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Title: **Antibiogram Analysis of *Salmonella paratyphi A* Isolated from Gall Bladder Patients in District Peshawar, Pakistan**

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Antibiogram Analysis of *Salmonella paratyphi A* Isolated from Gall Bladder Patients in District Peshawar, Pakistan

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Abstract

Salmonella paratyphi A harbors gall bladder in the human body. It serves as a site of persistence for *Salmonella paratyphi A*. It is an enteric pathogen which has become resistant to many drugs. Therefore, the current study was designed for the identification and antibiogram analysis of *S. paratyphi* A, isolated from the gall bladder patients undergone cholecystectomy. It included 250 samples of bile, stone, and tissue of patients. The samples were cultured on blood, macConkey, and *Salmonella Shigella* media. Further identification was carried out by morphological oxidase test and Analytical Profile Index (API) strips, followed by antibiogram analysis of the isolates. In the current study, twenty-eight (11.2%) *paratyphi* A were isolated including 10 (10%) from male patients and 18 (12%) from female patients. Furthermore, 96 samples were found to be positive for miscellaneous growth including 53 with *S. typhi* (21.2%), 13 with *Escherichia coli* (5.2%), 09 with *Klebsiella* (3.6%), 07 with *Providencia* (2.8%), 05 with *Pseudomonas* (2%), 03 with *Proteus* (1.2%), and 06 with *Staphylococcus aureus* (2.4%). The distribution and susceptibility pattern of *S. paratyphi* A isolates was checked in different types of clinical specimens including bile, stones, tissue, bile/stones, bile/tissue, stones/tissue, and bile/stone/tissue. *S. paratyphi* A was distributed as follows: bile (11), stones (5), tissue (3), bile/ stones (4), stones/ tissue (1), bile/tissue (1), and bile/stones/tissue (3). The results of the antibiogram analysis found that the isolates of *Paratyphi* A were resistant to sulfamethoxazole 23 (82.14%), cefixime 23 (82.14%), ceftriaxone (rocephin) 20 (71.42%), augmentin 19 (67.85%), and azithromycin 18 (64.28%). The increased susceptibility of these isolates was towards meronem 28 (100%), imipenem 28 (100%), cefoperazon + sulbactam (sulzone) 25 (89.28%), and amikacin 23 (82.14%). The current study signifies the use of the most susceptible and effective antibiotic options for gall bladder

diseases complicated by *S. paratyphi* A, which showed resistance to ceftriaxone (rocephin), cefixime, sulfamethoxazole, azithromycin, and augmentin, while sensitivity to meropenem, imipenem, cefoperazone + sulbactam (sulzone), and amikacin. It makes the latter a better choice for treatment against the gall stone disease complicated with *S. paratyphi* A infection.

1. Introduction

Salmonella is the genus of the family Enterobacteriaceae, species *enterica* that is further subdivided into two subspecies *enterica* and *bongori. Salmonella paratyphi* A is the human serovar of subspecies *enterica*. Morphological characteristics reveal it to be a facultative anaerobe and intracellular organism plus a gram-negative rod without spore, size 0.2 -1.5 x 2-5 µm. *Paratyphi* A is chemoorganotrophic and showed motility using peritrichous flagella [1, 2]. The gall bladder is a pear-shaped organ located beneath the liver on the right side. It performs the role of storing bile secreted and formed by the liver [3]. Bile is normally sterile by its bacteriostatic property, the flow of bile, secretion of IgA, and mucus that prevents the adhesion of bacteria to the surface of the lumen, and main biliary duct [4]. The epithelial cells of the gall bladder serve as a niche for the *Salmonella* species. This mechanism reveals the intracellular presence of *Salmonella* spp. within the gall bladder [5]. Evidence showed that certain bacteria are also present in the bile fluid and in the form of biofilms on gall stones defining them as other possible niches [6].

