MICROBIOLOGICAL PATTERN IN BILIARY TRACT INFECTION
AT CHO RAY HOSPITAL IN 2021

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Summary

Background: MBiliary tract infection is a common cause of bacteremia with high morbidity and mortality, particularly in older patients with co-morbid disease or when there is a delay in diagnosis and treatment. This study is undertaken to determine the microbiological pattern and antibiotic resistance in biliary tract infection at Cho Ray Hospital in 2021.

Subjects and methods: NA retrospective study was conducted from January 2021 to December 2021 at Cho Ray hospital. Bile samples were cultured on Blood agar, Mac-conkey agar. Bacterial strains were identified by the MALDI-TOF MS system and made susceptibility testing on the VITEK 2-compact system of Biomerieux. The antibiotic susceptibility were determined by minimum inhibitory concentration (MIC) according to CLSI.

Results: Out of 595 bile samples sent for aerobic culture and susceptibility testing, 203 (34.1%) were sterile and 392 (65.9%) were found to be culture positive. Of the bile culture positives, 129 (21.7%) were polymicrobial. The most prevalent bacteria isolates were Escherichia coli 33.8% (102), Klebsiella pneumoniae 22.2% (67), Enterococcus spp. 16.6% (50), Pseudomonas aeruginosa 7.9% (24). The ESBL rate of Escherichia coli was 42.2%, while this rate of Klebsiella pneumoniae was 9%. The vancomycin resistant rate of Enterococcus spp. was 8.3%. In Pseudomonas aeruginosa, among beta-lactams, imipenem had the highest resistant rate (45.8%). No resistance to colistin had been reported.

Conclusion: In biliary tract infections, about one-third cases had negative-culture results and about one-fifth cases had polymicrobial infection. The common pathogens in biliary tract infections were Escherichia coli, Klebsiella pneumoniae, Enterococcus spp. and Pseudomonas aeruginosa. The resistance rate to commonly used antibiotics in treatment was quite high.

Keywords: Biliary tract infection, ESBL.

INTRODUCTION

Biliary tract infection is a common cause of bacteremia with high morbidity and mortality, particularly in older patients with co-morbid disease or when there is a delay in diagnosis and treatment. Therefore, the majority of biliary tract infections require empiric antibiotic therapy and early biliary drainage, before culture results are available. The choice of empiric antibiotic treatment is often based on the resistance pattern of the organism and the causative agent. The most common causative agents of biliary tract infections are intestinal bacteria such as Escherichia coli, Klebsiella pneumoniae, Enterococcus spp. However, the microbiological pattern of the infection can change over the years and multidrug-resistant bacteria appear increasingly. Thus understanding the causative agent and the situation of antibiotic resistance in biliary tract infections is very important, in order to choose appropriate empiric antibiotics to increase...
treatment effectiveness, limit mortality and prevent drug resistance from increasing.

**Objectives of the study:** Describe microbiological pattern in biliary tract infections. Determine antimicrobial resistance rates of common bacteria in biliary tract infections.

**SUBJECTS AND METHODS**

**Subjects:** 595 bile specimens were cultured and isolated at Cho Ray Hospital from January 2021 to December 2021.

**Methods:** Retrospective, cross-sectional study. Bile specimens were cultured on Macconkey and blood agar. The agars were incubated at 35 - 37°C (blood agars were incubated in 5% CO₂ supplemented medium) and monitored every 18 - 24 hours. We did not culture the obligate anaerobic bacteria.

**Identification:** Bacterial strains were identified by the MALDI-TOF MS system or the VITEK-2 compact system of Biomerieux.

**Antibiotic susceptibility testing** based on the technique of determining the minimum inhibitory antibiotic concentration (MIC) using the automated system VITEK-2 compact of Biomerieux or by the disc diffusion method.

The MICs were then analyzed using both the CLSI M100 standard (2021) to categorize them as either susceptible, intermediate or resistant.

