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Isolation, identification, characterization and antibiotic susceptibility of *Vibrio cholera* during 1998-99.

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Abstract

Vibrio Cholera, causative agent of acute gastrointestinal disease or cholera is a natural inhabitant of aquatic environment. Cholera is endemic disease in Latin America, Southern Asia and parts of Africa, where poor sanitation and seasonal outbreaks are particularly associated with seasonal outbreaks. Large number of outbreaks of Vibrio cholera gastroenteritis in Asian countries indicates the need to evaluate the prevalence of that pathogenic species in different regions of Asia. This study was conducted to ascertain the prevalence and antibiotic resistance of *Vibrio cholera* in the endemic areas of Pakistan. Samples were collected from epidemic cell of National Institute of Health (NIH) during the time period of July 1998 to 1999, on the basis of reported cases of gastroenteritis/ cholera infections. A total of 172 isolates were collected from the 303 stools and vomitus samples of infected patients and their sensitivity to 18 antimicrobial agents were determined by disk diffusion method. All the isolates of *Vibrio cholera* showed 100% resistance to streptomycin and trimethoprim/ sulfamethoxazole throughout the study period. The O139 strain isolated from water was resistant to streptomycin and Kanamycin. In contrast Norfloxicin were found to be very effective with only 4% resistance rate during 1998 while Tobramycin showed the best results with only 1% resistance as compared to resistance percentage of Tetracycline 10%, Erythromycin 16%, Chloramphenicol 17%, Cefamendol 40%, Ampicillin 58%, Nalidixic acid 66%, Nitrofurantion 95% during 1999. The comparison of antibiotic sensitivity showed almost similar pattern of antibiotics sensitivity with little variations due to geographical barriers. Furthermore, the trends of increased resistance to antibiotics indicate that indiscriminate use of antimicrobial agents during hospitalization and self-medication contributed to the emergence of drug resistance in the prevalent strain of *Vibrio cholera*.

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Introduction

Cholera is a gastroenteritis infections resulting in millions of deaths worldwide caused by enterotoxin-producing *Vibrio cholera* (Berger, 2017). It remains a perennial health problem most of the developing countries presenting in sporadic, endemic, epidemic proportions and resulting in significant mortality and morbidity. Cholera has affected approximately 3–5 million people worldwide and resulting in 28,800–130,000 deaths per year (Hatti-Kaul and Mattiasson, 2016; Lozano *et al.,* 2013). Now it is categorized as a pandemic disease, rarely found in developed world (Reidl and Klose, 2002).

The death rate was greater than 3 million a year in the early 1980s. Still it is difficult to determine the exact numbers of cases of infection, due to misconception of having negative impact on tourism of a country (Sack and Bradley, 2006). *Cholera* infection mostly occurs as both chronically and outbreaks having status of both endemic and epidemic. Globally, areas with high risk of disease include south-east Asia and Africa with a death rate of 5% which may increase up to 50% among patients who do not prefer treatment (Dobre *et al.,* 2014). From 1984 -2014, approximately 13,000 cases of Cholera infection were reported to the Cholera and Other Vibrio Illness Surveillance (COVIS) (Crowe *et al.,* 2016). According to WHO each year from 2007-2011, >100 000 cases of cholera was reported by 20 African countries with annual case-fatality ratios (CFRs) from 2.22% to 2.95% (Mintz and Tauxe, 2013).

The Centers for Disease Control and Prevention (CDC) reported that consumption of undercooked oysters and see food increased the rate of infection since year 2001, specifying the need of practical measures to control human *Vibrio* infections (Centers for Disease Control and Prevention, 2010).

The cholera epidemic in Nigeria in 2010, with 41,787 reported cases with a case fatality rate (CFR) of 4.1%, was one of the biggest outbreaks in Nigeria, with 80% of reported cases attributed to children and women (Adagbada *et al.,* 2012; Dalhat *et al.,* 2014).

Vibrio cholerae is transmitted to humans through contaminated food and water. In Pakistan Diarrhea infection rate is found to be about 15% (Wikipedia, The Free Encyclopedia, 2017).

The Ministry of Health in Pakistan reported 99 cases of *Vibrio cholera 01* in different regions of the country. These cases of infections were confirmed by the laboratory of National Institute of Health until 30 September 2010 since after flood rains.