S. paratyphi A is the causative agent of paratyphoid A fever. The disease is transmitted through the oral-fecal route. The predisposing factors for the disease are poor sanitary conditions and overpopulation. Preventive and control measures for *paratyphi* A include careful handling of drinking and eating habits accompanied by maintaining personal hygiene. These precautionary measures are the only way of preventing this disease. There is no vaccine available for this disease and even the vaccines used against typhoid fever are not effective against *paratyphi* A. The progress of the disease is systemically reaching the liver, gall bladder, spleen, bone marrow, intestine, and lymphatic system [7]. It reaches the gall bladder either through blood or from bile by retrograde spreading [8]. After colonization, the chronic carriers shed the microbes occasionally in the lumen of the intestine and body wastes (feces). The persistence and transmission of the disease are due to fecal shedding and colonization of the organ by the causative agent. The fecal shedding and colonization of the organ serve as a central dogma of the disease. Blockage of biliary tracts is favorable for bacterial infection. These conditions can cause death if left untreated [9]. Furthermore, epidemiological reports reveal its widespread in Asian countries. Throughout the world, about 16 million new cases and 25000 deaths are recorded annually [10]. In addition to socioeconomic status other basal infectious diseases such as malaria fever, have the chance of acquiring the infection caused by *Salmonella.* Worldwide, *typhi* serovar was considered the most prevalent but recently, *paratyphi* A has been reported as the top prevalent drug by researchers [11]. The worldwide data relating to the bacteriology, mechanisms of carriage and prevalence of the infection showed that it's sparsely spread. The chance of being carriers increases with an increase in the age and

specifically in female gender. The recent present data of *Salmonella* chronic carrier state follow the epidemiology of the gall stones and other diseases associated with the gall bladder [12].

Antibiotic therapy is one of the choices for the treatment of enteric fever [13]. The species of *Salmonella* showed a high level of susceptibility to most of the drugs being used [14]. However, a steady increase in the resistance to one or more antibiotics has been shown by those species. The continuous antibacterial pressure is the main reason for this emerging resistance [15]. Multidrug-resistant (MDR) phenotypes have been increasingly described among *Salmonella* spp. worldwide according to the World Health Organization (WHO) report on infectious disease [8]. The multi-drug resistance in *Salmonella* is conferred through mobile genetic vehicles like plasmids, transposons, and integrons spreading via horizontal or vertical transfer [16]. Efflux pump is the main factor conferring resistance to a wide range of antibiotics including those used against *Salmonella* and other members of the *Enterobacteriaceae* family. *Salmonella* has been reported to show an increased frequency of resistance to antibiotics like chloramphenicol, tetracycline, and β-lactam [17]. Along with resistance to ciprofloxacin due to its immense use it also confers resistance to nalidixic acid globally in *typh*i and *paratyphi* A [18]. The last treatment option for enteric fever failed when resistance to the higher-generation cephalosporins (cefuroxime, ceftazidime, and cefotaxime) appears [19]. The other choice of drug for the disease is Azithromycin but it also has reported resistance in India and other states [20]. The antibiogram analysis of the blood isolates *S. enterica* is useful in the prediction of better treatment options for enteric fever. The resistance acquired to those antibiotics is due to mobile genes passing through generations in pathogens [21]. After the failure of former treatment options synthetic group is devised that includes fluoroquinolones and quinolones. This group is broad-spectrum antibiotics commonly used in clinical medicine. These antibiotics showed the best activity against gram-negative rods. In recent years, the resistance toward quinolones has increased. This condition further worsens the hope for treatment and results in a significant load of mortality and hospitalization [22, 23].

Therefore, for the better treatment of *S. paratyphi* A associated gallstone infection the assessment of the antibiogram is mandatory. The current study was designed for the identification and antibiogram analysis of *S. paratyphi* A isolated from gall bladder patients undergoing cholecystectomy in Peshawar, Pakistan.

2. Materials and Methods

2.1 Study Population and Sample Collection

The study was ethically approved by the ethical committee of Abasyn University Peshawar, Pakistan, and was carried out from November 2020 to November 2021. A total number of 250 gall bladder samples were collected from the patients' undergone cholecystectomy at different hospitals and private clinics in Peshawar, Pakistan. The proper consent form was signed by the patients which were included in this study. Among these patients, 150 were females and other 100 were males. All patients were 15 years-55 years of age, and even older than 55 years of age were also included in the sample collection. The samples were collected in sterile disposable wide-mouth bottles. Immediately after collection, samples were sent to the laboratory for further processing.

2.2 Culturing and Sub-Culturing of Samples

Bile, gallstones, and tissue samples were cultured on blood, MacConkey, and *Salmonella Shigella* (*SS*) media. Gall stones were crushed with a sterile blade and bile samples were streaked directly on the media surface followed by incubation at 37°C. Similarly, tissue was homogenized first and then cultured on those media and incubated at 37°C [24].

2.3 Identification of Bacterial Isolates

Morphological and physiological identification of the isolates were carried out through gram staining and other biochemical tests. The gram staining of isolates depicted them to be either gram-positive or gram-negative. To study the physiological properties of isolates oxidase tests were performed and 10S API (Analytical Profile Index) strips were used for further confirmation of the bacterial isolates.