**RESULT**

**Microbiological pattern of biliary tract infection**

Of the 595 bile samples collected, 203 samples (34.1%) were negative (no bacteria growth) and 392 samples (65.9%) were positive (Table 1).

<table>
<thead>
<tr>
<th>Result</th>
<th>Sample number (n = 595)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>392 (65.9%)</td>
</tr>
<tr>
<td>1 type of bacteria</td>
<td>214 (36%)</td>
</tr>
<tr>
<td>2 type of bacteria</td>
<td>44 (7.4%)</td>
</tr>
<tr>
<td>Only gram-negative bacteria</td>
<td>22</td>
</tr>
<tr>
<td>Only gram-positive bacteria</td>
<td>2</td>
</tr>
<tr>
<td>Both gram-negative and gram-positive bacteria</td>
<td>20</td>
</tr>
<tr>
<td>Polymicrobial infection</td>
<td>129 (21.7%)</td>
</tr>
<tr>
<td>Only yeast</td>
<td>5 (1.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>203 (34.1%)</td>
</tr>
</tbody>
</table>

In 302 bacterial strains isolated from bile samples, the gram-negative bacteria group accounted for 80.8% (244 strains), while the Gram-positive bacteria group accounted for a lower proportion of 19.2% (58 strains).

The most common bacteria were *Escherichia coli* (102; 33.8%), following by *Klebsiella pneumoniae* (67; 22.2%), *Enterococcus* spp. (50; 16.6%), *Pseudomonas aeruginosa* (24; 7.9%).

<table>
<thead>
<tr>
<th>Infectious agents</th>
<th>Rate (%)</th>
<th>Infectious agents</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>33.8</td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>1.0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>22.2</td>
<td><em>Proteus</em> spp.</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp</td>
<td>16.6</td>
<td><em>Serratia marcescens</em></td>
<td>0.7</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>7.9</td>
<td><em>Staphylococcus aureus</em></td>
<td>0.7</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila/caviae</em></td>
<td>5.6</td>
<td><em>Chryseobacterium indologenes</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>3.6</td>
<td><em>Morganella morganii</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>2.3</td>
<td><em>Raoultella ornitholytica</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp</td>
<td>2.0</td>
<td><em>Shewanella putrefaciens</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp</td>
<td>1.3</td>
<td><em>Shigella sonnei</em></td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 1. Microbiological culture results

Table 2. Distribution of organisms isolated from bile specimens
Figure 1. Antimicrobial resistant rate of *E. coli* (n = 102 isolates)

*Klebsiella pneumoniae* (*K. pneumoniae*) isolates were high-level resistant to beta-lactam combinations (over 40%), fluoroquinolones (over 50%) and carbapenems (over 46%).

Figure 2. Antimicrobial resistant rate of *K. pneumoniae* (n = 67 isolates)

The rate of resistance to vancomycin in *Enterococcus* spp. were 8.3%. The resistance rate to other antibiotics such as ciprofloxacin, high-level gentamicin (HLGR) and high-level streptomycin (HLSR) were over 20%.
The resistance rate to beta-lactams in *Pseudomonas aeruginosa* (*P. aeruginosa*) varied from 25 - 46%, resistance rate to imipenem was highest (45.8%). The rate of resistance to levofloxacin was higher than ciprofloxacin (45.8% versus 37.5%). No resistance to colistin was detected.
DISCUSSION