These infections were reported sporadically from different geographical regions of the country including flood-affected areas of Khyber Pakhtunkhwa, Punjab and Sindh (Guo *et al.,* 2017). Aquatic environments are the best habitat of *Vibrio cholera* (Islam *et al.,* 1993).

The primary symptoms of disease include painless and profuse diarrhea and vomiting of clear fluid (Ansaruzzaman *et al.,* 2004). These symptoms generally appear after 0.5-5 days of bacterial ingestion (Azman *et al.,* 2012). An untreated patient with cholera infection may produce 10-20L of diarrhea per day. If the patient is not properly treated, it can lead to severe and life threatening electrolyte imbalance and dehydration (Baracchini *et al.*, 2016; Faruque *et al.*, 2004).

Antimicrobial therapy plays an important role to overcome the effects of diarrhea by reducing the duration of symptoms, excretion of vibrios in the feces therefore shortening the volume of diarrhea. The ciprofloxacin-trimethoprim combination have additive and synergistic effects against *Vibrio cholera* (Mandal *et al.*, 2012).

Keeping in view the risk factors and associated losses, the present study was conducted with the aim to know the occurrence of *Vibrio cholerae* responsible for diarrhea in Pakistan including regions of Gilgit, Rawalpindi and its adjoining areas and Afghanistan. Furthermore to engender information concerning the effectiveness of commercially available antibiotic against *Vibrio cholerae.*

Table 1. Zone diameter interpretive Chart.

Serial No.	Antimicrobial agent	Disc Potency	Resistant	Intermediate	Susceptible
1.	Ampicillin (AM-AMP)	10-25µg	≤ 13	14-16	≥ 17
2.	Cefamendol (MAN)	30µg	≤14	15-17	≥ 18
3.	Cefotaximin (CTX)	30µg	≤14	15-22	≥ 23
4.	Ceftriazone	30µg	≤13	14-20	≥ 21
5.	Chloramphinacol (CH)	30µg	≤12	13-17	≥ 18
6.	Ciproflaxcin	5µg	≤15	16-20	≥ 21
7.	Trimethoprim/ Sulfamethoxazole (SXT)	1.2µg	≤10	11-15	≥ 16
8.	Enoxicin (ENX)	23.75µg	≤		≥
9.	Erythromycin (E)	10µg	≤14	15-17	≥ 18
10.	Kanamycin (K)	15µg	≤13	14-16	≥ 17
11.	Nitrofurantion (FD)	30µg	≤14	15-16	≥ 17
12.	Nalidixic Acid (NA)	300µg	≤13	14-18	≥ 19
13.	Norfloxicin (NOR)	30µg	≤12	13-16	≥ 17
14.	Ofloxicin (OFX)	10µg	≤12	13-15	≥ 16
15.	Polymyxin-B (POL-B)	50µg	≤08	09-11	≥ 12
16.	Streptomycin (ST)	300µg	≤06	07-09	≥ 10
17.	Tetracycline (TET)	30µg	≤14	15-18	≥ 19
18.	Tobramycin (MN)	10µg	≤12	13-14	≥ 15

Table 2. Biochemical characterization of isolates of *Vibrio cholera*.

S. No	Test	Result	S. No	Test	Result
1	Gram Staining	Gram negative	8	Motility in distilled water	-ve
2	Shape	Comma shaped	9	Motility in NaCl 80g/L	-ve
3	Oxidase Test	+ve	10	Motility in NaCl 100g/L	+ve
4	Catalase Test	+ve	11	Motility in NaCl free peptone water	+ve
5	Urea hydrolysis	Negative	12	Agglutination with OI group Vibrio cholera antiserum	+ve
6	Indole Test	+ve	13	Hydrogen Sulfide production	-ve
7	Vogus proskeur test	+ve	14	Growth and color changes on Citrate	-ve

Materials and methods

Sampling of Vibrio cholerae

This cross-sectional research study was designed on patients with the sign and history of dysentery, diarrhea, and excessive dehydration between July 1998 to 1999. A total of 303 stool and vomitus samples were collected from Epidemic cell of National Institute of Health (NIH), Islamabad, Pakistan on the basis of reported cases of gastroenteritis/cholera infections in people from all over the country including Rawalpindi, Islamabad, Gilgit and Afghanistan.

