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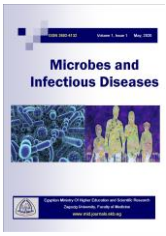

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Original article

Profiling of nosocomial infection in hepatic patients

Fatma Omar Khalil [*1], Tawfik Mohamed Abed El motaleb [1], Mervat Abed El-kareem [2], Islam Mohamed Elsheikh [3], Enas Mohamed Ghoneim [1]

1- Clinical and Molecular Microbiology and Immunology Department, National Liver Institute, Menoufia University, Shebin El-kom, Egypt

2-Hepatology and Gastrology Department, National Liver Institute, Menoufia University, Shebin El-kom, Egypt.

3- Infection Control Department, Directorate of Health Affairs, Ministry of Health Shebin El-kom , Egypt.

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Background: Nosocomial infection (NCI) is a problem with global concern due to increasing morbidity and mortality in hospitalized patients. Chronic liver disease increases the chance of NCI by suppressing cell and antibody mediated immunity. **Aim:** We aimed in this study to visualize the problem of NCI in hepatic patients regarding organisms causing it, pattern of resistance and possible device associated with its existence. **Methods:** End stage chronic hepatitis C patients who admitted to the National Liver Institute Hospital, Menoufia University, were enrolled in this observational study. Different samples from alleged sites further processed by conventional culture and sensitivity techniques and confirmed by VITEK2C2 system. Antibiotic resistance pattern of isolates was assessed. Pan drug resistance (PDR) *Acinetobacter baumannii* to Omp A, bap, and Csu E virulence genes was further processed by Multiplex PCR. **Results:** Gram- negative pathogens were significantly higher in CAUTI. The most predominant nosocomial organism was *Pseudomonas aeruginosa* which represent 19% (32 from 168), followed by Staph aureus that account for 17.86% (30 from 168), *Acinetobacter baumannii* signify 14.2%, *Klebsiella pneumonia* form 11.9%. MDR represents 123 isolates from 168 with 73.2% percentage, while XDR represents 23.8% of total isolates. Pan drug resistance *Acinetobacter baumannii* represents 5 from 24 isolates. It was associated with CAUTI and CLBSI but non-significant. 4 PDR isolates show Prescence of Omp A, Csu E, and bap biofilm forming genes. **Conclusions:** MDR NCI in hepatic patients need more attention regarding rational use of antibiotics especially with appearance of PDR *Acinetobacter baumannii* carrying Omp A, Csu E, and bap biofilm forming genes.

Introduction

Nosocomial infection (NCI) in hepatic patients complicate existing liver disease and increase the chance for development of liver failure together with widespread drug resistant nosocomial infections making effective treatment a challenge [1]. Nosocomial infection precipitate hepatic encephalopathy (HE), acute kidney injury (AKI), acute-on-chronic liver failure (ACLF) and mortality in cirrhosis [2]. Nosocomial ESBL-producing Enterobacteriaceae (ESBL-E), *Enterococcus faecium* (*E. faecium*), Multi drug resistant (MDR) bacteria including the non-fermentative Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa (P. aeruginosa)* and methicillin-resistant *Staphylococcus aureus* (MRSA) are not easy to treat. The extreme drug-resistant nosocomial bacteria (XDR) like the carpabenamase-producing *Klebsiella pneumoniae (K. pneumoniae)* and the vancomycin resistant enterococci (VRE) have recently been diagnosed among cirrhotic patients [1].

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* *Corresponding author:* Fatma Omar Khalil
E-mail address: fatma.khalil@Liver.Menofia.edu.eg

Nosocomial infection represents the higher rate of failure to empirical treatment, failure to design treatment protocols, poor response to available lines with increased mortality rates [3]. Nosocomial infection has been classified into 14 different types. Out of these, the incidences of device associated NCIs (DA-NCI) are the most common in healthcare settings which include central-line associated bloodstream infections (CLABSI), catheter-related bloodstream infections (CRBSI), catheter associated urinary tract infections (CAUTI), ventilator-associated pneumonia (VAP), and surgical site infections (SSI) [4]. Most of NCI are biofilm-based infections that is treated with high-dose antibiotics and if symptoms persist, a surgical replacement could be done to reduce further complications to patient health [5].

