academicJournals

Vol. 7(41), pp. 4857-4861, 11 October, 2013
DOI: 10.5897/AJMR2013.5959
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Influence of multi drug resistance Gram negative bacteria in liver transplant recipient

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Accepted 20 September, 2013

**Infection is a common cause of morbidity and mortality after liver transplantation, which are often caused by Gram negative bacteria and the most frequently occurring infectious complications after liver transplantation (LT). The aim of this study was to investigate, incidence, pathogenic spectrum, and risk factors for bacteria due to multidrug resistant (MDR) Gram-negative bacteria, and its impact on mortality after LT. In total, 150 consecutive patients who underwent liver transplantation between January 2012 and March 2013, 115 isolates of bacteria and 10 isolates fungal from 80 patients. MicroScan® microbiology and API20 were used to identify the all isolates. Bacterial infections represented the most frequent event (92.0%) and fungal infections (8.0%). The presumed sources of infection were the Urine (41 events, 51%), followed by the bail drain (14 events, 17.5%). Infections of the sputum, ascetic fluid, and blood site accounted for 13.8, 5.0 and 6.3%, respectively. Antibiotics the most susceptible antibiotic against *Pseudomonas aeruginosa* was colistin (94.1%). Patients with Gram-negative bacilli after liver transplantation show a significantly worse prognosis. Gram-negative bacteria after liver transplantation have been a major problem in our center.**

**Key words**: Gram-negative bacteria, multi drug resistance, liver transplantation, mortality.

## INTRODUCTION

Infection is a common cause of morbidity and mortality after liver transplantation (LT). The effect of infections in LT is higher compared with recipients of other organs. Bacterial infections are among the most relevant causes of morbidity and mortality after organ transplantation. The high prevalence of multidrug-resistance among bacterial pathogens causing infections in solid organ transplant recipients is an additional concern. In a recent report, infections with multidrug-resistant Gram negative bacteria were associated with higher mortality among liver transplant recipients (Shi et al., 2009). Emergence of nosocomial infections, especially *Acinetobacter baumannii, Pseudomonas aeruginosa* with multidrug resistance have become a major problem among transplant recipients in hospital settings (Diab et al., 2002, 2004; Patel et al., 2010). These infections can result in a wide range of complications, including bacteremia, pneumonia, urinary tract infection and peritonitis. The aim of this study was to investigate the clinical characteristics, mortality, and outcomes among liver transplant recipients with Gram-negative infection. Over the past 30 years, colistin use has been limited due to concerns regarding its toxicity along with the development of newer antibiotics with better safety profiles (Jain et al., 2004). However, the increasing

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**Table 1**. Microorganisms distribution of 125 isolates in 80 patients after liver transplant (L T) recipients.

| Microorganisms isolated | Total number of Microorganisms isolated (%) |
|---|---|
| **Bacteria** | **115 (92%)** |
| *Pseudomonas aeruginosa* | 34 (29.6%) |
| *Klebsiella pneumonia* | 25 (21.7%) |
| *Escherichia coli* | 24 (20.9%) |
| *Acinetobacter* spp | 13 (11.3%) |
| *Staphylococci* | 12 (10.4%) |
| *Coagulase-negative staphylococcus* | 5 |
| *Staphylococcus aureus* | 7 |
| *Enterobacter* spp | 5 (4.3%) |
| *Aeromonas hydrophila* | 2 (1.7%) |
| | |
| **Fungi** | **10 (8%)** |
| *Candida species* | 7 (70%) |

incidence of multidrug-resistant *A. baumannii* in addition to a lack of new antimicrobial agents has reawakened interest in the utilization of colistin due to its good activity against this organism.

## MATERIALS AND METHODS

In total, 150 consecutive patients (50 female and 100 male) who underwent liver transplantation between January 2012 and March 2013 at the Ain Shams University Specialized Hospital, Cairo, Egypt, were enrolled in this study. Patients range age from 18 to 70 years. Specimens included blood, pus, tissues and body fluids, urine, sputum and peritoneal dialysate.

