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Study of Bacteriological Profile and Antibiogram of Bile in Patients with and Without Gall-Stones

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A B S T R A C T

This is a prospective study done in 2 tertiary care hospitals and a super-specialty hospital attached to a medical college. 50 patients undergoing cholecystectomy were included in the study. Patients with even a single macroscopic gall-stone anywhere in the biliary tract were included in Category-A and the rest in Category-B. The aim of the project was to find out the difference, if any, in the bacteriological profile and antibiogram of bile in patients in these two categories which cover most of the causes of cholecystitis, and help in better management of these patients especially in places where facilities to drain and culture bile are not available. The bile collected was immediately taken to microbiology department and inoculated into Selenite-F broth and BHI broth and incubated at 37ºC. Cultures were performed on sheep blood agar and MacConkey agar. Bacterial identification was done based on standard biochemical reactions and antibiogram performed as per CLSI guidelines 2014. 68% of Category-A patients had positive culture in contrast to 80% in Category-B. Category-A was dominated by females in their 5th and 6th decade while Category-B had males in their 6th and 7th decade. *Klebsiella* and *E.coli* were equally frequent in Category-A patients while the Category-B was dominated by *E.coli*. *Enterococcus* was third most common (13.5% of all positive cases). *Pseudomonas*, Gram negative non fermenters (GNNF) covered the rest. Cholangiocarcinoma was more prone to bacterial invasion than peri-ampullary carcinoma. Only around 8.1% of the cases are associated with poly-microbial infection. There seemed to be some association between biliary stricture and GNNF. Antibiogram was almost similar in the two categories with overall in vitro sensitivity being maximum with Amikacin (81%), followed by Piperacillin-tazobactum (77%), and Gentamicin (64%). Cefepime (13%), Ampicillin (22%), Amoxicillin-Clavulanate (23%), Cefotaxime (27%), Ciprofloxacin (29%) showed poor sensitivity.

Introduction

Once considered the disease of West and affluent, today gall-stones and other gall-bladder diseases are establishing themselves in developing countries also[1]. Infection of gall-bladder and its complications is an important cause of morbidity and mortality in these patients[2]. The pathogenesis of bile infection is incompletely understood, with the prevailing theories not fully explaining all the observations[3].

Acute cholangitis refers to inflammation of the biliary ductal system from bacterial or non-bacterial infection, usually in the setting of biliary obstruction[4]. The main factors in the pathogenesis of the acute cholangitis are biliary tract obstruction, elevated intra-luminal pressure and infection of bile[5]. The bile is normally sterile but in the presence of obstruction, the chances of cholangitis increases[2].

The micro-organisms are proposed to enter the biliary tree from duodenum, through portal vein, etc., though with varying levels of certainity[2]. There are some studies on microbiological profile of bile in gall bladder diseases which have shown that the risk for infection development varies with the primary condition[6,7,8]. Thus, different gall bladder diseases differ in probability of developing infection and also the micro-organisms involved in it, depending on the route of entry of micro-organisms. There are only a few, if any, microbiological studies on various gall bladder diseases individually, excepting gall stones. Thus there is a need of data for infections secondary to other causes, which might help the doctor use a more appropriate antibiotic specific to patient's condition. But since infection is usually due to gall-stones – 60-80% of the times[3,9], and that the other causes like obstructing primary tumours, gall-bladder carcinoma, benign strictures, primary stenosing cholangitis, parasites and hemobilia[2], altogether constitute only 20-30% of the cases, microbiological study of individual problems is difficult, and less accurate.

This analysis inspired us to attempt on finding the common bacterial flora in patients with other gall-bladder conditions. We grouped the patients into two, one with gall-stones and one without gall-stones but with some other underlying gall-bladder conditions, which would include all other risk factors for infection mentioned above. We found the common microbial flora in the two groups and the antibiotic susceptibility patterns, and thus helping in judicious use of antibiotics.

Materials and Methods

This prospective study on bile was carried out in two tertiary care hospitals and a super-specialty hospital attached to Bangalore Medical College and Research Institute, over a period of two months in 2017. Total of 50 patients diagnosed with cholecystitis and undergoing cholecystectomy were included in this study which comprised of 25 patients having gall-stones, labeled as Category-A and 25 patients having no gall-stones but other pathology which was responsible for their cholecystitis, labeled as Category-B.