Acinetobacter baumannii is a biofilm forming superbug with increased risk of infection and increasing rates of antimicrobial resistance, including colistin, the last-resort antibioticC:\Users\muhammad\Downloads\Profiling of Nosocomial infection in hepatic patients.docx -_ENREF_6 [6]. Its tendency to form biofilm on biotic and abiotic surfaces has contributed to most nosocomial infections [7]. Biofilms are matrix encased aggregates of microorganisms that protect it and enhance resistance to various antibiotics [8]. Biofilm formation is a complex process employing many factors that include the aggregation substance, adhesion of collagen, expression of pili, and iron acquisition [9]. The biofilm-associated protein (*bap*) encoded by the *bap* gene plays an important role in intercellular adhesion, accumulation of bacterial cells, and establishment of biofilm [7,10]. The genes are clustered together in the form of a *csu* operon, is a member of the usher-chaperone assembly system; the products of which form a pilus-like bundle structure in *A. baumannii* that play a vital role in biofilm formation [8].The Csu pili and biofilm-associated proteins (*BAPs*), BAP-like proteins (BLPs) and the outer membrane proteins (*OMP*) identified in *A. baumannii*, *Omp A* contribute to the formation and maintenance of biofilms, which enable the persistence of *A. baumanii* under environmental threats and contribute to intrinsic antimicrobial resistance [11]. We aimed in this study to outline nosocomial infecting pathogens; types, resistance pattern and if biofilm forming genes incriminated in this resistance pattern in hepatic patients.

Methods

The present study was performed at National Liver Institute Hospital, Menoufia University, for 12 months from 1st January 2020 to 31st December 2021. Two hundred-seven Patients having advanced chronic liver disease/cirrhosis of liver and culture-positive HA bacterial infections were included in the study who are admitted at ICU wards of Hepatology and hepatobiliary surgery departments. NCI infections are typically developed in a patient after 48 hours of hospital entrance, 3 days after discharge or 30 days post -surgical intervention [12].

Informed consent was obtained from all the patients. This work was approved by the Medical Ethical Committee of The National Liver Institute, Menoufia University. The diagnosis of advanced chronic liver disease was established by history taking, clinical examination, biochemical investigations, and imaging studies. Exclusion criteria include patients with community acquired (CA) infections, HIV positive patients, patients on immunosuppressive therapy, other infections; fungal, viral infections and patients having malignancy. As in **figure (1)**; From 207 patients two -hundred forty-seven samples were taken from blood, urine, catheters, drains, surgical sites, endotracheal tube swabs, central venous lines, and sputum. Fresh single samples from each site under complete a septic condition from hepatic patients diagnosed with nosocomial infections. The samples transmitted immediately to microbiological laboratory for culture and antibiotic sensitivity testing using standard microbiological techniques. We excluded any samples considered as community acquired infection.

The blood culture was performed in a semi-automated BacT/Alert (Biomerieux, France). Further microscopic, culture, identification of microorganisms and sensitivity testing were done to samples by conventional methods on blood agar, Muller Hinton agar, mannitol salt agar, MacConkey agar, nutrient agar (HiMedia, India). Further confirmation was done by Vitek 2 Compact System (Biomerieux, France). A reference strain *of E. coli* ATCC 8739 was used to confirm the antibiotic resistance pattern. Multi drug resistant bacteria is known as resistance to at least one antibiotic in three or more antimicrobial classes, XDR bacteria is defined as bacterial isolates remain susceptible to only one or two categories and pan drug resistance

(PDR) is termed as resistance to all agents in all antimicrobial categories [13].

Detection of biofilm forming *A. baumannii*:

The detected PDR *A. baumannii* was further processed for biofilm production by culture on congo red agar (Hi media, India) as in **figure (1).** congo red is a secondary diazo dye that can be used as a pH indicator, due to a color change from blue to red at pH 3.0–5.2, and is an indicator of the presence of amyloid fibrils [14]. Plates were streaked using sterile loops and incubated for 48 h at 37 °C. The morphology and color of the resulting colonies were then assessed. On Congo red agar, a positive result for biofilm formation was indicated by black colonies with a dry crystalline consistency. Biofilm forming colonies shows crystal violet ring formation **(Figure 2).** Genetic detection of biofilm genes via DNA extraction and extraction of *OMP A, bap, Csu E* biofilm resistance genes were performed by multiplex PCR.