Samples were collected in sterile containers with proper labelling and data sheet and transported to Bacteriology Laboratory of Public Health Division under aseptic conditions for further processing and analysis.

Isolation, identification and characterization of Vibrio cholerae

For the purpose of isolation of pathogens, the stool samples were inoculated on the nutrient agar plates by swabbing and incubated for 24 hours at 37°C.

The colonies obtained on nutrient agar were further purified by streaking on Mac Conkey's agar, Blood agar (BA), Thiosulfate citrate bile salts sucrose (TCBS) agar plates and incubated at 37°C for 24-48 hours. The purified organisms were selected for further analysis (Sperandio *et al.,* 1995). Identification was accomplished on the basis of results of microscopic examination of stained smear through Gram staining and cultural appearances and characteristics on different media.

Table 3. In vitro susceptibility of clinical Isolates of *V.cholerae* to different antimicrobial agents during 1998.

S. No	Antimicrobial agents	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
1.	Kanamycin	14	---	7.14	92.86
2.	Streptomycin	26	100	----	----
3.	Trimethoprim/ Sulfamethoxazole	26	100	----	----
4.	Chloramphenicol	22	----	9.1	90.9
5.	Erythromycin	26	----	100	----
6.	Norfloxicin	26	3.85	----	96.15
7.	Nalidixic acid	26	33.85	----	96.15

Table 4. In-vitro susceptibility (%) of clinical isolates of *Vibrio cholerae* to different antimicrobial agents during 1999.

S. No	Antimicrobial agents	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
1.	Tetracycline	131	9.92	13.74	76.34
2.	Norfloxicin	140	----	0.71	99.29
3.	Nitrofurantion	95	94.74	4.21	1.05
4.	Ceftriazone	53	----	5.66	94.34
5.	Ciprofloxicin	143	----	6.29	93.71
6.	Cefotaxime	53	----	11.32	88.68
7.	Ofloxicin	142	----	----	100
8.	Erythromycin	89	15.73	83.15	1.12
9.	Chloramphenicol	134	17.16	70.15	12.69
10.	Nalidixic acid	140	65.71	21.71	13.57
11.	Streptomycin	53	100	----	----
12.	Trimethoprim/Sulfam ethoxazole	54	100	----	----
13.	Ampicillin	124	58.06	30.65	11.29
14.	Cefamendol	81	39.51	19.75	40.74
15.	Kanamycin	4	----	50	50
16.	Tobramycin	131	0.76	10.69	88.55

Different biochemical test were performed to characterize the pathogen such as oxidase, Voges-Proskauer, methyl red, and triple iron sugar tests, hemolysis pattern and motility test (Barua, 1992; Cheesbrough, 1981; Srinivasan *et al.,* 2011)(Ansaruzzaman *et al.,* 1996; Cheesbrough, 1981; Noguerola and Blanch, 2008).

Antibiotic sensitivity testing

Antibiotic susceptibility was determined by the agar disc diffusion method also known as Kirby-Bauer method(Bauer *et al.,* 1966)against 18 commonly used antibiotics by using the Muller-Hinton agar medium. This method is commonly used to determine the in vitro efficacy of commonly used antibacterial agents by measuring the specific length of the zone of inhibition, which is produced due to diffusion of agent into the medium surrounding the disc.

Table 5. Locality wise in vitro susceptibility of clinical isolates of *Vibrio cholerae* to different antimicrobial agents during 1998.

Antimicrobial agents	Rawalpindi (adjacent areas)				Afghanistan			
	Number of isolates tested	Resistant (%)	Intermediate (%)	Susceptible (%)	Number of isolates tested	Resistant (%)	Intermediate (%)	Susceptible (%)
Kanamycin	9	---	7.14	88.89	5	----	----	100
Streptomycin	21	100	----	----	5	100	----	----
Trimethoprim/ Sulfamethoxazole	21	100	----	----	5	100	----	----
Chloramphenicol	20	----	9.1	100	2	----	100	----
Erythromycin	21	----	100	----	5	----	100	----
Norfloxicin	21	4.76	----	95.24	5	----	----	100
Nalidixic acid	21	4.76	----	95.24	5	----	----	100

The name of used antibacterial agents with their specific concentration per disc and the diameter of zone of inhibition are shown in Table 1.

