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Original Article

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Antibiotics susceptibility test patterns of bacterial species isolated from the human gallbladder bile of Sudanese patients

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Abstract

Objectives This study was carried out to determine the antibiotics susceptibility test patterns of bacterial species isolated from the human gallbladder bile. These species were belonging to six different bacterial genera four are Gram- negative and two are Gram - positive. ***Methods*** A total of 100 bile specimens from 100 patients (88 females and 12 males), were examined in this study. The mean age of the patients was (46.22) years, with a range value from 23-86 years. The antibiotics susceptibility tests were done to all bacterial isolates using Kirby - Baur disc diffusion method. ***Results*** Most bacterial isolates were found sensitive to many antibiotics. The results of sensitivity tests showed that there is resistance observed in some species including *Pseudomonas* spp., *Salmonella* spp., and *Citrobacter freundii*. Enterobacteria are the commonest bacteria examined in our study. Interestingly, all resistant strains isolated belonged mostly to bacterial species known to cause nosocomial infections.

Keywords: susceptibility tests; bile specimen's; bacterial isolates; infected gallbladders; resistant strains

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Introduction

Morbidity and mortality due to septic complications caused by pathogenic bacteria in patients undergoing elective or emergency cholecystectomy are one of the main problems to those who are working in the health care services. Bacterial resistance to most currently used antibiotics, encountered in open or laparoscopic cholecystectomy, drew the attention of surgeons, gastroenterologists, and a microbiologist working in this field.

The importance of performing antibiotics susceptibility test to bacterial species isolated from gallbladder bile lies in the fact that appropriate antibiotic can be administered in the event of positive culture to prevent complication like septicaemia[1]. In recent years, development of resistant strains and lower susceptibility of anaerobic Gram – negative rods have made antibiotics therapy difficult and complicated [2]. However, Rerknimitr *et al.* [3] observed an excellent responses to quiolones against Gram- negative bacteria isolated from human gallbladder bile, as well as, there were highly responses to vancomycin against Gram- positive bile isolates.

Bacterial infections of the gallbladder, particularly in female subjects, are increasing nowadays. Survey studies to determine antibiotics susceptibility patterns of bacterial species involved in such infections were performed in some countries of the world [4,5,6], nevertheless, such studies have not been performed in the Sudan.

Epidemiological data for bacterial infections of the gallbladder in the Sudanese population is still lacking. The purpose of this research study is therefore, to throw light on the patterns of antibiotics sensitivity of bacteria isolated from gallbladder bile of Sudanese population, using a variety of approaches and a number of techniques.

AL-Harbi *et al.* [7] in Saudi Arabia, examined the Susceptibility of some bacterial species (*E. coli* and *P. aeruginosa*) isolated from gallbladder bile to antibiotics. They were found that all isolates were sensitive to amikacin and imipenem. The sensitivity of the nine strains of *E. coli* was: ampicillin 44.4% (4/9), cefuroxime 88.8% (8/9); all were sensitive to ceftriaxone, ceftazidime, gentamicin, amikacin and imipenem. The three strains of *P. aeruginosa* were sensitive to ceftriaxone, ceftazidime, gentamicin, amikacin and imipenem.

Material and Methods

The 100 bile specimens studied were collected from three different operating theatres including; Omdurman Teaching Hospital, Ibn Sena Hospital and Sudan Private Clinic. Six bacterial species were recognized in the present study, four of them are Gram- negative bacteria and the two are Gram-positive species.

Antibiotics Sensitivity Testing for Isolated Bacteria

The Kirby–Baur Disk diffusion technique was used, as described by Cheesbrough [8].

Preparation of Sensitivity Test Medium

Petri dishes each containing 20 ml of Muller and Hinton Agar were sterilized and used as the sensitivity test medium. The media were allowed first to settle and solidify and then plates were dried for 5 minutes in an incubator at 37℃ to remove excess moisture from the medium surface and the cover plate.

Preparation of Inocula and Inoculation

The inocula were prepared by emulsifying 4-8 colonies of the test organism in small amount of sterile Nutrient Broth (3ml). In order to prevent further growth, the inocula were diluted and standardized and were not allowed to stand more than 15 to 20 minutes before inoculation onto the sensitivity test plates. A sterile cotton swab was dipped into the diluted culture or suspension and then rotated. The swab was then pressed against the side of the tube to remove excess fluid and then stroke across the medium [8].

