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.

Subbjects and methods: NA retrospective study was conducted from January 2021 to Demcember 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 mininum 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.[2,8]. However, the microbiological pattern of the infection can change over the years and multidrug-resistant bacteria appear increasingly[2], thus understanding the causative agent and the situation of antibiotic resistance in biliarytract infections is very important, in order to choose appropriate empiric antibiotics to increase

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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% CO_2 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 1. Microbiological culture results

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

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

Table 2. Distribution of organisms isolated from bile specimens

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

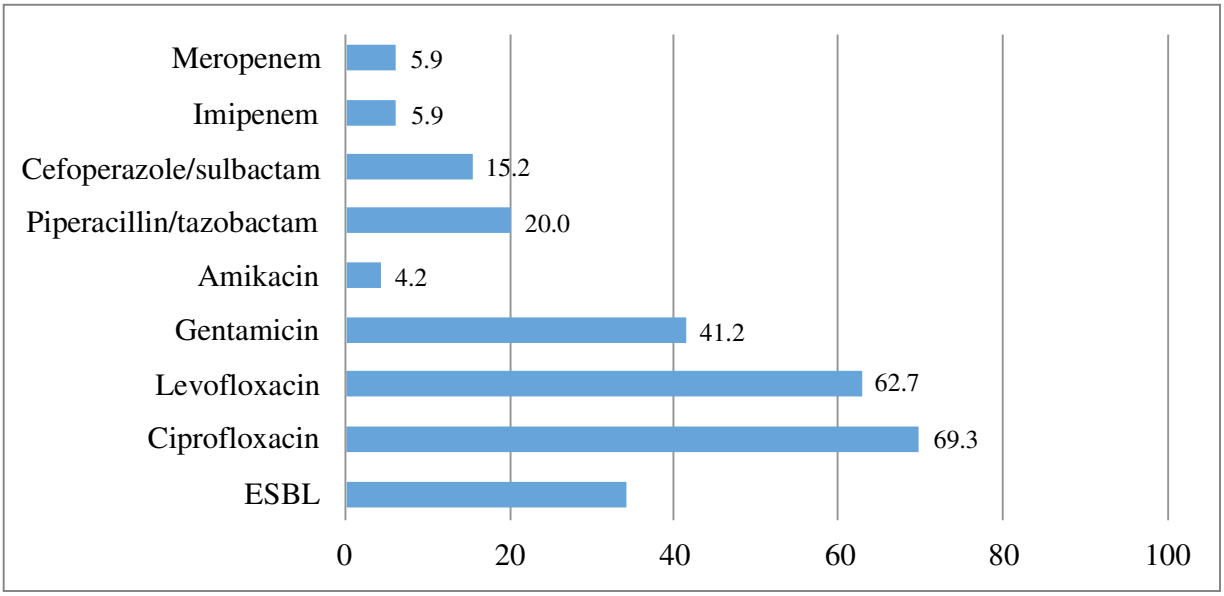

Figure 1. Antimicrobial resistant rate of *E. coli* (n = 102 isolates)