5ml of bile sample was collected in a sterile container with permission of the operating surgeon using standard precautions, and was appropriately labelled to include the patient's name and the presence or absence of gall-stones, to avoid mixing of the data. It was immediately taken to the Microbiology department and 1ml of the bile was inoculated into BHI broth and another 1ml of bile was inoculated into Selenite F broth. Then, direct streaking of bile on 5% sheep blood agar and MacConkey agar medium was done and maintained at 37^{o}C overnight.

After 6 hrs of incubation in Selenite F broth, sub-culture on blood agar was done. And after overnight incubation in BHI broth, subcultures were done in both MacConkey and Blood agar culture media.

The isolates were identified by standard biochemical reactions[10] and were subjected to antibiotic susceptibility testing as per CLSI guidelines 2014[11]by modified Kirby-Bauer disk diffusion technique on Muller Hinton agar.

Results and Discussion

Category-A

Out of 25 patients belonging to this group, 19 (76%) were females and 6(24%) were males (Table 1). The average age was 51 years (females - 47 years; males - 58 years). 68% of the cases were positive for bacterial growth. *E. coli* and *Klebsiella* were the most frequently isolated bacteria accounting for 41.17% and 35.29% of the positive cases respectively. Together they were responsible for 52% of all the cases or 76.47% of the culture positive cases. Pseudomonas, other Gram negative non fermenters and *Enterococcus* covered the rest of the positive cases. Poly-microbial growth was less frequent (4%). Resistance was common among bacteria for Ampicillin (14%), Amoxicillin-Clavulanate (9%), and Cefepime (14%). Bacteria were sensitive to Amikacin (81%), Piperacillin-Tazobactum (78%) and Gentamicin (69%). Co-trimoxazole (45%) and Ertapenem (50%) also showed relatively favorable sensitivity pattern.

Two cultures yielded Pseudomonas which were multi-drug resistant. One was resistant to Gentamicin, Ciprofloxacin, Amikacin, Imipenem, Cefepime, Aztreonam, Ceftazidime, Ticarcillin and Piperacillin, and sensitive only to Polymyxin-B. The other case where pseudomonas was isolated was resistant to Cefotaxime, Amoxicillin-Clavulanate, Piperacillin-Tazobactum, Cefepime, Aztreonam, Ceftazidime, and Piperacillin, but was sensitive to Imipenem.

Category-B

Out of 25 patients belonging to this group, 8 (32%) were females and 17 (68%) were males (Table 1). The average age was 57 years (females - 49 years; males - 60 years). This category is composed of peri-ampullary carcinoma, Cholangiocarcinoma, biliary stricture, carcinoma of gall-bladder, mass in the head of pancreas. The age-wise distribution of these cases is depicted in Figure 2. 80% of the cases were positive for bacterial growth. Case-wise distribution of micro organisms and antibiotic sensitivity is given in Table 2. *E. coli* was very much more frequent than *Klebsiella* in the ratio of 3.25:1 and were together responsible for 85% of the positive cases. Gram negative non fermenters and *Enterococcus* covered the rest of the positive cases. Poly-microbial growth was not infrequent (8%).Resistance was common among organisms for Ampicillin (27%), Cefotaxime (11%), Amoxicillin-Clavulanate (31%) and Cefepime (13%). The organisms were sensitive to Amikacin (80%), Piperacillin-Tazobactum (76%), and Gentamicin (60%).

The comparison of bacterial isolation pattern and antibiotic susceptibility pattern in Category A and B is depicted in Table 3 and Figure 1 respectively.

Total of 50 patients were included in this study which comprised of 25 patients having a gall-stones and 25 patients having no gall-stone but having other pathology which was responsible for their cholecystitis. Those with gall-stones were labelled category-A patients and the others as category-B patients. The median age of patients was 54 years. Male:Female ratio was 0.85:1.

Patients belonging to category A had a median age of 51 years with female to male ratio being 3.16:1. Thus, gall-stone disease is predominantly a disease of females in 5^{th} and 6^{th} decade of their lives. The culture positivity was seen in 68% of the cases, slightly higher from other studies probably because ours is a tertiary care hospital and also because we were able to inoculate and streak the sample within an hour. Manan *et al.*,[3] (2014) had an