The strains had been isolated on blood agar, desoxycholate citrate agar, thiosulphate citrate bile salts sucrose agar tests for growth at 30, 37°C were performed in brain heart infusion broth in a water bath. Sheep blood (5%) agar plates were used to detected hemolysis, gelatin stab method was used to test for gelatin liquefaction. The susceptibility tests were read after overnight incubi-tion, and the tests for hemolysis and growth at 30, 37°C were read on the first and second day. For gelatin liquefaction the incubation temperature was 22°C. Antimicrobial susceptibility was determined by the Kirby- Bauer disk diffusion method (Selim, 2011; Selim et al., 2012, 2013). The interpretive criteria followed the latest National Committee for Clinical Laboratory Standards (NCCLS) recommend-dations (NCCLS, 2002). Identified at least to genus level by MicroScan® microbiology, biochemical tests and API 20E system (bioMe´rieux, France), in a previous study and cryopreserved in 20% glycerol at -80°C. Working cultures were maintained in Luria Bertani (LB) agar and broth. We collected data from hospital and operative records of each liver transplantation recipient, including perioperative demographic and clinical characteristics (that is, age, gender, Child-Pugh), operative variables (that is, operation time, blood loss) and clinical events with 6 months post transplant (that is, duration of initial intubation, intensive care unit (ICU) stay, reoperation, dialysis, and rejection).

### Statistical analysis

The data was analyzed using Chi-square test, Z-test and two way classification of ANOVA.

## RESULTS AND DISCUSSION

In total, 150 patients (median age 40 years) were included in this study and contributed a total of 64.4, 80 patients positive infection, events 70 cases are negative. 125 isolated, bacterial infections represented the most frequent (115 events, 92%). followed by fungal infections (10 events, 8.0 %).

### Bacterial infections

Among the bacterial infections, the most frequent pathogen was *Pseudomonas aeruginosa* with 34 isolates (29.6%).The second most common isolated organism was *Klebsiella pneumonia* (25 events, 21.7.0%), followed by *E. coli* (24 events, 20.9%), *Acinetobacter baumannii* (13 events, 11.3%), *Staphylococcus* spp (12 events, 10.4%), *Enterobacter* spp (5 events, 4.3%), *Aeromonas* spp (2 events, 1.7%) The majority of the bacterial infections occurred during the first 6 months, with the highest incidence of infection during the first 30 days (30 events/ month), Table 1.

### Fungal infections

In total, 10 events of fungal infections were documented. In 7 cases (70.0%), Candida species were isolated (5 events of *Candida albicans* and 2 cases with *Candida krusei*).. Infections with *Aspergillus* accounted for 30 %of fungal infections (3 cases of *Aspergillus fumigatus*). One case with pulmonary involvement was documented during the first 30 days.

### Manifestation of infection

The most common site of pathogen isolation overall was the urine (41 events, 51%), followed by the bail draine

**Table 2.** Postoperative Gram negative bacteria isolates from various sites in 80 patients after LT.

| Site | *Pseudomonas aeruginosa* 34 in (25) | *Klebsiella pneumonia* 25 in (20) | *E.coli* 24 in (20) | *Acinetobacter* spp. 13 in (9) | *Aeromonas hydrophila* 2 in (2) | *Enterobacte* Spp. 5 in (4) | Total |
|---|---|---|---|---|---|---|---|
| Urine | 16 (15) | 13(11) | 12(11) | 2(2) | 1(1) | 2(1) | 46(41) |
| Bile drain | 9 (5) | 3(2) | 4(3) | 3(2) | 1(1) | 1(1) | 21(14) |
| Sputum | 4(2) | 5(4) | 3(3) | 2(1) | None | 1(1) | 15(11) |
| Fluid | None | 2(1) | 2(1) | 3(2) | None | None | 7(4) |
| Blood | 3(2) | 1(1) | None | 1(1) | None | 1(1) | 6(5) |
| Abdominal | 2(1) | 1(1) | 3(2) | 2(1) | None | None | 8(5) |
| Total | 34(25) | 25(20) | 24(20) | 13(9) | 2(2) | 5(4) | 103(80) |

Total = isolates of bacteria/number of patients.