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

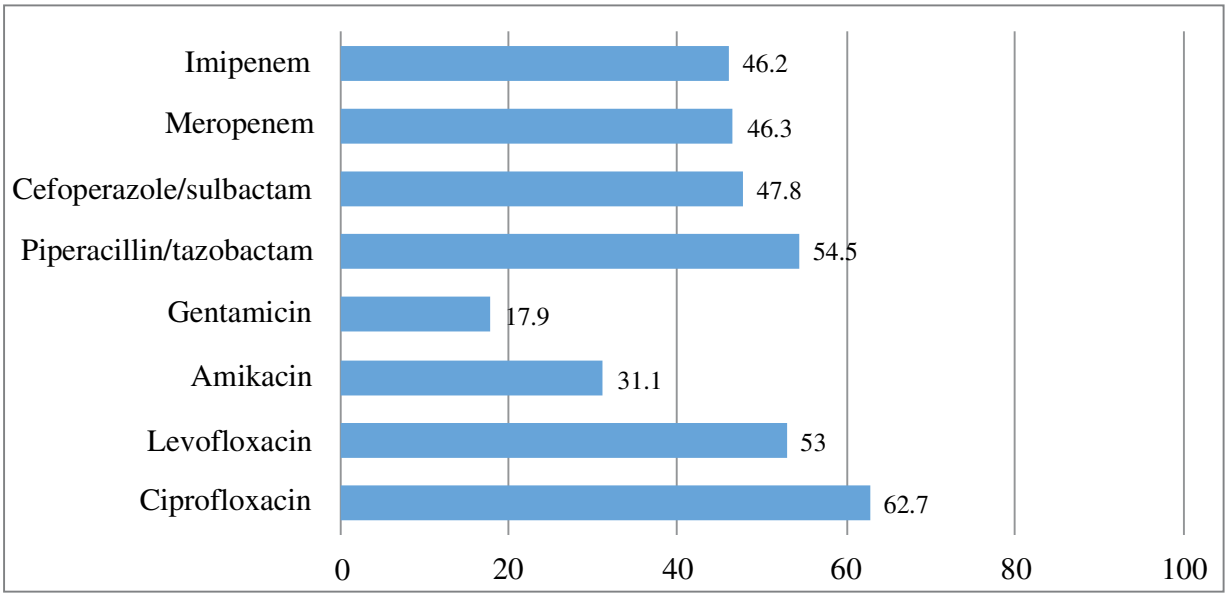

Figure 2. Antimicrobial resistant rate of *K. pneumoniae* (n = 67 isolates)

The rate of resistance to vancomycin in Enterococcus spp. were 8.3%. The resistance rate to other antibiotics such as ciprofloxacin, high-levelgentamicin (HLGR) and high-level streptomycin (HLSR) were over 20%.

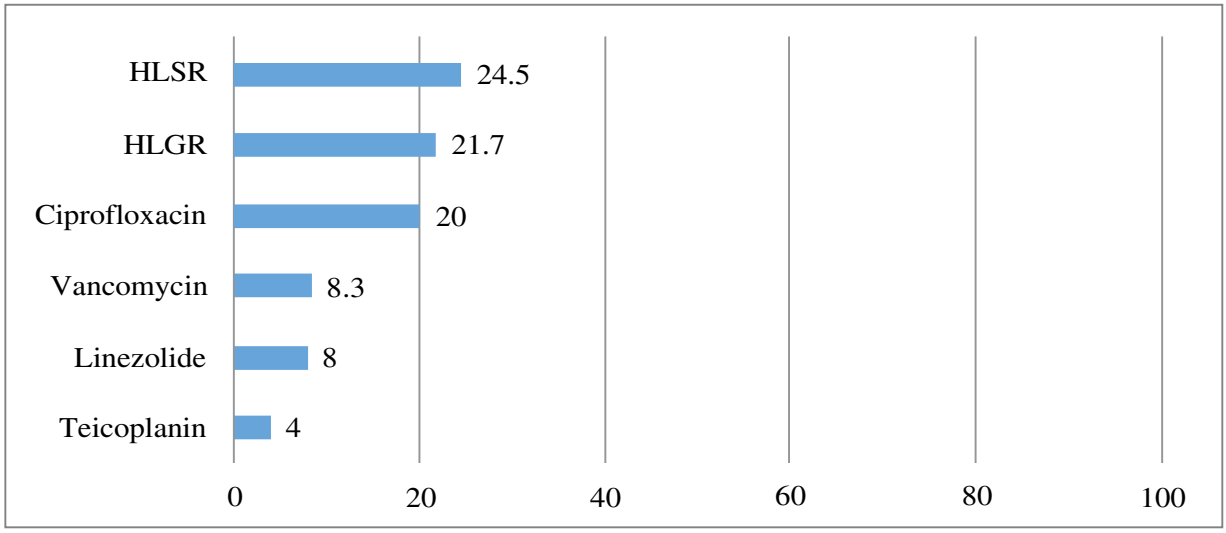

Figure 3. Antimicrobial resistant rate of *Enterococcus* spp. (n = 50 isolates)

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.