2.4 API (Analytical Profile Index)

The API 10 S strip consisted of 10 microtubes covered with dehydrated substrates for different biochemical tests namely glucose (GLU), ornithine decarboxylase (ODC), nitrogen dioxide (NO_2), Lysine decarboxylase (LDC), hydrogen sulfide (H_2S), urease (URE), tryptophan deaminase (TDA), indole (IND), oxidase (OX), citrate (CIT), arginine (ARA), and ortho-nitrophenyl-galactopyranoside (ONPG). The media was reconstituted with the addition of the bacterial suspension to these microtubes. On incubation, bacterial metabolism produced color changes that were either immediate or appeared with the addition of reagents. The combined result of all tests identified the organism by using specific codes or profile indexes as mentioned in the manual. The humid environment was created in the incubation box (lid and tray) by filling its wells with 3ml of distilled water. The strips were then placed in the incubation box. The bacterial suspension was formed in a tube by inoculating a large colony of the bacterium into a 0.85 % NaCl solution. The suspension was made homogenous and without clumps of floating bacteria. The strip was held at a little angle above the top of the table. The bacterial suspension was inoculated into the tubes of the strip with a sterile pipette (the tip of the pipette was placed against the side of the cupules to avoid the formation of bubbles at the base of the tube). The bacterial suspension was filled in both tubes and cupules of the CIT test having boxes around their names. The bacterial suspension was filled in only tubes of LDC, ODC, H_2S, and URE, while sterile mineral oil was filled in cupules to ensure anaerobiosis. The bacterial suspension was filled in only tubes for the remaining tests. Then the incubation box was kept for 24 hours at 37 °C for incubation. Results were recorded with the help of a reading table as positive or negative, after 24 hours of incubation.

2.5 Antibiotic Sensitivity Test

Antibiogram analysis was carried out through the Kirby-Bauer disc diffusion protocol as discussed in the Clinical and Laboratory Standards Institute (CLSI, 2019). Bacterial dilution was prepared and compared with Mac Farland 0.5 standard. Dilution was poured and spread over on Mueller Hinton agar media. Antibiotic discs were placed over the media with help of forceps. It was kept at 37ºC for 24 hours in an incubator. A scale was used to measure the zone of inhibition. Different concentrations of antibiotics were used (Table 1).

Table I. Concentration and Sensitivity Pattern of Different Antibiotics

#	Antibiotics	Abbreviation	Concentration (µg)	Sensitivity (mm)		
				S	I	R
A	**β-Lactamase Inhibitor**					
1.	Augmentin	AUG	10/ 20	18	14-17	13
B	**Carbapenem**					
1.	Meropenem	MEM	10	23	20-22	19
2.	Imepenem	IMP	10	23	20-22	19
C	**Fluroquinolones**					
1.	Ciprofloxacin	CIP	5	31	21-30	20
2.	Moxifloxacin	MOR	5	24	21-23	20
3.	Levofloxacin	LEV	5	17	14-16	13
D	**Cephalosporin**					
1.	Cefixime	CTX	30	14	4	82
2.	Ceftriaxone (Rocepin)	CRO	30	21	14-20	13
3.	Cefoperazon+sulbactam (Sulzone)	SCF	105	15	12-14	11
E	**Folate pathway inhibitor**					
1.	Sulfamethoxazole	SMZ	100	16	11-15	10
F	**Macrolides**					
1.	Azithromycin	AZM	15	18	14-17	13
G	**Aminoglycosides**					
1.	Gentamycin	CN	10	15	13-14	12
2.	Amikacin	AK	30	15	13-14	12

S= Sensitive, I= Intermediate, R= Resistance

3. Results

In the current study, we screened a total of n=250 gall bladder samples out of which n=124 were positive. Among these, n=28 (11.2%) showed the positive growth of *S. paratyphi* A. About n=96 samples showed miscellaneous growth including *S. Typhi* n=53 (21.2%), *E. coli* n=13 (5.2%), *Klebsiella* n=09 (3.6%), *Providencia* n=07 (2.8%), *Pseudomonas* n=05 (2%), *Proteus* n=03 (1.2%), and *Staphylococcus aureus* n=06 (2.4%). The remaining n=126 samples were found negative (Figure I). We divided our specimens into bile, stones, tissue, bile/stones, bile/tissue, and bile/stones/tissue. The number of all *paratyphi* A isolated from respective specimens was distributed as follows: n=11, n=05, n=03 from bile, stones, and tissue respectively, while n=04, n=01, n=01, n=03 were the number found in bile/stones, stones/tissue, bile/tissue, and bile/stones/tissue, respectively (Table 3). *S. paratyphi* A was identified by analytical profile index (API) strips. The observed results of the analytical profile index (API) strip showed that glucose (GLU), ornithine decarboxylase (ODC), and nitrogen dioxide (NO_2) were positive, while other tests as Lysine decarboxylase (LDC), hydrogen sulfide (H_2S), urease (URE), tryptophan deaminase (TDA), indole (IND), oxidase (OX), citrate (CIT), arginine (ARA), and ortho-nitrophenyl-galactopyranoside (ONPG) were negative for *S. paratyphi* A (Table 2).