Results

Isolation, identification and characterization of Vibrio cholerae

During 1998-1999, Specimens from 303 patients with diarrhea were cultured. A total of 170 *Vibrio cholera* strains were isolated from 303 stool and vomitus samples. Among these 170 cholera positive patients, 88 were males, 75 were female and 7 were of unknown gender.

During the two year period, 15.66% isolates were from children under the age of five years. 28% of isolates were from young child of 5-20 years and 8% from the age of over 60s. Results obtained from different biochemical tests showing the presence of *Vibrio cholerae* are shown in Table 2.

Seasonal and annual trend is illustrated in figure 1. It is observed that Cholera infections most commonly occur during the monsoon or flood season from June -August, when it is likely the contamination of water drinking sources with infested fecal material.

The reported data of two years 1998-1999 revealed that there is high risk of infection during the rainy season including July-September.

Susceptibility Testing

Strains variations were observed in susceptibility of *Vibrio cholerae* with increasing resistance to commonly used antimicrobial agents during the two year (1998-1999) period.

Norfloxicin and Nalidixic acid were found to be most effective drug against *Vibrio cholerae*, only 3.85% isolates were resistant to both antibiotics while Kanamycin and Chloramphenicol showed resistance rate of 7.14% and 9.1% respectively. Erythromycin was less effective because all the isolates showed resistance to it. Furthermore, Streptomycin and Trimethoprim/Sulfamethoxazole showed high resistance rate and least effectivity (Table 3).

During 1999, most of the isolates of *Vibrio cholerae* were appeared to be as multi-drug resistant. Norfloxicin and Ofloxicin were found to be the most effective drug against multi-drug resistant isolates. Ceftriazone and Ciprofloxacin are also observed to be effective drugs with intermediate resistance rate of 5.66% and 6.29% respectively.

Chloramphenicol, Erythromycin and Nitrofurantion proved to be less effective antibiotics because less than 15% isolates were susceptible to them while in case of Streptomycin and Trimethoprim/Sulfamethoxazole, all the tested isolates were resistant (Table 4).

Locality wise susceptibility pattern

During 1998 all the isolates were obtained from Afghanistan, Rawalpindi and its adjacent areas as shown in Table 5. Nalidixic acid Norfloxicin and kanamycin were observed to be effective drugs for the isolates of Afghanistan with 10.05% susceptibility rate as compared to Rawalpindi and its adjacent areas where all the isolates were immediately resistant to Chloramphenicol while Erythromycin, trimethoprim/ Sulfamethoxazole and streptomycin were found totally resistant to both regions.

Table 6. Locality wise in vitro susceptibility of clinical isolates of *Vibrio cholerae* to different antimicrobial agents during 1999.

Antimicrobial agents	Gilgit				Afghanistan				Rawalpindi (adjacent area)			
	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
Tetracycline	53	----	3.77	96.23	19	5.26	5.26	89.47	59	20.34	25.42	54.24
Norfloxicin	53	----	----	100	18	----	----	100	69	----	1.45	98.55
Nitrofurantion	8	100	----	----	21	100	----	----	66	92.42	6.06	1.51
Ceftriazone	53	100	5.66	94.34	---	----	----	----	78	----	----	----
Ciprofloxicin	48	---	----	100	17	----	5.88	94.12	----	----	10.26	89.74
Cefotaxime	53	----	11.32	88.68	----	----	----	----	68	----	----	----
Ofloxicin	53	----	---	100	21	----	----	100	65	----	----	100
Erythromycin	8	12.5	87.5	----	16	6.25	93.75	----	65	18.46	80	1.54
Chloramphenicol	53	20.75	77.36	1.89	15	20	33.33	46.67	67	13.43	73.13	13.43
Nalidixic acid	53	71.70	28.30	----	21	85.31	9.52	4.76	66	54.56	18.18	27.27
Streptomycin	53	100	----	----		----	----	----	----	----	----	----
Trimethoprim/Sulfamethoxazole	33	100	----	----	21	100	----	----	40	100	----	----
Ampicillin	45	64.44	28.89	6.67	20	35	50	15	59	61.02	25.42	13.56
Tobramycin	45	0.76	8.89	88.55	13	----	30.77	69.23	70	----	8.87	91.43
Cefamendol	----	----	----	----	12	60	33.33	16.67	69	37.68	17.39	44.93

During 1999, all the isolates were susceptible to Norfloxicin, Ciprofloxacin and Ofloxicin with 100% susceptibility rate as demonstrated in Table 6.