**Table 3**. Antibiotic resistance profiles of predominant Gram-negative bacilli in 80 recipients after LT.

| Antimicrobial agents | *Pseudomonas aeruginosa* (n=34) | *Klebsiella pneumonia* (n=25) | *E. coli* (n=24) | *Acintobacter* spp (n=13) | *Enterobacter* spp (n=5) | *Aeromonas hydrophila* (n=2) |
|---|---|---|---|---|---|---|
| Amoxicillin-clavulanate | 17.6%(6/34) | 20%(5/25) | 16.7%(4/24) | 92.3%(12/13) | 40%(2/5) | 0%(0/2) |
| Ampicillin-sulbactam | 23.5%(8/34) | 20%(5/25) | 20.8%(5/24) | 100%(13/13) | 20%(1/5) | 0%(0/ |
| Piperacillin-tazobactam | 11.8%(4/34) | 16%(4/25) | 20.8%(5/24) | 100%(13/13) | 60%(3/5) | 100%(2/2) |
| Ceftriaxone | 17.6%(6/34) | 28%(7/25) | 25%(6/24) | 92.3%(12/13) | 20%(1/5) | 50%(1/2) |
| Cefepime | 20.6%(7/34) | 16%(4/25) | 16.7%(4/24) | 84.6%(11/13) | 20%(1/5) | 50%(1/2) |
| Ceftazidime | 17.6%(6/34) | 16%(4/25) | 16.7%(4/24) | 100%(13/13) | 20%(1/5) | 50%(1/2) |
| Imipenem | 94.1%(32/34) | 80%(20/25) | 83.3%(20/24) | 100%(13/13) | 100%(5/5) | 100%(2/2) |
| Meropenem | 88.2%(30/34) | 88%(22/25) | 83.3%(20/24) | 100%(13/13) | 100%(5/5) | 100%(2/2) |
| Gentamicin | 44.1%(15/34) | 52(13/25) | 62.5%(15/24) | 100%(13/13) | 40%(2/5) | 100%(2/2) |
| Amikacin | 11.8%(4/34) | 8%(2/25) | 8.3%(2/24) | 100%(13/13) | 20%(1/5) | 0%(0/2) |
| Netilmicin | 14.7%(5/34) | 20%(5/25) | 8.3%(2/24) | 92.3%(12/13) | 40%(2/5) | 50%(1/2) |
| Colistin | 5.9%(2/34) | 72%(18/25) | 29.2%(7/24) | 7.7%(1/13) | 20%(1/5) | 0%(0/2) |

Values in the parentheses are number (n) of antibiotic resistance isolates/number of total isolates tested, respectively.

(14 events, 17.5%). Infections of the sputum, blood and ascetic fluid site accounted for 13.8, 6.3 and 5.0%, respectively. Other common sites of infection included the abdominal (6.3%) (Table 2).

## Antimicrobial resistance of main Gram-negative bacilli

The antibiotic resistance patterns of predominant Gram negative bacilli were listed in Table 3. The most susceptible antibiotic against *p. aeruginosa* was colistin (94.1%). Imipenem was the most resistance antibiotic against all isolates (80 to 100%).

All antimicrobial agents showed relative low susceptibility against *Acinetobacter* spp except colistin. Piperacillin/tazobactam (84%) high antibiotic susceptibility to *K. pneumonia*.

## Phenotypic identification

All isolates were subjected to simplified phenotypic tests as described in Table 4. Briefly, tests for growth at 30, 37°C were performed in brain heart infusion broth in a water bath. Sheep blood (5%) agar plates were used to detected hemolysis, gelatin stab method was used to test for gelatin liquefaction. The susceptibility tests were read after overnight incubation, and the tests for haemolysis and growth at 30, 37°C were read on the first and second day. For gelatin liquefaction the incubation temperature was 22°C.

## DISCUSSION

Studies of infectious complications after liver transplantation exist in the literature (Piselli et al., 2007).

**Table 4.** Phenotypic characteristics of Gram negative bacteria isolates from patients after LT.

| Parameter | *Pseudomonas aeruginosa* N=34 | *Klebsiella pneumonia* N=25 | *E.coli* N=24 | *Acinetobacter* spp. N=13 | *Aeromonas hydrophila N=2* | *Enterobacter* spp. *N=5* |
|---|---|---|---|---|---|---|
| Hemolysis | + | - | - | 2 | **1** | - |
| Lipase | + | - | - | - | - | - |
| Proteolytic | + | - | - | - | - | - |
| Gelatinase | + | - | - | - | - | - |
| Catalase | + | + | + | + | + | + |
| Congo red | 30 | 23 | 20 | 11 | 2 | 5 |

N= number of isolates.

However, most of these data are older than 10 years and all of the studies were performed in the United States and Europe. Therefore, we analyzed the incidence, presentation, and risk factors of infections in liver transplant recipients in Egypt population treated between 2012 and 2013, and compared our findings with data worldwide.