Microbiological characteristics of biliary tract infection

Biliary tract infection often occurs in patients with biliary obstruction, the causative agent is bacteria from the intestine or, rarely, from the portal vein. Of 302 isolates, the most frequently identified pathogens were gram negative organisms (80.8%), mostly residing in the intestinal tract, leading by E. coli. Other studies also showed that gram-negative bacteria were the common cause of biliary tract infections, leading by E. coli with the rate from 30 to 44.4%\(^1\,^2\,^9\). However, research of Rupp in Germany showed that gram-positive bacteria, led by Enterococci, were the cause of biliary tract infections\(^7\). Some studies had also found that the rate of biliary tract infection caused by gram-negative bacteria tends to decrease slightly and gram-positive bacteria tends to increase slightly\(^9\). This result may be due to the choice of empiric antibiotic therapy, which mainly covered gram negative bacteria. In addition, previous studies have found biliary tract infection to be infected in 9 - 42% of patients who underwent elective laparoscopic cholecystectomy, the causative agent was P. aeruginosa\(^2\). In our study, P. aeruginosa was the 4th leading agent (7.9%). Other studies also reported that P. aeruginosa was one of the 5 common pathogens causing biliary tract infection with the rate varied from 2 to 9%\(^2\,^6\,^9\). In particular, we found that Aeromonas was the 5th leading agent (5.6%), while many studies recorded a low rate of biliary tract infections caused by Aeromonas\(^8\). This may be due to differences in the microbiological characteristics all over the world.

The study also showed that 21.7% of the bile samples were infected with polymicrobial. Kruis T. et al (2020) reported 69% biliary tract infections caused by polymicrobial\(^6\), while this rate in Ballai’s study (2019) was 19.26%\(^2\).

Therefore, it requiresthe use of broad-spectrum antibiotics that can cover both gram negative and gram positive organism and possibly anaerobes. Ballai’s study (2019) reported 35.75% of anaerobic cultured bile samples showed positive results, mainly Bacteroides fragilis\(^2\). Our study had the limitations that we didn’t perform the isolation culture of absolutely anaerobic bacteria.

Antimicrobial resistance pattern

Beta-lactams were the empiric treatment before the culture results were available. Of the Enterobacterals that often caused biliary tract infections, the resistance rate to beta-lactams agents varied from species. The prevalence of ESBL producing E. coli were 42.2%, most of these strains were often cross-resistant with other antibiotic groups such as fluoroquinolones, aminoglycosides. However, E. coli were quite susceptible to antibiotics active against ESBL-producing strains such as beta-lactam combination agents (cefoperazole/sulbactam, piperacillin/tazobactam) and carbapenems, the resistance rate to these agents were only less than 20%. Meanwhile, K. pneumoniae had a quite high resistance rate to beta-lactam antibiotics, especially the carbapenems (over 45%). Carbapenem resistant strains of K. pneumoniae were often due to the carbapenemase producing, which were emerging as a dangerous pathogens, because they can resistance to many antibiotics (including colistin), make difficult in treatment and can transmit resistance genes to others. Ballai’ et al also reported carbapenem resistance rate of K. pneumoniae in biliary tract infection were over 45%\(^2\) In P. aeruginosa, the resistance rate to antipseudomonal beta-lactams varied from 25 to 45.8%, in which imipenem had highest resistant rate (45.8%) and lowest resistant rate to ceftazidime (25%). We founded that the rate of resistance to beta-lactam agents in gram-negative
bacteria in biliary tract infection was different, so the empiric treatment antibiotics should be based on the disease, the underlying condition and the causative agent. The resistance rates of empiric antibiotics should not exceed 20%, and although *P. aeruginosa* is one of the common pathogens causing biliary tract infections, it is not necessary to use antipseudomonal activity antibiotics until available evidence of microbiological culture results, except in patients with multiple high-risk factors or healthcare-associated infections.

Fluoroquinolones are also commonly used antibiotics for inpatients or outpatients. In recent years, in Vietnam and around the world, the resistant rate of this antibiotic tends to increase, especially in the *Enterobacterals* 

The resistance rate to other antibiotics such as ciprofloxacin, high-level gentamicin and high-level streptomycin were relatively low (at 20%, 21.7% and 24.5% respectively). So combination therapy can be used when a biliary tract infection caused by *Enterococcus* spp. is suspected.

**CONCLUSION**

In biliary tract infections, about one-third cases had negative culture results and about one-fifth cases had polymicrobial infection. The common pathogens in biliary tract infections were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* spp. and *Pseudomonas aeruginosa*. The resistant rate to commonly used antibiotics in treatment was quite high. The fluoroquinolone agents should not be used as the initial treatment antibiotic due to the high resistance rate.

**REFERENCES**