Pan drug resistance *A. baumannii* DNA was extracted and purified using the gene JET™ genomic DNA purification kit (Thermo Fisher Scientific, USA).The primers in the table (1) were used for Amplification and detection of target DNA that was done by denaturation at (95°C for 15 min), DNA denaturation at 94°C for 30 sec for 30 cycles, annealing at 60°C for 90 sec , primer extension (72°C for 1 min), and final extension (72°C for 10 min). Agarose gel Electrophoresis was performed (Fermentas, Lithuania) with ethidium bromide (Sigma, USA). The products were visualized by UV transilluminator by 50 bp DNA ladder [15].

Statistical analysis

Collected data were collected, tabulated, and analyzed by statistical package for the social sciences (SPSS, version 22; SPSS Inc., Chicago, Illinois, USA) software. Mean and standard deviation, number and percentage was done at 5% level of significance.

Results

The present study included 207 chronic liver disease patients with mean age of (49.5 ± 11.9) years with significant NCI in male patients (130(62.8%)). Of 247 samples, 68% (168) indicate growth of NC pathogens; 32% (54) were Gram positive bacteria and 68% (114) were Gram negative bacteria.

In **table (2)** NCI by Gram negative pathogens was significantly increased in patients with hepatic coma ($p<0.05$). also, it increased in Spontaneous bacterial peritonitis (82.1%) which was non- significant. While Gram positive NCI was increased in hepatic patients with marked ascites (66.7%). Infection was significantly higher in patients with repeated courses of antibiotics, diabetics, on parenteral nutrition and in those underwent cholecystectomy ($p<0.05$)

In **table (3)** device associated nosocomial infections in hepatic patients was illustrated; gram negative nosocomial pathogens were significantly higher in CAUTI. Catheter-related bloodstream infections shows Gram positive pathogen predominance which was non-significant.

In **table (4)** The most predominant nosocomial organism was *P. aurignosa* which represent 19% (32 from 168), followed by *Staph aureus* that account for 17.86% (30 from 168), *A. baumannii* signify 14.2%, *K. pneumoni*ae form 11.9%. MDR represents 123 isolates from 168 with 73.2% percentage, all *Burkholderia cepacia, Enterobacter, Streptococci* and *Proteus* spp were MDR while XDR represents 23.8% of total isolates with 37.5% of *Acinetobacter*, 34% of *Pseudomonas* and 33.3% of *S. aureus* isolates are XDR. Pan drug resistance *A. baumannii* represents 5 from 24 isolates. It was associated with CAUTI and CLBSI but non-significant.

The MDR pathogens were resistant to ampicillin, amikacin, ampicillin/sulbactam, amoxicillin /clavulanic, cefepime, cefotaxime, ceftazidime, ceftriaxone, gentamycin, piperacillin and sensitive to ciprofloxacin, imipenem, meropenem, trimethoprim, colistin and vancomycin. XDR pathogens were sensitive to colistin in gram negative isolates and trimethoprim in Gram positive isolates.

Table 1. Primer sequence used in detection of virulence gene.

	Base Sequence	product length (bp)	reference
outer membrane proteins (*OmpA*)			[16] **Zeighami et al.**
Forward primer	GTTAAAGGCGACGTAGACG	578	
Reverse primer	CCAGTGTTATCTGTGTGACC		
biofilm-associated proteins (*bap*)		184	[17] **Farajzadeh et al.**
Forward primer	TGCTGACAGTGACGTAGAACCACA		
Reverse primer	TGCAACTAGTGGAATAGCAGCCCA		
chaperon-usher pilus (*csu E*)			
Forward primer	CATCTTCTATTTCGGTCCC	168	[18] **Khoshnood et al.**
Reverse primer	CGGTCTGAGCATTGGTAA		

Table 2. Clinical presentation and risk factors in NCI infected hepatic patients.