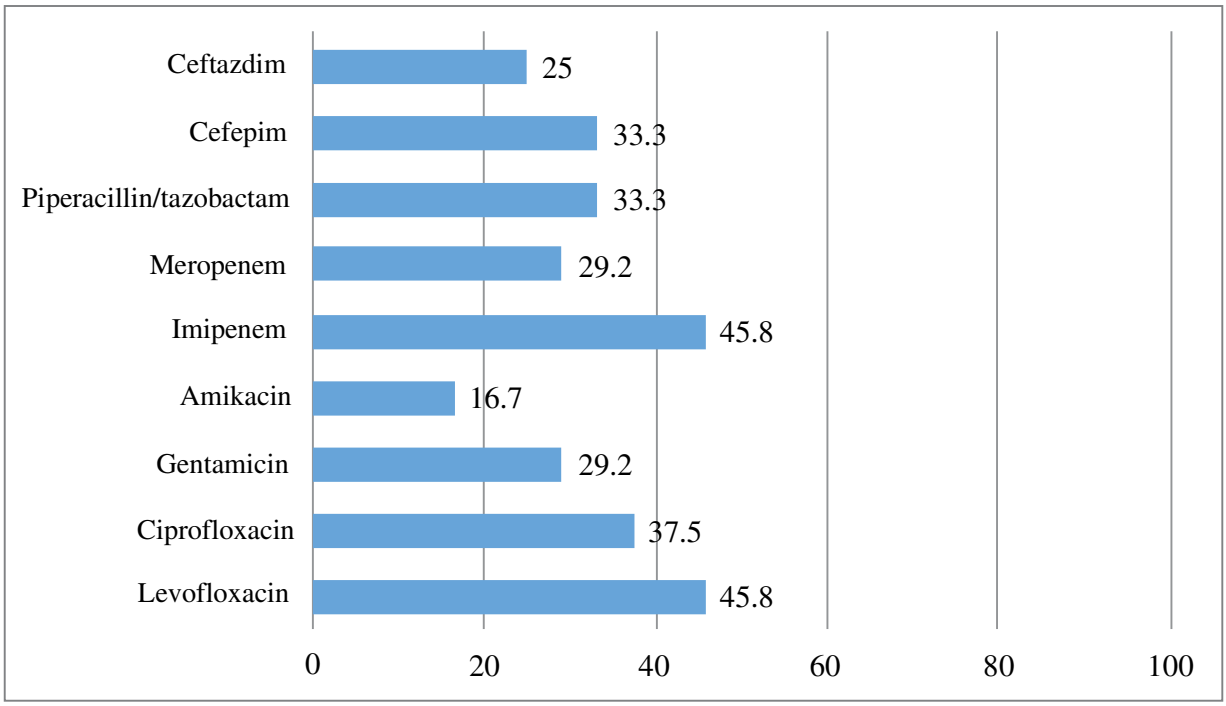

Figure 4. Antimicrobial resistant rate of *P. aeruginosa* (n = 24 isolates)

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 ourstudy, *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*[4]. 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 causedby 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 resistancepattern

Beta-lactams were the empiric treatment before the culture results were available. Of the Enterobacterals that often caused biliary tract infections, the resistancerate 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 rateto these agentswere only less than 20%. Meanwhile, *K. pneumoniae* had a quite high resistancerate 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 infectionwas different, so the empiric treatment antibiotics should be based on the disease, the underlying conditionand 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[5].

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*[2]. In ourstudy, the resistance rate of fluoroquinolone antibiotics in *E. coli* and *K. pneumoniae* were over 50%. In *P. aeruginosa*, this rate were lower, but also at 37.5 - 45.8%. Therefore, fluoroquinolonesshould only be used when the patient have susceptibility testing result or are allergic to beta-lactam agents[5].

Enterococcus spp. was one of the important causes of biliary tract infection, especially in critically ill patients with comorbidities, and vancomycin should be used until available culture results[5]. In our study, the rate of biliary tract infection caused by *Enterococcus* spp.was 16.6% (ratio of *E. faecalis* and *E. faecium* is 1.8:1). The vancomycin resistance rate was 8.3% (mainly *E. faecium*), in these cases linezolide can be used, because all of them were still susceptible to linezolide. Many studies reported that the prevalence of vancomycin resistance in *Enterococcus* spp. is increasing, especially in *E. faecium*, even *E. faecium* have appeared resistant to vancomycin, teicoplanin and linezolide, making difficult in treatment. The resistance rate toother antibiotics such as ciprofloxacin, high - level gentamicin and high - level streptomycim 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.

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