Tetracycline was found to be the 2nd most effective drug in Gilgit, Afghanistan and Rawalpindi with susceptibility rate of 96.23%, 89.47, and 54.25% respectively. Similarly Tobramycin was found to be the 3rd most effective drug in Rawalpindi, Gilgit and Afghanistan with susceptibility rate of 91.43%, 88.89%, 69.23% respectively while Cefamendol, Ampicillin, Nalidixic acid and Chloramphenicol were proved to be less effective because more than 50% of the isolates showed resistance against these drugs. Furthermore all the isolates were resistant to Trimethoprim/Sulfamethoxazole and Nitrofurantion.

Temporal Changes in Antibiotic Susceptibility of Vibrio cholerae

Temporal Changes in antibiotic susceptibility of Vibrio cholera from July 1998 to October 1999 were also studied. Isolates of 1999 were found to be most resistant to chloramphenicol and Nalidixic acid with 88.43% and 87.31% respectively as compared to resistance pattern of isolates obtained during 1998 with resistance rate of 3.85% and 9.1% respectively. Kanamycin was found to be 2nd most resistant drug with 50% and 7.14% resistance rate during 1999 and 1998 respectively. No significant antibiotic susceptibility changes were observed in isolates of *Vibrio cholera* against Norfloxicin, Streptomycin, Trimethoprim, Sulfamethoxazole and Erythromycin within two year period (Table 7).

Table 7. Temporal changes in antibiotic susceptibility among Vibrio cholera strains isolated during 1998-1999.

Antimicrobial agent	Resistance (%) during 1998	Resistance (%) during 1999
Kanamycin	7.14	50
Streptomycin	100	100
Trimethoprim/Sulfamethoxazole	100	100
Chloramphenicol	9.1	87.31
Erythromycin	100	98.88
Norfloxicin	3.85	0.71
Nalidixic Acid	3.85	88.43

Antibiotic susceptibility of Isolates from Water Sample

Two isolates of vibrio cholera were separated from water sample, O139 and Non-O1. Both strains of *Vibrio cholera* were susceptible to Chloramphenicol, Norfloxicin, Nalidixic acid, Kanamycin, Trimethoprim/Sulfamethoxazole, Enoxicin and Tobramycin. Both strains were immediately resistant to Erythromycin and Streptomycin (Table 8).

Discussion

Results obtained from random sampling of patients showed an incident rate of 46% and 54% in women and male respectively which is similar to previous findings (Sheikh *et al.,* 1997). This incidence rate was also reported in several other studies (Mahalanabis *et al.,* 1994; Nitsure *et al.,* 1997). The high rate of infection in male population is due to outdoor activities and exposure to contaminated environment. With regard to seasonal variation, it has been demonstrated that most of the cases occur during summer (Wilcox *et al.,* 1992). In the present study maximum isolates of 120 were isolated in the month of August followed by 29 in July 11 in June. Similar reports were also shown in previous data (Sheikh *et al.,* 1997; Nitsure *et al.,* 1997).

According to Deb *et al.,* (1979) the source of infection could be the rainfall in abundant summer season when the average temperature ranges between 25-40°C, that is the optimal range of temperature for the survival of Vibrio cholerae. Cholera is also known as disease of young children.Over the last two years18.9% of the isolates were from the children under the age of 5 years.

The 33.6% of isolates were from children of 5-20 years (Deb *et al.,* 1979). However according to Sheikh *et al.,* (1997), 44% of the isolates were from the children under the age of 5years, 12% from young of 5-20 years and 12% from patients over the age of 60 years (Sheikh *et al.,* 1997), The first characterized isolates of O139 strains were from Madras in January 1992 (Albert, 1996). *Vibrio cholerae* O1 biotype ELT reappeared in 1993 and became the dominant serotype and replace the O139, established itself in Karachi in 1993 (Fisher-Hoch *et al.,* 1993) and disappeared in 1996. It is proposed that the dramatic decrease in prevalence of *Vibrio cholerae* O139 were associated with changes for example in colonization factor that determine long term persistence in aquatic environment (Faruque *et al.,* 1997).