Clinical presentation	**Nosocomial infected patients (207)**		***p* value**
	Gram positive pathogens (n=54)	**Gram negative pathogen (n=114)**	
Spontaneous bacterial peritonitis (56)	10(17.9%)	46(82.1%)	
hepatic coma (32)	0(0.0%)	32(100%)	**<0.001***
jaundice (18)	6(33.3%)	12(66.7%)	
marked ascites (30)	20 (66.7%)	10(33.3%)	
Hepatosplenomegaly (12)	4(33.3%)	8(66.7%)	
Surgical wound infection (14)	10(71.4%)	4(28.6%)	
bile duct injury (4)	0(0.0%)	4(100.0%)	
malignant obstructive jaundice (2)	0(0.0%)	2(100.0%)	
watery diarrhea +fever (6)	4(66.7%)	2(33.3%)	
Risk factors	**Nosocomial infected patients (207)**		
	Gram positive pathogens (54)	**Gram negative pathogens (n=114)**	***P* value**
Diabetes Mellitus (DM)	27(50.0%)	66(57.89%)	**0.04***
Repeated antibiotic intake	10(18.5%)	24(21.05%)	**0.003****
Hypertension (HTN)	8(14.8%)	10(8.7%)	0.09
Cholecystectomy	6(11.1%)	12(10.5%)	**0.02***
Steroid intake	2(3.7%)	6(5.26%)	0.5
Parenteral nutrition	1(1.85%)	6 (5.26%)	**0.01***

Table 3. Types of devices associated nosocomial infections in hepatic patients.

	Gram positive pathogens (n=54)	**Gram negative pathogens (n=114)**	
Catheter associated urinary tract infection (CAUTI)	5 (9.26%)	34 (29.8%)	**0.04***
Surgical site infection (SSI)	15 (27.8%)	34 (29.8%)	
Catheter related blood stream infection (CRBSI)	20(37.04%)	14(12.28%)	
Central line blood stream infection (CLABSI)	10(18.5%)	20 (17.54%)	
Ventilator associated pneumonia (VAP)	4(7.4%)	12(10.5%)	

Table 4. Pattern of microorganisms detected in infected hepatic patients.

Organism	**Nosocomial pathogen**	**MDR**	**XDR**	**PDR**	**Total**
	Positive (n=168)	**(123)**	**(40)**	**(5)**	**(168)**
Pseudomonas aeruginosa	32	21(65.6%)	11(34.4%)	0	32
Staph aureus	30	20(66.7%)	10(33.3%)	0	30
Acinetobacter baumannii	24	10(41.7%)	9 (37.5%)	5(20.8%)	24
Klebsiella pneumoni	20	16(80.0%)	4(20%)	0	20
E-coli	16	14(87.5%)	2(12.5%)	0	16
Staph. epidermidis	16	14(87.5%)	2 (12.5%)	0	16
Burkholderia cepacia	12	12(100.0%)	0	0	12
Enterococci	6	5(83.3%)	1(16.7%)	0	6

Figure1. *Acinetobacter baumannii* colonies; right; forming black biofilm forming colonies

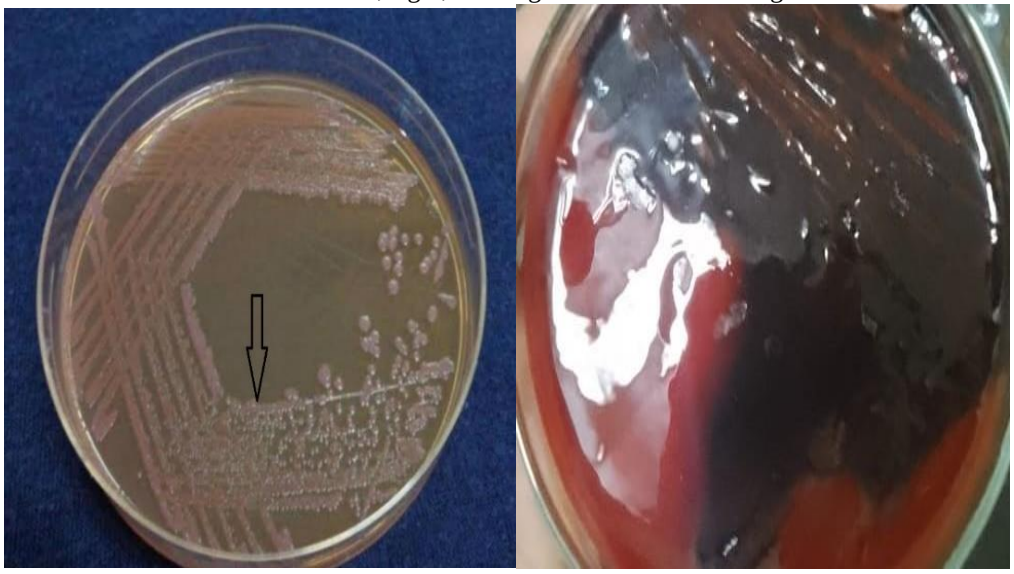


Figure 2. Crystal violet ring test; positive for biofilm-forming *A. baumannii.*

Figure 3. Distribution of nosocomial samples.