isolation rate of 58.73% while Capoor *et al.,*[6](2008) had an isolation rate of 32%. Only one case showed a polymicrobial growth. *E. coli* and *Klebsiella* dominated the scene in this category with each of them almost equally frequent covering 76.47% of all the positive cultures. This finding is in consistency with findings of Capoor *et al.,*[6](2008) and Manan *et al.,*[3](2014). Two cases gave growth of Pseudomonas which was resistant to almost all the drugs with only a drug or two being spared. The organism showed significant resistance to ampicillin with only 14% of the cultures being sensitive to it. This was also noted in the studies of others. Amoxicillin-Clavulanate also showed very low efficacy, with only 9% of the cultures being sensitive to it. The sensitivity rates with Cefepime and Ciprofloxacin were also very low, with only 14% and 22% of the cases yielding sensitive organism respectively. Amikacin (81%) and Piperacillin-Tazobactum (78%) were the most effective antibiotics for this category of patients. Gentamicin (69%) also showed favorable sensitivity patterns. This is in contrast to the findings of Manan *et al.,*[3] (2014) and Shenoy *et al.,*[7](2014) who found that most bacteria were sensitive to cephalosporins, indicating the regional variation of resistance pattern. Even Capoor *et al.,*[6] (2008) found Piperacillin-Tazobactum very sensitive. According to Wu *et al.,*[12], the stone may be acting as a source of infection with the bacteria trapped in the centre. In such cases, it becomes imperative to remove the stones prior to antibiotic therapy.

Patients belonging to Category-B had a median age of 57 years with female to male ratio being 0.47:1. Thus aculculous cholecystitis requiring surgical intervention is usually a disease of males in the 6th and 7th decade of their lives. This category is composed of peri-ampullary carcinoma, Cholangiocarcinoma, biliary stricture, carcinoma of gall-bladder, mass in the head of pancreas, etc. All these cases are associated with some form of biliary obstruction and biliary stasis probably indicating biliary stasis as the main cause for bacterial invasion. Based on their study findings, Yusoff *et al.,*[2] (2003)also concluded that biliary stasis was responsible for infection. The culture positivity was noted in 80% of the cases with 2 cases showing multi-bacillary growth representing 8% of the total number of cases or 10% of the positive cases. This is again expectable based on the consideration that these pathologies were responsible for a long period of obstruction of biliary tract. *E. coli* alone dominated the scene with *E. coli* to *Klebsiella* isolation frequency being 3.25:1 with *E. coli* alone contributing to 65% of the positive cases. *Klebsiella* accounts for 20% of the positive cases. *Enterococcus* was responsible for 15% of the positive cases. Also, two cases yielded Gram negative non-fermenters.

The commonest pathology in this group was Peri-ampullary carcinoma accounting for 10 cases or 40% of the cases in this group. It showed male preponderance (M:F=7:3).70% of the cases yielded positive culture but not a single case was associated with multi-bacillary growth. *E. coli* dominated the scene with *E. coli* representing 86% of the positive cases. *Klebsiella* represented 14% of the positive cultures. So, compared to the 80% isolation rate as a whole in this category, it was less frequently associated with bacterial isolation.

On the other hand, cholangiocarcinoma which represented 24% of cases in this category, there was 100% bacterial isolation rate with 33.3% cases showing multi-bacillary growth. So, it is relatively more prone to develop bacterial infection. This probably is because of the early obstruction of the biliary tract as compared to peri-ampullary carcinoma and

hence again indicating that obstruction is the main cause for bacterial invasion. 100% of the cases showed presence of *E. coli*. One case additionally had *Klebsiella*, and the other had *Enterococcus*. Male to female ratio was 5:1.

Gall-bladder carcinoma accounted for 3 cases in this category. All the three were females in their 6th decade. This was associated with culture positivity in all the 3 cases. Two cases yielded *Klebsiella* and one case yielded *E. coli*. Biliary stricture as mentioned had Gram negative non fermenter in both the cases.

One case of metastatic pancreatic head mass yielded *Enterococcus*. Another case of non-neoplastic head mass yielded *E. coli*. One case of carcinoma of head of pancreas yielded no growth.

We had also got a case of Caroli's disease which was diagnosed on surgical table which yielded no growth.

If it is obstruction that is responsible for bacteria to invade biliary tree, then there should have been similar isolation pattern with and without gall-stones.

This cannot be used to explain why *E. coli* is thrice as common as *Klebsiella* in patients without gall-stones.

If we think that prolonged obstruction causes *E. coli* to take over *Klebsiella* then similar finding would have been seen in Category-A patients. Those who had prolonged obstruction or long history of symptoms would show selectively *E. coli*. But we didn't find any such selection. It is possible that malignancy induced immune-depression may be responsible for selective *E. coli* dominance.

Resistance was common among bacteria for Ampicillin (27%), Cefotaxime (11%), Amoxicillin-Clavulanate (31%) and Cefepime (13%). Bacteria were sensitive to Amikacin (80%), Piperacillin-Tazobactum (76%), and Gentamicin (60%).

