-Negative Bacteria. 1–11.
120. Karam MRA, Habibi M, Bouzari S. Relationships between Virulence Factors and Antimicrobial Resistance among Escherichia coli Isolated from Urinary Tract Infections and Commensal Isolates in Tehran, Iran. Osong Public Health Res Perspect. 2018 Oct;9(5):217-224. doi: 10.24171/j.phrp.2018.9.5.02. PMID: 30402376; PMCID: PMC6202021.
121. Sundaresan, A. K., Vincent, K., Babu, G., Mohan, M., & Ramakrishnan, J. (2022). Journal of Global Antimicrobial Resistance Association of sequence types , antimicrobial resistance and virulence genes in Indian isolates of Klebsiella pneumoniae : A comparative genomics study. Journal of Global Antimicrobial Resistance, 30, 431–441.

https://doi.org/10.1016/j.jgar.2022.05.006

122. Popoiag, R. E., Panaitescu, E., Suceveanu, A. I., Suceveanu, A. P., Micu, S. I., Mazilu, L., Parepa, I., Voinea, F., Costea, D. O., Enache, F., & Fierbințeanu-braticevici, C. (2021). Spontaneous bacterial peritonitis mortality trends of cirrhotic patients in the last decade in Constanta County. 1–6. https://doi.org/10.3892/etm.2021.10164
123. Kim JJ, Tsukamoto MM, Mathur AK, Ghomri YM, Hou LA, Sheibani S, Runyon BA: Delayed paracentesis is associated with increased in-hospital mortality in patients
124. Ding, X., Yu, Y., Chen, M., Wang, C., Kang, Y., & Lou, J. (2019). Causative agents and outcome of spontaneous bacterial peritonitis in cirrhotic patients : community-acquired versus nosocomial infections. 1–8.
125. Cheong HS, Kang CI, Lee JA, Moon SY, Joung MK, Chung DR, Koh KC, Lee NY, Song JH, Peck KR. Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. Clin Infect Dis. 2009;48(9):1230–6.
126. Fernández J, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, Rodés J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. Hepatology. 2002;35(1):140–8.
127. Song JY, Jung SJ, Park CW, Sohn JW, Kim WJ, Kim MJ, Cheong HJ.Prognostic significance of infection acquisition sites in spontaneous bacterial peritonitis: nosocomial versus community acquired. J Korean Med Sci. 2006;21(4):666–71.
128. Chaulk J, Carbonneau M, Qamar H, Keough A, Chang HJ, Ma M, Kumar D, Tandon P. Third-generation cephalosporin-resistant spontaneous bacterial peritonitis: a single-Centre experience and summary of existing studies. Can J Gastroenterol Hepatol. 2014;28(2):83–8.
129. Park YH, Lee HC, Song HG, Jung S, Ryu SH, Shin JW, Chung YH, Lee YS, Suh DJ. Recent increase in antibiotic-resistant microorganisms in patients with spontaneous bacterial peritonitis adversely affects the clinical outcome in Korea. J Gastroenterol Hepatol. 2003;18(8):927–33.
130. Heo J, Seo YS, Yim HJ, Hahn T, Park SH, Ahn SH, Park JY, Park JY, Kim MY, Park SK, et al. Clinical features and prognosis of spontaneous bacterial peritonitis in Korean patients with liver cirrhosis: a multicenter retrospective study. Gut Liver. 2009;3(3):197–204.
131. Macnaughtan J, Jalan R. Clinical and pathophysiological consequences of alterations in the microbiome in cirrhosis. Am J Gastroenterol 2015; 110:1399-410; quiz 1411.
132. Bhattacharya, C., Das-mondal, M., & Gupta, D. (2019). Annals of Hepatology Infection in cirrhosis : A prospective study. Annals of Hepatology, 18(6), 862–868. https://doi.org/10.1016/j.aohep.2019.07.010