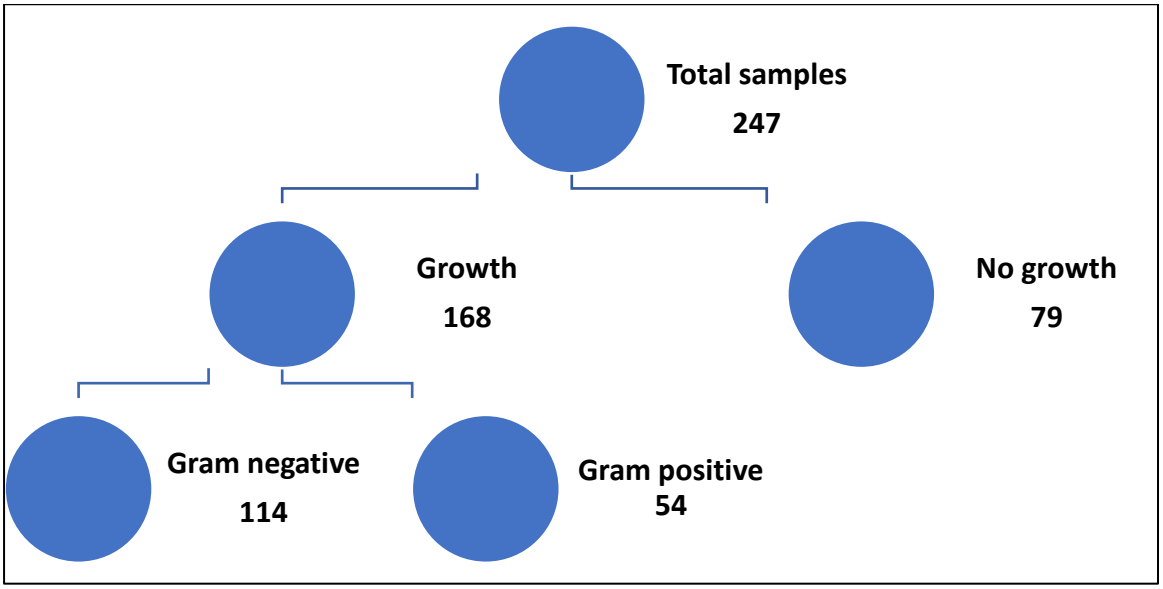
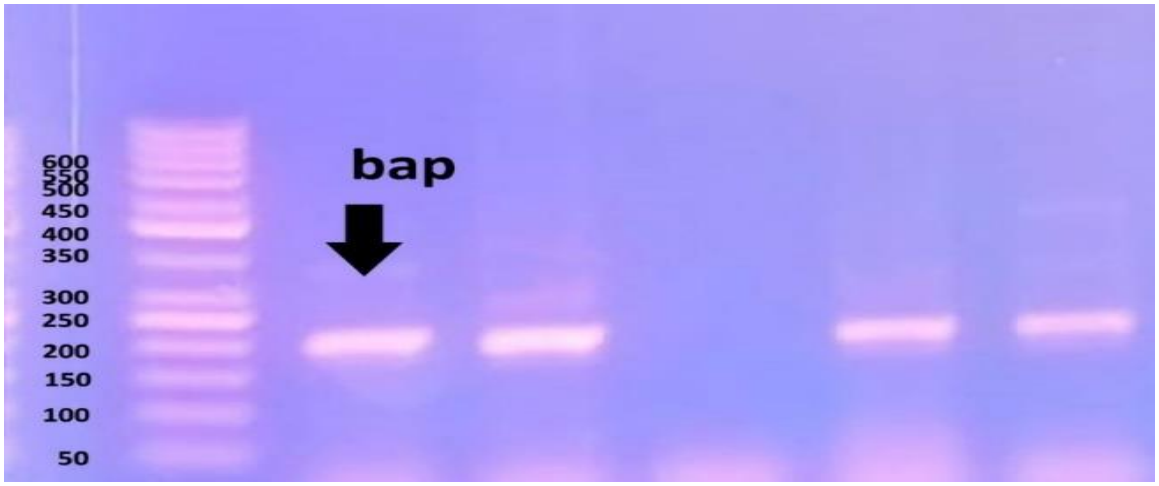