Application of Sensitivity Discs

Multi discs, each containing 8 different antibiotics (Axiom laboratories, New Delhi, India) and two single disks (Difco Laboratories, Detroit, U. S .A) were used. The discs were removed from their containers with the help of sterile forceps and were then carefully placed on the surface of the inoculated medium. They were pressed gently with the forceps to make complete contact with the medium. After incubation for 24 hours at 37 ℃ aerobically, the cultures were examined for zone of inhibition of bacterial growth around the respective disks.

Zones Readings

The diameter of zone of inhibition for each antibiotic was measured to the nearest millimeter (mm) using a measuring ruler (Appendix-3). The diameter of the zone was measured and compared with the zone diameter shown in the table of (NCCLS) [9] to determine whether the tested organism is resistant, intermediate, or susceptible to the tested antibiotics as explained by Koneman *et al.*[10].

Results

Antibiotics sensitivity testing to bacterial species isolated from bile

Bacteria were isolated from 40 out of 100 bile specimens cultured with an overall incidence of 40%.Three Salmonellae were isolated form bile of acalculous gallbladders. The most prevalent bacteria isolated were *E.coli*, which was isolated from 24 out of the 100 bile specimens. *Staphylococcus aureus* and Pseudomonas were less frequently isolated from bile specimens showing frequencies of 4 (4%) each. Other species including *Salmonella spp.* and *Listeria monocytogenes* were isolated in 3 occasions. The less frequently bacterial isolates observed in the present study is *Citrobacter freundii* which were isolated in two cases only (2%) from the total bacterial isolates (Table 1).

The susceptibility of the various bacterial species and strains to antibiotics was tested, using the disc diffusion method, as previously described by Cheesbrough [8]. Ten antibiotics were used to test the sensitivity of those bile bacterial strains (Table 2 and 3).

The antibiotics included in this study were Amoxicillin/Clavulanic acid, Cefuroxime, Tetracycline, Ceftizoxime, Ofloxain, Gentamycin, Ciprofloxacin, Piperacillin, Cefotaxime and Ampicillin / Sulbactam. Two of them were presented in a combined form. All bile bacterial isolates were tested against the ten antibiotics. Results were interpreted by measuring the diameters of inhibitory zones, according to NCCLS. The result was expressed as either the bacterial strain was sensitive (S), resistant (R) or intermediately sensitive (Int) to the test antibiotic.

The number of bacterial species sensitive or resistant to an antibiotic were recorded and expressed as a percentage of the total number of the same species isolated. Table 2 & 3 shows results of antibiotics sensitivity test for Gram-positive and Gram-negative bacteria species, *respectively*. All *Staphylococcus aureus* strain isolated in this study were sensitive to Amoxicillin / Clavulanicacid, Cefuroxime, Ceftizoxime and to Ciprofloxacin. The same strains were resistant to Tetracycline. Some of the strains were also intermediately sensitive to Gentamycin, Piperacillin, and Cefotaxime and to Ampicillin/ Sulbactam (Table2).

Table 1: Frequencies and percentages of bacteria isolated from positive gallbladder's bile cultures

Bacteria isolated	Frequency	percentage %
No growth	60	60
Escherichia coli	24	24
Staphylococcus aureus	4	4
Pseudomonas spp.	4	4
Salmonella spp.	3	3
Listeria monocytogenes	3	3
Citrobacter freundii	2	2
Total	100	100

All the three species of *Listeria monocytogenes* isolated were sensitive to Tetracychine, Gentamycin, Ofloxacin, Ceftizoxime and to Ciprofloxacin, and resistant to Amoxicillin / Clavulanic acid and Piperacillin (Table 2). Twenty four strains of *Escherichia coli* were isolated in the present study. The strains showed variations in their sensitivity to the different antibiotics. No antibiotic was found sensitive to all strains while no antibiotic was resistant by all strains (Table 3). However, more than 70% of the isolated strains were sensitive to Amoxicillin/ Clavulanic acid , Tetracycline , Ceftizoxime, Ciproflxacin and Ampicillin /Sulbactam. 58.3% of the strains isolated were resistant to Piperacillin. All the four strains of *Pseudomonas* spp. isolated were resistant to Amoxicillin / clavulanic acid, Tetracychine, Piperacillin and also to Cefotaxime. The same four strains were sensitive only to Ofloxacin (Table 3).