There was not much difference in the antibiotic sensitivity pattern of the isolated bacteria from the two cases as a whole as is evident from Figure 1. Morever, there was no significant difference in antibiotic sensitivity pattern of the *E. coli* (Fig. 2) and *Klebsiella* (Fig. 3) separately from the two categories.

The antibiotic sensitivity patterns of the bacteria isolated from cholangiocarcinoma and peri-ampullary carcinomais shown in Figure 4.

Putting it all together, 74% of the cases were associated with positive culture. Shenoy *et al.*,[7] (2014) had an isolation rate of 54% while Claesson B *et al.*,[8](1984) had an isolation rate of 72%.54% of the positive cases gave *E. coli*. 27% of the cases were positive for *Klebsiella*.

So, *E. coli* was overall twice as frequent as *Klebsiella*. Other bacteria included *Enterococcus* (13.5%), Gram negative non-fermenter (8.1%), *Pseudomonas* (5.4%). Polymicrobial growth was seen in 8.1% of the cases.

This in contrast with very high polymicrobial infection rate of 69.5% in study of Sahu *et al.*,[9]and31.5% in study of Shenoy *et al.*,[7](2014).

Overall, Amikacin (81%), Piperacillin-Tazobactum (77%), and Gentamicin(64%) showed favourable sensitivity. Cefepime (13%), Ampicillin (22%), Amoxicillin-Clavulanate (23%), Cefotaxime (27%), Ciprofloxacin (29%) showed poor sensitivity (Fig. 5 and 6). This was in consistency with M K Sahu*et al.*,[9] who also found significant resistance of bacteria to third generation Cephalosporins and Ciprofloxacin.

Table.1 Age-wise distribution of the cases in Category A and B

Age range of the patients	Category-A		Category-B	
	Male	Female	Male	Female
20-30 years	0	5	0	1
31-40 years	0	3	1	1
41-50 years	2	3	1	2
51-60 years	1	2	3	3
61-70 years	3	3	7	1
71-80 years	0	1	4	0
>80 years	0	2	1	0
Total	6	19	17	8

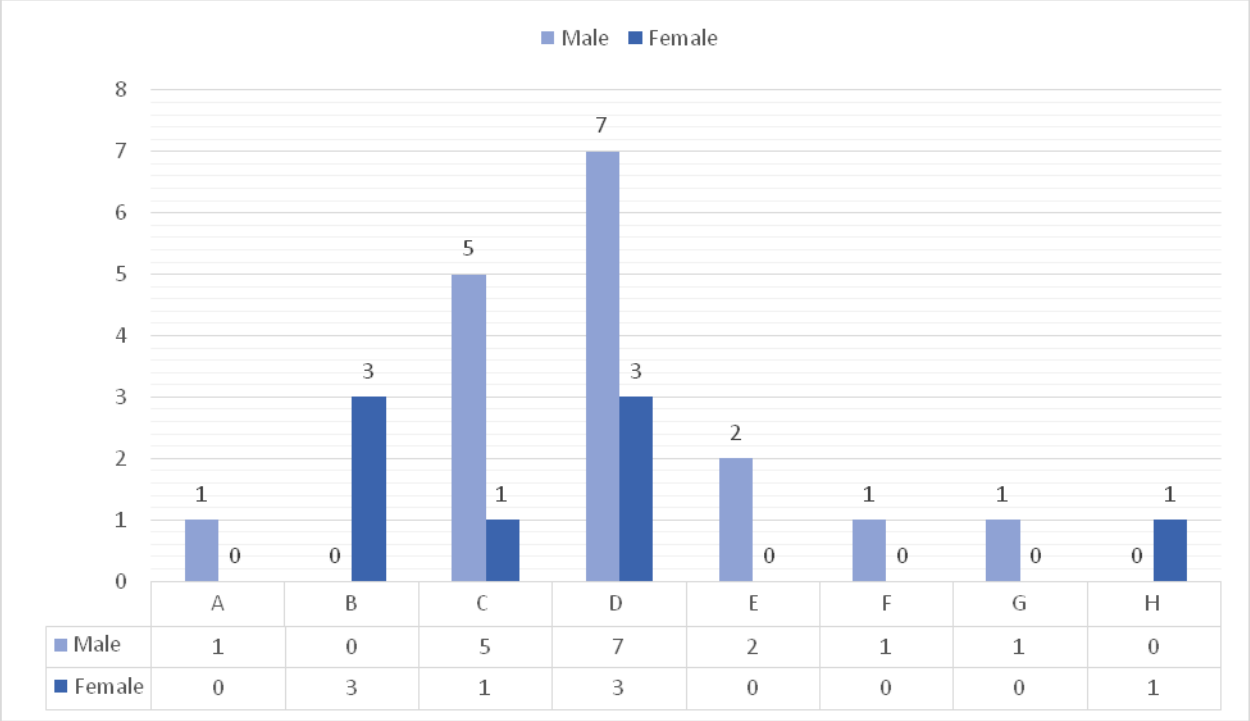
Table.2 Case-wise distribution of bacterial growth among Category – B