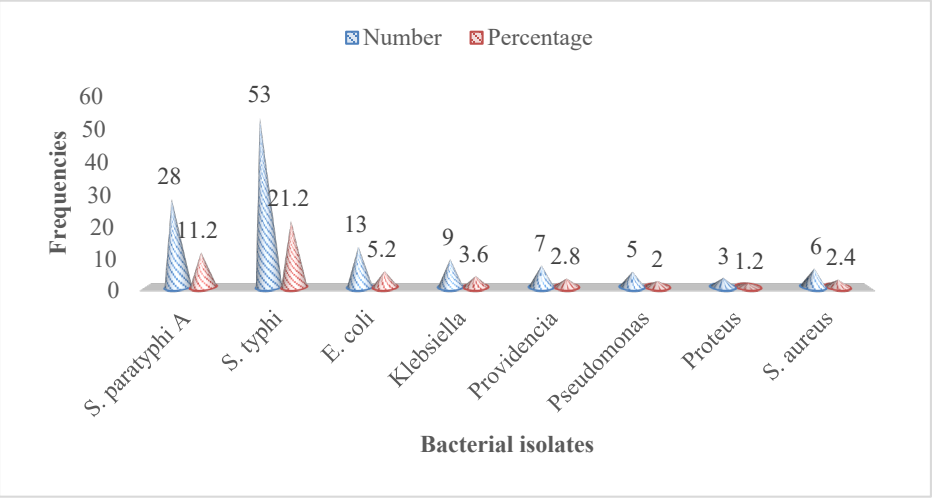

Figure 1. Prevalence of Different Bacterial Isolates from Gall Bladder Samples

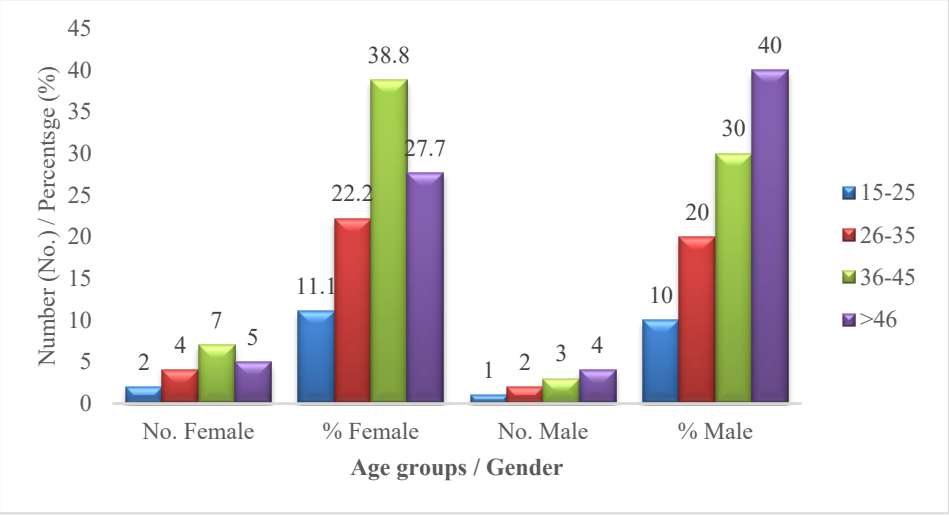

Figure 2. Gender and Age-wise Distribution of *S. paratyphi* A in Gall Bladder Samples

Out of 28 (11.2%) positive cases of *S. paratyphi* A, 18 (12%) patients were female and 10 (10%) were males. The age-wise distribution in females showed the following trend: 02 (11.11%) cases were found positive from the age group 15-25, 04 (22.22%), from the age group 26-35, 07 (38.88%), and from the age group 36-45, while 05(27.77%) from 46 and above the age group. The trends of positive cases were as followed in males: 01(10%) from the 15-25 age group, 02 (20%) from the 26-35 age group, 03 (30%) from the 36-45 age group, while 04 (40%) from 46 and above cases (Figure 2).