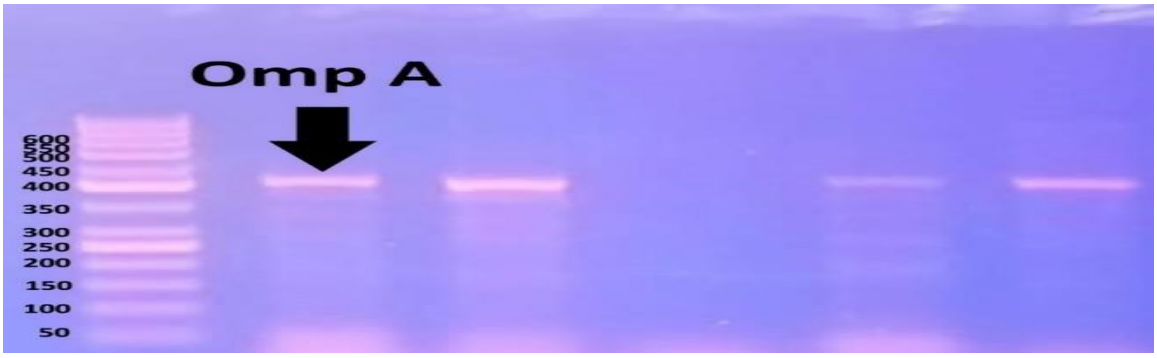
Figure 4.Agarose gel electrophoresis for bap gene detection.



In this figure, gel electrophoresis using 50bp ladder shows bands at 186 bp for bap gene that detected in *Acinetobacter* sample no: 1,2,4, and 5.

Figure 5. PDR *Acinetobacter* gel electrophoresis Csu E gene was detected at 168 bp in lane 1,2,4,5.

Figure 6. OmpA gene detection in PDR *Acinetobacter baumannii* in lane1,2,4 and 5 at 578 bp.



Discussion

Nosocomial infections are without a doubt one of the major hazards when managing female as well as male patients with end-stage liver disease. Early diagnosis, immediate treatment and in particular the development of prophylactic measures are key challenges to improve patients' outcome [3]. Nosocomial infections could lead to considerably higher mortality rates, length of the hospital stays and costs, and represent a serious public health concern worldwide. Besides, the unreasonable use of antibiotics could lead to get resistant to different antibiotics and create limited therapeutic options, increased risks of treatment failure and poor patient management [19]. In this study, we aimed to study the magnitude of NCI among critically ill hepatic patients.

In this study, chronic liver disease male patients (130(62.8%)) with mean age of (49.5 ± 11.9) years had significant increase in incidence of NCI. This was in agree with **Penrice et al.** who mentioned that 66% men, and the median age was 48 (21-83) [20]. On the other hand, **Griemsmann et al.** found no major differences in the incidence and outcome of nosocomial infections between male and female patients [3].

The current study confirms higher prevalence of Gram- negative pathogens as etiological agents of NCI than Gram positive pathogens. This was in accordance with results obtained by **Ding et al.** [21]. Gram-negative bacteria were the major pathogens involved in the cirrhotic patients. The strains isolated from the patients with nosocomial infection displayed higher drug resistance than those isolated from patients with community acquired infection. Compared with community acquired SBP, nosocomial SBP had a poorer outcome [21].

In the present study, NCI by Gram negative pathogens was significantly increased in patients with hepatic coma (p<0.05). also, it increased in spontaneous bacterial peritonitis (82.1%) which was non- significant. While gram positive NCI was increased in hepatic patients with marked ascites (66.7%).

This was in agree with **Shi et al.** who mentioned that regarding clinical symptoms, two groups exhibited obvious difference, fever, abdominal pain, hepatic encephalopathy, vomiting or diarrhea, and septic shock were more frequently observed in the nosocomial group [22]. Also, **Choudry et al.** found that Gram-positive organisms were most commonly isolated in patients with spontaneous bacterial peritonitis and bacteremia,

whereas Gram-negative bacteria were most prevalent in urinary tract infections and pneumonia [23].

According to the current study, infection was significantly higher in patients with repeated courses of antibiotics, diabetics, on parenteral nutrition and in those underwent cholecystectomy ($p<0.05$). Similar results mentioned by author who mentioned that excessive antibiotic intake and diabetes increases hospital acquired infections [24].