All the strains of Salmonella spp. isolated were sensitive to Ceftizoxime, Ciprofloxacin and to Ampicillin / sulbactam.

The same strains were resistant to Cefuroxime and Tetracycline while intermediately sensitive to Cefotaxime (Table 3). The two strains of Citrobacter freundii were only sensitive to Ampicillin / Sulbactam and resistant to Cefuroxime, Tetracycline and Piperacillin (Table 3). Relatively, more resistant bacterial strains were encountered only among *Pseudomonas* spp, *Salmonella* spp and *Citrobacter freundii* isolated from bile specimens showing frequencies of 4 (4%) each.

Table 2: Antibiotics susceptibility patterns of Gram-positive bacteria isolated from gallbladder bile

Tested Antibiotics	Bacterial isolates tested			
	Staphylococcus aureus		*Listeria monocytogenes*	
	No.of bacterial isolates	% from total isolates	No. of bacterial isolates	% from total isolates
Amoxicillin /Clavulanic acid	S 4	100%	R 1 Int. 2	33.33 % 66.66 %
Cefuroxime	S 4	100%	S 1 Int. 2	33.33 % 66.66 %
Tetracycline	R 4	100%	S 3	100 %
Gentamycin	S 3 Int. 1	75 % 25 %	S 3	100 %
Ofloxacin	S 2 Int. 2	50 % 50 %	S 3	100 %
Ceftizoxime	S 4	100%	S 3	100 %
Ciprofloxcin	S 4	100%	S 3	100 %
Piperacillin	R 1 Int. 3	25 % 75 %	R 1 Int. 2	33.33 % 66.66 %
Cefotaxime	R 1 Int. 3	25 % 75 %	S 1 Int. 2	33.33 % 66.66 %
Ampicillin/ Sulbactam	S 2 R 1 Int. 1	50 % 25 % 25 %	S 1 Int. 2	33.33 % 66.66 %

Key: - **S**: sensitive R: Resistant **Int**.: Intermediate

Table 3: Antibiotics susceptibility patterns of Gram-negative bacteria isolated from gallbladder bile

Tested Antibiotics	Bacterial isolates tested			
	Escherichia coli	**Pseudomonas spp.**	**Salmonella spp.**	***Citrobacter freundii***
	No. and % of Bacteria from total isolates	No. and % of Bacteria from total isolates	No. and % of Bacteria from total isolates	No. and % of Bacteria from total isolates
Amoxicillin /Clavulanic acid	S 17 70.87% R 1 4.2 % Int 6 25%	R 4 100%	S 1 33.3% R 1 33.3% Int.1 33.3%	Int 2 100%
Cefuroxime	S 15 62.5% R 3 12.5% Int. 9 25%	S 2 50% R 1 25% Int 1 25%	R 3 100%	R 2 100%
Tetracycline	R 17 70.8% Int 7 29.2%	R 4 100%	R 3 100%	R 2 100%
Gentamycin	S 12 50% R 7 29.2% Int.5 20.8%	R 2 50% Int 2 50%	S 1 33.33% R 3 66.66%	S 1 50% Int.1 50%
Ofloxacin	S 15 62.5% R 9 37.5%	S 4 100%	S 2 66.66% Int 1 33.33%	S 2 100%
Ceftizoxime	S 20 83.3% Int. 4 16.6%	S 2 66.66% R 1 33.33%	S 3 100%	S 1 50% Int.1 50%
Ciprofloxacin	S 21 87.5% R 3 12.5%	S 2 50% R 2 50%	S 3 100%	S 1 50% R 1 50%
Piperacillin	S10 41.66% R 14 58.3%	R 4 100%	R 2 66.66% Int 1 33.33%	R 2 100%
Cefotaxime	S 8 33.33% R 4 16.66% Int 12 50%	R 4 100%	Int 3 100%	Int. 2 100%
Ampicillin/ Sulbactam	S 17 70.8% Int 7 29.2%	S 2 50% R 2 50%	S 3 100%	S 2 100%

S: sensitive, R: resistant, **Int.**: intermediate, **spp.**: four unidentified species of the genus Pseudomonas were isolated and three species of Salmonella.