Table 2. Biochemical Characteristics of *S. paratyphi* A by API strip

Tests	Active Ingredients	Reaction Enzymes	Results		Results
			Negative	Positive	
ONPG	2- nitrophenyl-B D galactopyranosie	B-galactosidase (ortho-nitrophenyl-B D-galactopyranoside)	Colorless	Yellow	negative
GLU	D Glucose	Fermentation/ oxidation (glucose)	Blue/ blue green	Yellow/ yellow-grey	positive
ARA	L- Arabinose	Fermentation/ oxidation (Arabinose)	Blue/ blue green	Yellow	negative
LDC	L- Lysine	Lysine decarboxylase	Yellow	Red/ orange	negative
ODC	L- Ornithine	Ornithine decarboxylase	Yellow	Red/ orange	positive
CIT	Trisodium citrate	Citrate utilization	Pale green / Yellow	Blue green/blue	negative
H_2S	Sodium thiosulphate	H_2S production	Colorless/ grayish	Black deposits/ thin line	negative
URE	Urea	Urease	Yellow	Red/ orange	negative
TDA	L- tryptophane	TryptophaneDeaminase	Yellow	Reddish brown	negative
IND	L- tryptophane	Indole production	ColorlessPale green / Yellow	Pink	negative
OX	Phenylenediamie	cytochrome- oxidase	Colorless	Purplish blue	negative
NO_2	GLU tube	NO_2 production	Yellow	Red	positive

3.1 Antibiogram Assay of *S. paratyphi* A

The antibiogram analysis of *S. paratyphi* A was performed by the disc diffusion method. Following classes of antibiotics such as beta-lactamase inhibitors, carbapenems, fluoroquinolones, cephalosporins, folate pathway inhibitors, macrolides, and aminoglycosides were utilized in the study for the determination of the multi-drug resistant (MDR) species. The Zone of inhibition was determined for the effectiveness of 13 utilized drugs. The zone of inhibition categorized drugs as sensitive(S), intermediate (I), and/or resistant (R). The analysis showed following susceptibility trend: Augmentin S: 05(17.85%) I: 04(14.28%) R: 19(67.85%), Meronem S: 28 (100%) I: 0(0%) R: 0(0%), Imepenem S: 28 (100%) I: 0(0%) R: 0(0%), Ciprofloxacin S: 15 (53.57%) I: 04 (14.28%) R: 09 (32.14%), Moxifloxacin S: 18(64.28%) I: 02(7.4%) R: 08(25.87%), Levofloxacin S: 13(46.42%) I: 5(17.85%) R: 10(35.71%), Cefixime S: 04(14.28%) I: 01(3.57%) R: 23(82.14%), Ceftriaxone (Rocepin) S: 06(21.42%) I: 02(7.14%) R: 20(71.42%), Cefoperazon+sulbactam (Sulzone) S: 25(89.28%) I: 02(7.14%) R: 01(3.57%), Sulfamethoxazole S: 03(10.71%) I: 02(7.14%) R: 23(82.14%), Azithromycin S: 8(28.57%) I: 02 (7.14%) R: 18(64.28%), Gentamycin S: 12 (42.85%) I: 06(21.42%) R: 10(35.71%), Amikacin S: 23(82.41%) I: 3 (10.71%) R: 2 (7.14%) (Table 3, Figure 3).

Table 3. Antibiotic Susceptibility Pattern of *S. paratyphi* A Concerning the Type of Clinical Specimens

S. No.	Antibiotics	Bile n=11			Stone n=05			Tissue n=03			Bile/Stone n=04			Bile/Tissue n=01			Stone/Tissue n=01			Bile/Tissue/Stone n=03		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1.	**Augmentin**	1	2	8	0	1	4	2	0	1	0	0	4	1	0	0	1	0	0	0	1	2
2.	**Meronem**	11	0	0	5	0	0	3	0	0	4	0	0	1	0	0	1	0	0	3	0	0
3.	**Imepenem**	11	0	0	5	0	0	3	0	0	4	0	0	1	0	0	1	0	0	3	0	0
4.	**Ciprofloxacin**	7	2	2	2	1	2	2	0	1	1	1	2	1	0	0	0	0	1	2	0	1
5.	**Moxifloxacin**	9	0	2	1	1	3	2	1	0	2	0	2	1	0	0	1	0	0	2	0	1
6.	**Levofloxacin**	4	1	6	1	1	3	2	0	1	3	1	0	1	0	0	0	1	0	2	1	0
7.	**Cefixime**	1	0	10	1	0	4	1	0	2	1	0	3	0	0	1	0	0	1	0	1	2
8.	**Ceftriaxone(Rocepin)**	0	0	11	1	0	4	1	0	2	3	0	1	0	0	1	0	1	0	1	1	1
9.	**Cefoperazon+sulbactam (Sulzone)**	9	1	1	4	1	0	3	0	0	4	0	0	1	0	0	1	0	0	3	0	0
10.	**Sulfamethoxazole**	1	1	9	0	0	5	1	0	2	0	1	3	0	0	1	0	0	1	1	0	2
11.	**Azithromycin**	3	1	7	1	0	3	1	0	2	1	1	2	0	0	1	1	0	0	1	0	2
12.	**Gentamycin**	4	2	5	2	0	3	2	0	1	2	1	1	0	1	0	1	0	0	1	2	0
13.	**Amikacin**	10	1	0	4	1	0	2	1	0	3	0	1	1	0	0	1	0	0	2	0	1