This study showed that device associated nosocomial infections in hepatic patients caused by Gram negative nosocomial pathogens were significantly higher in CAUTI. **Jahani-Sherafat et al.** mentioned that CAUTI was also the most common DA-HAI [25]. In this study, CRASBI shows Gram positive pathogen predominance which was non-significant. Comparable results obtained by other authors [3]. On the other hand, **Gustot et al.** found that Spontaneous bacterial peritonitis (SBP) is often identified as the most frequent infection in cirrhosis, followed by urinary tract infection and pneumonia [26].

The higher proportion of infected patients among cirrhotic compared with non-cirrhotic patients likely reflects their greater susceptibility to bacterial infection because of the immunological deficiencies associated with cirrhosis [26].

In this study, the most predominant nosocomial organism was *P. aurignosa* which represent 19% (32 from 168), followed by *S. aureus* that account for 17.86% (30 from 168), *A. baumannii* signify 14.2%, *K. pneumoni* form 11.9%. on the other hand, **Hassan et al.** found that most common organism was (49.1%). *E. coli* (21.1%) followed by MRSA (15.8%) were the commonest bacteria [27].

In the present study, we found that, MDR bacteria represents 123 isolates from 168 with 73.2% percentage, all *Burkholderia cepacia, Enterobacter, Streptococci* and *Proteus* spp were MDR bacteria. The MDR bacteria were resistant to ampicillin, amikacin, ampicillin/sulbactam, amoxicillin /clavulanic, cefepime, cefotaxime, ceftazidime, ceftriaxone, gentamycin, piperacillin and sensitive to ciprofloxacin, imipenem, meropenem, trimethoprim, colistin and vancomycin. XDR bacteria were sensitive to colistin in Gram negative isolates and trimethoprim in Gram positive isolates.

Comparable results obtained by [28] who mentioned that based on Gram staining, with the majority of Gram-negative pathogens being MDR bacteria ($p = 0.005$).on the other hand, **Hassan et al.** Multi- drug resistant organisms were reported in 52.6% of the isolates [27]. Multidrug resistant bacterial infections constitute a prevalent, growing, and complex healthcare problem in patients with decompensated cirrhosis and acute-on-chronic liver failure across Europe, negatively impacting on prognosis. Strategies aimed at preventing the spread of antibiotic resistance in cirrhosis should be urgently evaluated [29]. Clinical impact and cost/effectiveness of measures should be evaluated such as epidemiological surveillance (regular assessment of potential carriers of MDROs through rectal and nasal swabs during hospitalization)

This study shows, XDR bacterial isolates represented 23.8% of total isolates with 37.5% of *Acinetobacter*, 34% of *Pseudomonas* and 33.3% of *S. aureus* isolates are XDR. Similar results obtained by some authors [30,7,19].

In the current study, PDR *Acinetobacter baumannii* represents 5 from 24 isolates (20.8%). On the other hand, one author mentioned that the results of the antibiotic susceptibility test of *A. baumanii* revealed that the resistance rates of all strains were >55% against all the tested antibiotics [8].

The current study entailed that 4 of PDR *A. baumannii* produce biofilm virulence genes *Omp A, Csu E,* and *bap* This result agreed with **Yang et al.** who mentioned that; the multiple drug resistant isolates usually provided a higher biofilm formation [8]. Biofilm formation and antibiotic resistance levels may vary among sites and the key factors responsible for this resistance may differ. Regarding resistance, the primary evidence indicates that conventional mechanisms cannot explain the high resistance to antibacterial agents associated with biofilms [31]. Several mechanisms considered key factors in the high resistance of biofilms have been explored: (a) limited diffusion, (b) enzyme-caused neutralizations, (c) heterogeneous function, (d) slow growth rate, (e) persistent (non-dividing) cells, and (f) biofilm phenotype adaptive mechanisms [31,32].

The development of novel therapeutic strategies as phage therapy, nanoparticle therapy and photodynamic therapy for the eradication of preformed biofilms which can help tackle biofilm associated *A. baumannii* infections is required for

successful treatment outcomes. Understanding the key factors behind function and regulation of the biofilm machinery of *A. baumannii* and other nosocomial pathogens will provide insight to develop novel approaches to combat nosocomial infections.

Conflict of interest

We declare that we have no conflict of interest.

Financial disclosures: nothing to declare.

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