Discussion

Difficulties were practiced in the treatment of *Salmonella typhi* carriers, as the organisms are stored in the gallbladder [11] [12] [10] and administered antibiotics cannot reach the gallbladder in adequate amounts [13]. Practically, in vivo treatment of bacterial cholecystitis with antibiotics is not satisfactory [14]. Prophylactic treatment with antibiotics is usually adopted before cholecystectomy to prevent the risks of sepsis [13]. In the present study the sensitivity of bacteria recovered from bile to antibiotics was tested using ten different types of antibiotics. This was done to check the availability of antibiotic resistant strains among those bile strains.

The three species of *Staphylococcus aureus* isolated in the present study were sensitive to Amoxicillin / Clavulanic acid, Cefuroxime, Ceftizoxime and to Ciprofloxacin. The same strains were resistant to Tetracycline. Similar results were reported by Darko and Archampong [15] in Ghana and by Al-Harbi *et al.* [7] in Saudi Arabia. Some of those strains of *Staphylococcus aureus* were intermediately sensitive to Gentamycin, Piperacillin, and Cefotaxime and to Ampicillin /Sulbactam which agrees with the finding of Darko and Archampong [15]. *Listeria monocytogenes* strains were sensitive to Tetracycline, Gentamycin, Ofloxacin, and Ceftizoxime and to Ciprofloxacin. The same strains were resistant to Amoxicillin / Clavulanic acid and to piperacillin. *Listeria species* are known to be sensitive to Tetracycline when they are recovered from human infections [10]. In biliary infections, the sensitivity of *Listeria species* to Tetracycline was not studied [10,12]. The twenty four strains of *E. coli* which were isolated in our study showed variations in their sensitivity to the different antibiotics tested. No antibiotic was found to be sensitive for all strains whereas no antibiotic was resisted by all strains. Nevertheless, more than 70% of the strains of *E. coli* isolated in the present study were found to be sensitive to Amoxicillin /Clavulanic acid, Tetracycline, Ceftizoxime, Ciprofloxacin and Ampicillin / Sulbactam. This agrees with the reports of Darko and Archampong [15]; Zsirka *et al.* [16] and AL-Harbi *et al.* [7]. However, our findings disagree with that reported by Petakovic *et al.* [17]. The four strains of *Pseudomonas* spp., which were

isolated in this study, were found to be resistant to Amoxicillin /Clavulanic acid, Tetracycline, Piperacillin and to Cefotaxime. The same species were sensitive only to Ofloxacin. It is known that *Pseudomonas* spp. were among those bacteria commonly causing nosocomial infections and were reported to be highly resistant to almost all antibiotics in current use [10,18]. They gained this character by their possessing R- genes in their plasmids [11]. All strains of Salmonellae recovered from bile in the present study were found to be sensitive to Ceftizoxime, Ciprfloxacin and Ampicillin / Sulbactam. The same strains were resistant to Cefuroxime and Tetracycline and intermediately sensitive to Cefotaxime. Those results were similar to that reported by Darko and Archampong [15]; AL-Harbi *et al.* [7] and Chang *et al.* [19]. *Citrobacter freundii* was isolated in two occasions in this survey study. The two strains were sensitive only to Ampicillin / Sulbactam and resistant to Cefuroxime, Tetracycline and Piperacillin. In our study, more resistant bacterial strains were encountered among *Pseudomonas* spp., *Salmonella* spp. and *Citrobacter freundii.*

Conclusion

Interestingly, most of bacterial species examined in this study were sensitive to many antibiotics. Resistant strains isolated belonged mostly to bacterial species known to cause nosocomial infections. Our study did not show precisely how bacteria could gain access into the gallbladder. It is obvious that those organisms may originate from an endogenous or an exogenous source, but their presence in the gallbladder protects them from the action of antibodies as well as antibiotics.

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