n= Number of *S. paratyphi* A isolates, S= Sensitive, I= Intermediate, R= Resistance

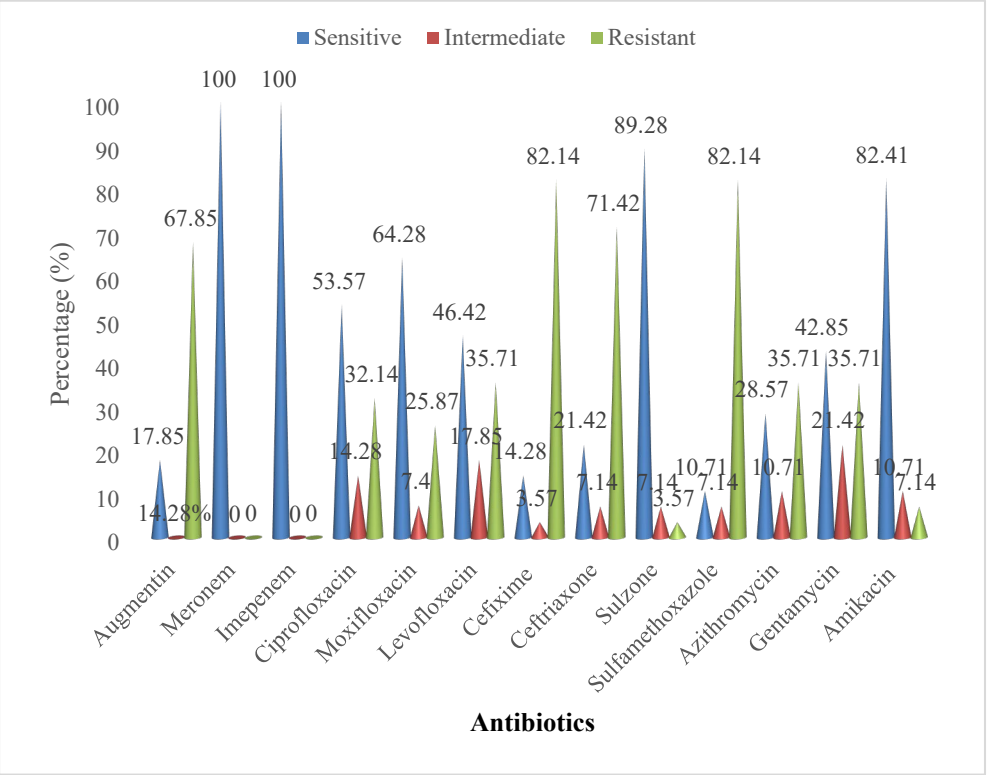

Figure 3. Antibiogram assay of *S. paratyphi* A

3.2 Antibiotic Susceptibility Pattern of *S. paratyphi* A Concerning the Type of Clinical Specimens

We evaluated the susceptibility pattern distribution against 13 antibiotics concerning types of clinical specimens in this study. The specimens were classified as bile, stones, tissue, bile/stones, bile/ tissue, stones/tissue, and bile/stone/tissue. The isolates of all clinical specimens were found 100% sensitive to Imipenem and Meropenem. The isolates of the bile specimen showed resistance to the following antibiotics in descending order: Ceftriaxone (100%), Cefixime (90.90%), Sulfamethoxazole (81.81%), Augmentin (72.72%), and Azithromycin (63.63%). Similarly, isolates of stone specimens were found completely resistant to Sulfamethoxazole (100%) followed by 90.90% resistance towards Augmentin, Cefixime, and Ceftriaxone, while 81.81% resistance was shown against Moxifloxacin, Levofloxacin, Azithromycin, and Gentamycin. Among the isolates of tissue Cefixime, Ceftriaxone and Sulfamethoxazole were showing 90.90% resistance. The following resistivity order was deduced from the antibiotics for the isolates of bile/stone specimens: Augmentin (100%), Cefixime, and Sulfamethoxazole (90.90%). Bile/tissue isolates showed complete resistance (100%) towards Cefixime, Ceftriaxone, Sulfamethoxazole, and Azithromycin. Ciprofloxacin, Cefixime,

and Sulfamethoxazole were the antibiotics being 100% resistant to isolates of stone/tissue specimens. Lastly, *S. paratyphi* A collected from bile/stone/ tissue specimens showed a 90.90% of resistance against Augmentin, Cefixime, Sulfamethoxazole, and Azithromycin. The details of all antibiotics used are described in Table 3.