Pathology	Number of cases	Median age	Percentage culture positivity	Bacterial profile	Sensitive antibiotics
Carcinoma of head of pancreas	1	52	0	-	-
Carcinoma of gall-bladder	3	55	100%	*Klebsiella*- 66.6% *E. coli*- 33.3%	Amikacin Piperacillin-Tazobactum Ertapenem
Cholangiocarcinoma	6	64	100%	Multibacillary in 33.3% *E. coli*- 100% *Klebsiella*- 16.6% *Enterococcus*- 16.6%	Amikacin Piperacillin-Tazobactum Cefotaxime Gentamicin
Peri-ampullary carcinoma	10	66	70%	*E. coli*- 86% *Klebsiella*- 14%	Amikacin Piperacillin-Tazobactum Ertapenem
Billiary stricture	2	63	100%	GNNF- 100%	Gentamicin Piperacillin-tazobactum
Metastatic pancreatic head mass	1	45	100%	*Enterococcus*- 100%	Ampicillin Ciprofloxacin
Non-neoplastic pancreatic head mass	1	31	100%	*E. coli*- 100%	Ampicillin Gentamicin Sensitive to almost all drugs.
Caroli's disease	1	24	0%	-	-

Table.3 Comparison of Bacterial isolation pattern in the two categories

Bacteria	Patients with gall-stones(n=25)	Patients without gall-stones (n=25)
E. coli	7 (28%)	13 (52%)
Klebsiella	6 (24%)	4 (16%)
Pseudomonas	2 (8%)	0 (0%)
GNNF	1 (4%)	2 (8%)
Enterococcus	2 (8%)	3 (12%)
Polymicrobial growth	1 (4%)	2 (8%)
Overall positive culture	17 (68%)	20 (80%)
No growth	8 (32%)	5 (20%)

Fig.1 Gender-wise distribution of the cases in Category B



A- Carcinoma of head of pancreas.
B- Carcinoma of gall-bladder.
C- Cholangiocarcinoma
D- Peri-ampullary carcinoma
E- Biliary stricture
F- Metastatic pancreatic head mass
G- Non-neoplastic pancreatic head mass
H- Caroli's disease.

Fig.2 Antibiotic sensitivity pattern of the two categories

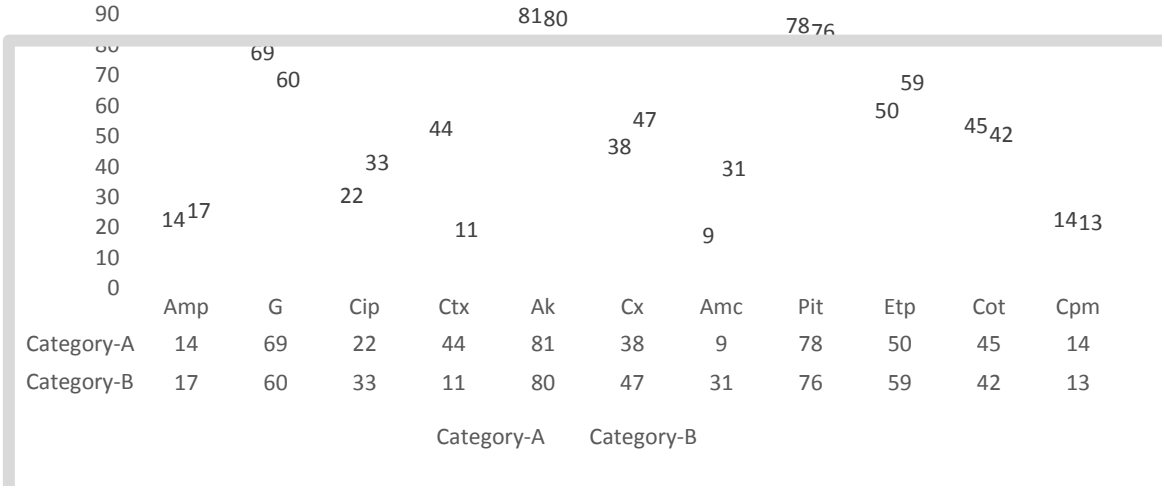


Fig.3 Comparison of antibiotic sensitivity of the *E. coli* in the two categories