The 1-year survival in our population was 89%, similar to that of other transplant groups. The incidence of infection during the period of 12 months was comparable to data by an Italian study (Garbino et al., 2005), who reported infections in 56% of patients during the first year, and somewhat lower than data surveyed by Kusne et al. (1998) from the University of Pittsburgh Medical Center between 1984 and 1985, who found infections in83% of the patients, or results from a Swiss single-center study, who reported that 80% of patients developed infections after liver transplantation (Garbino et al., 2005).

Bacterial infections were the most frequent event in our population, as found by other groups (9, 4). In our study, the proportion of bacterial events was higher (92.0%) in comparison with 47% observed in the Swiss study (Garbino et al., 2005), 48% reported by Torbenson et al. (1998), about 54% at the Pittsburgh Medical Center, or 57% in the Italian study (Piselli et al., 2007). Infection by Gram-negative enteric organisms was the main cause of bacterial infection in our population, which shows that the epidemiology of infections at our hospital is similar to those of Western Europe and the United States. In accordance with other studies (Piselli et al., 2007, Garbino et al., 2005), most bacterial infections occurred within the first month, and the bloodstream was the leading infection site. Other significant locations were urinary tract infections, and abdominal and surgical site infections.

Fungal infections accounted for 8.0% in our patients which compared with other studies that showed infection rates between 12 and 29% (Piselli et al., 2007). Pappas et al. (2006) reported a rate of 4% of invasive fungal infections during the first 100 days post transplantation. Most of the infections in our study were attributed to Candida species and peaked during the first month. In summary, liver transplant recipients within the first month are most susceptible to nosocomial infections similar to those seen in non-immunosuppressed surgical patients.

Positive cultures by routine investigations, obtained according to the protocols of harvesting systematic samples after the transplant, or the identification of a microorganism of the normal human flora were not taken into account, unlike some studies that included such events (Dawwas et al., 2007). Multiple screening cultures likely result in an over diagnosis of infection; however, screening can be the only early guidance for early treatment of infection.

The literature lacks randomized controlled studies for bacterial prophylaxis comparing the efficacy of different antibiotic regimens in transplant recipients. Most centers used amoxicillin-clavulanate or a second- or third-generation cephalosporin against bacterial infections. We used amoxicillin-clavulanate in combination with gentamicin, a standard prophylaxis, and reported an infection rate that was comparable with data worldwide. As described previously, most infections were caused by Gram-negative aerobic bacteria such as *E. coli*, which are inhabitants of the digestive tract.

However, the incidence of multiple-antibiotic-resistant bacteria is increasing, so the choice of prophylaxis also has to take into consideration the bacterial isolates that are commonly present in the center performing the transplantation. Therefore, it is difficult to compare our results with other studies reports regarding prophylaxis and infection rate, because many other variables affect infection rates. In one of the largest studies with more than 1200 patients, more than 8 types of antibiotics or antibiotic combinations were used for surgical prophylaxis (Asensio et al., 2008); however, the most important risk factor for surgical site infection was the choledochojejunal reconstruction (Asensio et al., 2008).

The recent study could confirm the data by other groups and supports the impact of post-transplant infections on morbidity and mortality of liver recipients. Infections were mainly of bacterial origin and occurred more frequently during the early post-transplant period. The bloodstream, abdomen, urinary tract, and drain were the most common sites of infection. Parenteral nutrition and a prolonged stay in the ICU were associated significantly with an increased infection rate. Early detection and treatment of infections is essential to obtaining a better outcome in

liver transplant patients. The choice of prophylactic regimen has to take into account the bacterial isolates that are commonly present in the center performing the transplantation. Our results provide additional insight into the risk of infection after liver transplantation and could motivate performance of new studies concerning the understanding and improved prophylaxis of post-transplant infections.

## Conclusion

Infection due to MDR Gram-negative bacilli are common after LT, and associated with allograft acute rejection, post-transplant reoperation, and abdominal infection. Patients with MDR Gram-negative bacillus have significantly worse prognosis, and are associated with pre LT bacterial infections, renal dysfunction, post- LT abdominal infection, and prolonged endotracheal intubation. The increasing isolates of MDR Gram-negative bacilli pose a great challenge for clinical treatment.

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