4. Discussion

The *S. paratyphi* A harbors the gall bladder in the human body. It serves as a reservoir and site of persistence for the organism [25]. It may be a direct cause or auxiliary organism being involved in gall bladder complications like cholelithiasis, Cholecystitis, cholangitis, acalculous and calculus cholecystitis, cholangiocarcinoma, and gall bladder cancer (GBC). This organism is an enteric pathogen which has become resistant to many drugs [26]. Cholelithiasis referring to gall stones formation is the main problem resulting in the other above-mentioned conditions. The other least frequent causes of developing these conditions are alteration and physical damage in biliary tracts and other parts of the organ. Various epidemiological support the persistence of the biofilms of *Salmonella* on gallstones and laboratory-based studies [27]. Acute cholangitis (AC) is another inflamed condition that develops because of the bacterial infection in the biliary ducts along with the formation of stones. It is the most fatal condition among other complications of the gall bladder [28].

The current study included a total of 250 patients who have undergone gall bladder surgery. Among the total population, 150 (60%) were female, while 100 (40%) were male patients. Bile, gall bladder tissue, and gall stones were sampled from patients for microbiological identification. *Paratyphi* A was isolated from 28 (11.2%) patients. API 10 E strips and oxidase test were used for the identification of *S. paratyphi* A. The prevalence of *S. Paratyphi* A was found to be 12 % (18) in females and 10 % (10) in males. About 96 samples showed growth of other organisms including: *S.* Typhi 53 (21.2%), *E. coli* 13 (5.2%), *Klebsiella* 9 (3.6. %), *Providencia* 7 (2.8%), *Pseudomonas* 5 (2%), *Proteus* 3 (1.2%), and *Staphylococci* 6 (2.4%). The 126 samples were found to be negative. In 2014, a study was performed on 126 patients with symptomatic gallstone disease. In 126 samples, female and male cases were 100 (79.37%) and 26 (20.63%), respectively. In this study 74 (58.73%) samples showed a growth percentage, whereas 52 (41.27%) showed no growth percentage. The trend of prevalence of organisms in the study was as follows: *Shigella* 8 (6.35%), *Salmonella* 16 (12.70%), *Klebsiella* 22 (17.46%), and *E. coli* 28 (22.22%). Another study by Capoor et al. [26] included the spectrum of biliary microflora in patients with or without cholelithiasis, acute cholangitis, or other biliary diseases. Bile, gallbladder, and gallstones were collected for microbiological and histopathological examination. The most commonly isolated organisms were *Escherichia coli* 11 (29.7%), *Klebsiella pneumoniae* 10(27%), *Citrobacter freundii* 03 (8.1%), and *S. enterica* 03 (8.1%). While in 2009 Khatari et al. [29] in their study included patients suffering from enteric fever. *S. paratyphi* A was isolated from about 12 patients. Thereby, this study signifies the relation between *Salmonella* persistence and acute cholecystitis. Whereas Latif et al. [30] used API 20 E and oxidase test for the identification of *paratyphi* A. The difference was noticed due to the availability of resources.

The age-wise distribution of *paratyphi* A revealed it to be most prevalent in the 36-

45 and above 45 age group in females and males, respectively, while the least number was seen in the 15-25 age group of both male and female patients. Manan et al. [28] also mentioned the age-wise distribution of various organisms being studied. According to this study, *Salmonella* was prevalent in the 41-50 age group and the least number was isolated from other included age groups. The age group distribution criteria was different from our study but included all the age groups mentioned in the current study. This study also highlighted the gender-wise distribution of *Salmonella,* while they included the age-wise distribution of all organisms being included. The previous literature revealed no such study which purely focused on the age and gender-wise distribution and culturing sensitivity of *paratyphi* A which was the center of concern in the contemporary study.