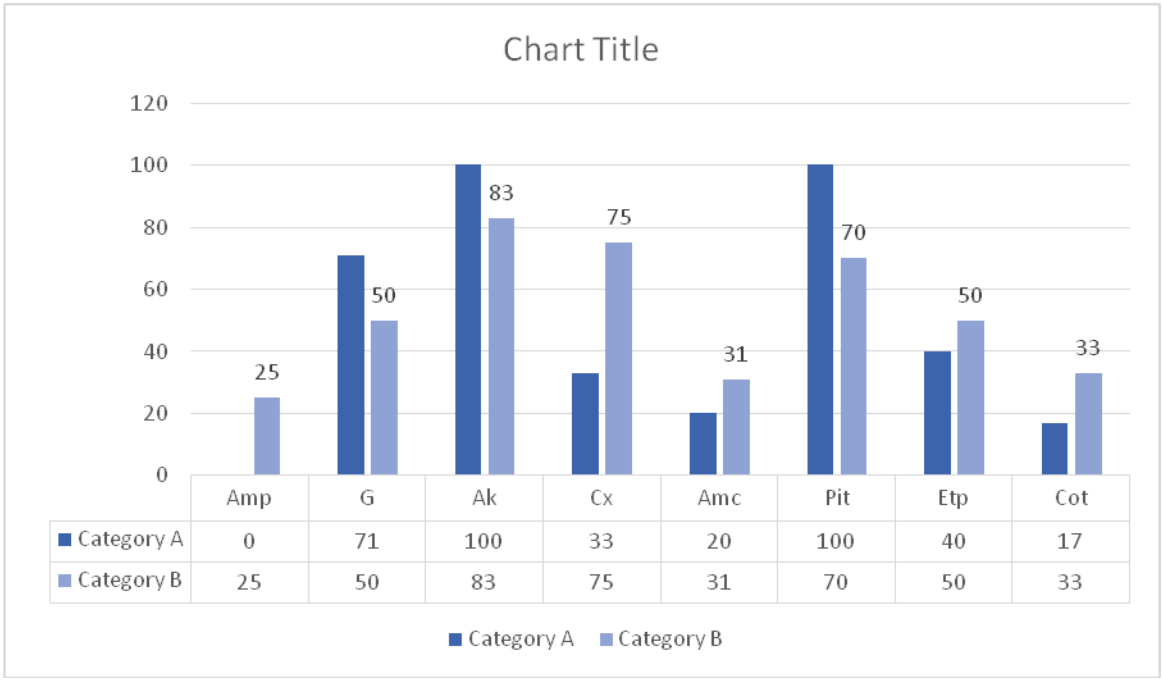


Fig.4 Comparison of antibiotic sensitivity of the *Klebsiella* in the two categories