In this study, an antibiogram analysis was performed of 28 *S. paratyphi* A on Mueller Hinton agar following the protocol of the Kirby Bauer disc diffusion method was mentioned in CLSI (2019) guidelines against 13 antibiotics. Different antibiotics were used in the current study which included: Augmentin, Meronem, Imipenem, Ciprofloxacin, Moxifloxacin, Levofloxacin, Cefixime, Ceftriaxone (Rocephin), Cefoperazon+sulbactam (Sulzone), Sulfamethoxazole, Azithromycin, Gentamycin, and Amikacin. Kaya et al. [31] examined bile samples of patients suffering from cholangitis microbiologically and tested the antibiogram analysis of the Gram-negative isolates with 11 antibiotics. Similar to our study Dutta et al. [32] interpreted the antibiogram pattern of the *paratyphi* A by disc diffusion protocol of Kirby Bauer mentioned in CLSI (2019) guidelines using about 17 discs such as Ampicillin, Amoxicillin/clavulanic acid, Amikacin, Aztreonam, Azithromycin, Cefotaxime, Cholorompheniсol, Ceftazidime, Ceftriaxone, Co-trimoxazole, Ciprofloxacin, Gentamicin, Levofloxacin, Nalidixic acid, Streptomycin, Ofloxacin, and Tetracycline.

According to the current study, *paratyphi* A isolates were found resistant to cephalosporins (Cefixime and Ceftriaxone), folate pathway inhibitors (Sulfamethoxazole), β-lactamase inhibitor (Augmentin), and macrolide (Azithromycin). Ahmad et al. [33] found Ceftriaxone to be resistant to the paratyphoid pathogen. Another study in 2014 by Pokharel et al. [19] demonstrated resistance of Sulfamethoxazole towards *S. paratyphi* A, while on contrary current study found ceftriaxone as an excellent agent in treating paratyphoid fever. In the study of Raji et al. [34], Augmentin was found variably resistant to the isolates of *S. paratyphi* A. In contrast to our study, Butler et al. [35] and Eslami et al. [27] showed Azithromycin is highly sensitive toward *paratyphi* A isolates. The reason behind the difference was over or misuse of Azithromycin in our city for the treatment of paratyphoid fever. While another study by Capoor et al. [36] provided proof that Azithromycin is resistant to *paratyphi* A. The study of Rizvi [37] illustrated Cefixime to be efficient in treating paratyphoid fever and as in our study it was found resistant. This change was due to the difference in environment and treatment options.

This study revealed that most isolates showed increased susceptibility for carbapenems (Meronem and Imepenem) followed by cephalosporin (Cefoperazon + sulbactam) fluoroquinolones (Ciprofloxacin, Moxifloxacinand Levofloxacin) and aminoglycosides (Gentamycin and Amikacin). Toh et al.

[38] in their study concluded that Imipenem and Amikacin were the most effective drugs being used against *Enterobacteriaceae*. Dongol et al. [18] found *paratyphi* A isolated from cholecystectomy to be sensitive toward Amikacin, Ciprofloxacin, and Gentamycin. Pokharel et al. [19] illustrated in their study that *S. paratyphi* A was susceptible to Imipenem. A study by Stass et al. [39] illustrated that Moxifloxacin was sensitive to both gram-positive and gram-negative organisms. Garg et al. [40] in their study proved that *paratyphi* A is susceptible to Cefoperazon + sulbactam and other gram-negative organisms. The current study included an innovative aspect which was checking the distribution of susceptibility patterns against 13 antibiotics concerning clinical samples. There was no previous literature available regarding this aspect which could be similar to our study. Samples were categorized as bile, stones, tissue, bile/stones, bile/ tissue, stones / tissue, and bile/stone/tissue. *S. paratyphi* A isolates from clinical samples were found completely sensitive to Meropenem and Imipenem. Every specimen showed complete, incomplete, or varied resistance against two common antibiotics Ceftriaxone and Sulfamethoxazole. The other antibiotics showing a high percentage of resistance were Augmentin, Cefixime, Ciprofloxacin, Levofloxacin, Moxifloxacin, Azithromycin, and Gentamycin.

4.1 Conclusion

The current study concluded that gall bladder diseases, especially gallstone disease (cholelithiasis), accompany bacterial infections resulting in further complications. The results of antibiogram analysis indicated that *S. paratyphi* A was mostly resistant to Ceftriaxone (Rocephin), Cefixime, Sulfamethoxazole, Azithromycin, and Augmentin. Increased sensitivity was shown to Meronem, Imipenem, cefoperazone + sulbactam (Sulzone), and Amikacin which made these drugs a better choice of treatment against the gallstone disease complicated with *S. paratyphi* A infection. Additionally, the maximum number of *paratyphi* A was isolated from bile followed by stone, bile/stone, tissue, and bile/stones/tissue specimens in descending order. However, the lowest number of isolates was found in stone/tissue and bile/tissue specimens.

Conflict of Interest

The authors declare no conflict of interest.

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