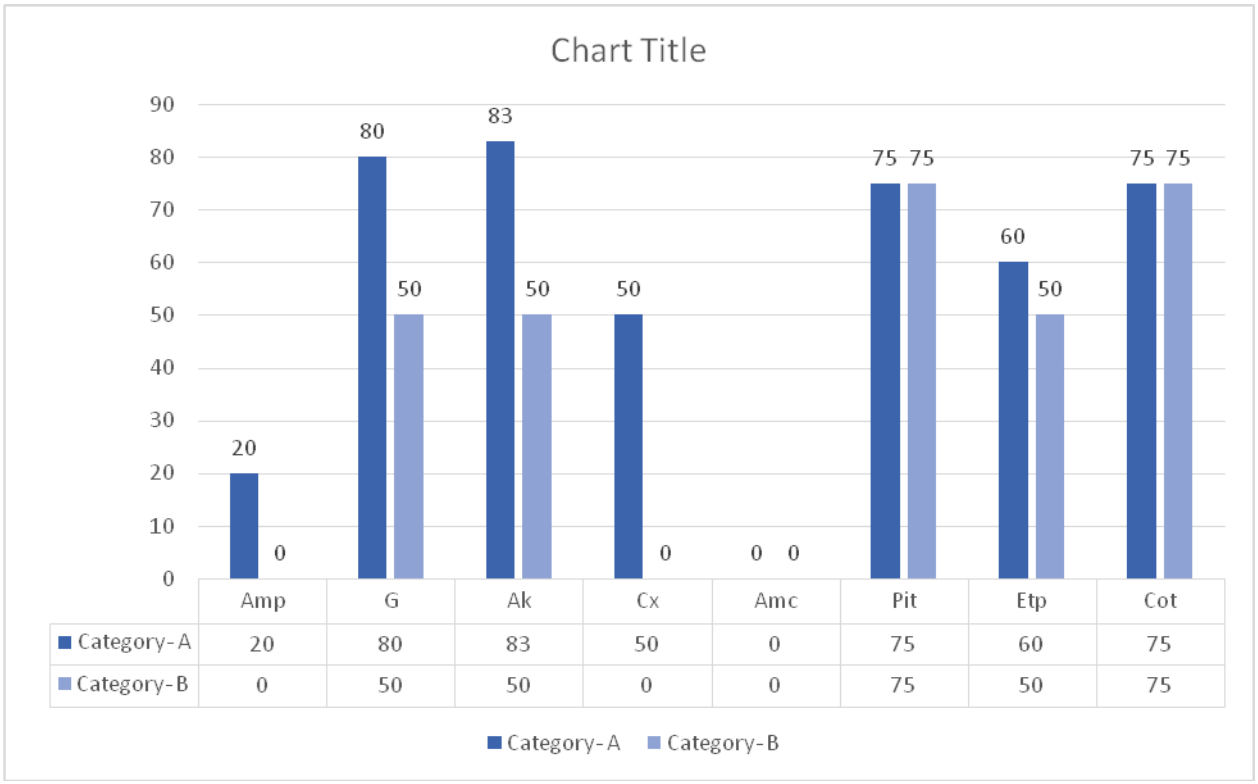


Fig.5 Comparison of antibiotic sensitivity pattern among peri-ampullary carcinoma and cholangiocarcinoma

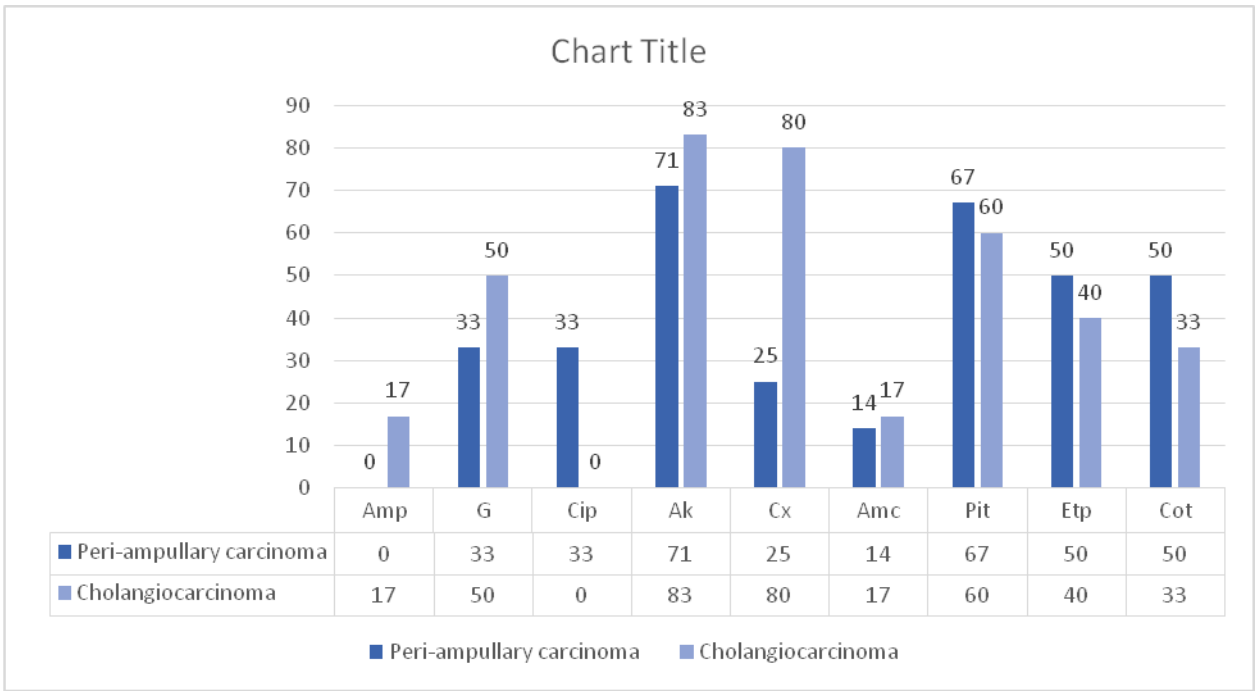
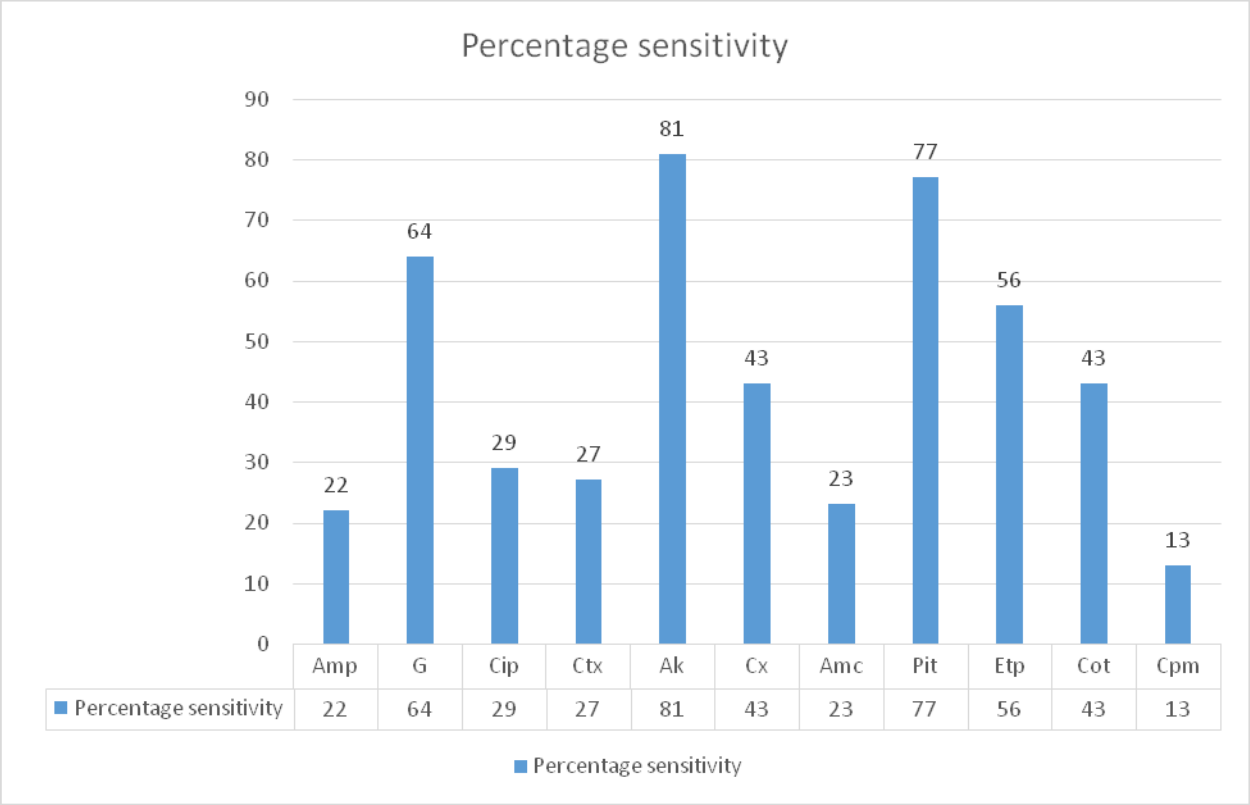


Fig.6 Overall antibiotic sensitivity pattern in gall-bladder diseases



In conclusion, 68% of the cases with gall-stones have a detectable bacterium in their bile, while 80% of the patients with aculculous cholecystitis requiring surgical intervention have some bacteria in their bile. The second group basically refers to malignancies of the hepato-biliary system and pancreas. Gall-stone disease is predominantly a disease of female in their 5th and 6th decade while malignancies of the biliary tract and pancreas are diseases of males in their 6th and 7th decade. *E. coli* is twice more common than *Klebsiella* overall. Inturn, *Klebsiella* is twice more common than *Enterococcus* overall. Patients with gall-stone have almost equal chance of *E. coli* and *Klebsiella* invasion while in the malignancies *E. coli* is thrice more commoner. Amikacin, Piperacillin-Tazobactum and Gentamicin are preferable antibiotics for cholecystitis. Cefepime, Ampicillin, Amoxicillin-Clavulanate, Ciprofloxacin, cefotaxime were usually ineffective. To tell about the association of biliary stricture with Gram negative non-fermenter would require a larger sample size.

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