Studies in Bangladesh indicated that the O139 serotype survived better than the O1 strain in aquatic environment (Islam *et al.*, 1993; Khan *et al.*, 1988; Sheikh *et al.,*
1997).

Antibiotic Susceptibility

Generally most of *Vibrio cholera* isolates are susceptible to Vibriocidals, Penicillin, Cephalosporin, Quinolone, Aminoglycosides, Lincosides, Antibiomimetics, Tetracycline, Sulfonamides and Co-trimoxazole but it is still a clinically significant pathogen due to its resistance to many agents. Widespread use and misuse of antibiotics imposes immense selective pressure for the emergence of antibiotic resistance in bacteria and as a consequence the development of antibiotic is inevitable (Gillepsie and Skurray, 1987). Effects to control infectious disease more comprehensively are undermined not only by socioeconomic conditions but also by nature of pathogenicity of pathogen. Isolates of infectious diseases have become so resistant to antimicrobial drugs by horizontal gene transfer that they are almost untreatable (Fuchs, 1998).

Table 8. Antibiotic susceptibility pattern of *Vibrio cholerae* strains isolated from water samples during 1998.

Strain	Date of isolation	O serotype	Antibiogram
NE 46	06-7-98	*Vibreo cholera* 0139	CH^s,NOR^s,NA^s, K^s, SXT^s, ENX^s, TOB^s, ST^R, E^I
NE 124	13-08-98	*Vibreo cholera* Non-O1	CH^S, NOR^S, NA^S, K^S,SXT^R, ENX^S, TOB^S, ST^S, E^I

CH= Chloramphenicol; Nor= Norfloxicin; NA= Nalidixic Acid; K= Kanamycin; SXT=Trimethoprim/ sulfamethoxazole; ENX= Enoxicin, TOB=Tobramycin, ST=Streptomycin, I= Intermediate resistant; S= Susceptible; R= Resistant.

In the present study strain variation was observed in the changing antibiograms of isolates over the period of observation with increasing resistance to commonly used antibiotics. The resistance strains have also been reported form Russia, India, Africa and South America (Mhalu *et al.,* 1979; Sundaram and Murthy, 1984; Tabtieng *et al.,* 1989; Ved'mina *et al.*, 1984; Weber *et al.,* 1994).

Among the penicillin group, Ampicillin was tested against 124 isolates, 41.94% strains were almost intermediately resistant and 58.06% were totally resistant. Ciortino*et al.,* (1995) observed that 16.5% of strains of *Vibrio cholera* were resistant to Ampicillin from around the world.Several studies were carried out in India and found a dramatic increase in resistance *of Vibrio cholerae* to Ampicillin between the period of 1992-94 (Mukhopadhyay *et al.,* 1995).
Aminoglycosides group including Streptomycin, Kanamycin and Tobramycin were also tested against *Vibrio cholera* isolates. All the isolates were totally resistant to Streptomycin while 88.55% of the total isolates were sensitive to Tobramycin while 83.33% isolates showed sensitivity to Kanamycin.

Indian strains of *Vibrio cholerae* were especially resistant to Streptomycin between 1992-94 (Mukhopadhyay *et al.,* 1995). Plasmid born resistant strains to Streptomycin and Kanamycin was found in *Vibrio cholerae* OI ELT or isolates involved in a major epidemic in 1985-86 in the Horn of Africa, same results were demonstrated by later studies in Ecuador. The sensitivity of Kanamycin in the present study is contradictory to results of Coppo *et al.,* (1995) and Weber *et al.,* (1994). The geographical barriers, medications of antibiotics uses and seasonal variations are the possible reasons of difference in resistance rate.

Chloramphenicol, a Lincosamides is very active against gram negative bacteria. In the present study 90.9% of isolates were sensitive to it during 1998 while only 12.69% of isolates showed sensitivity to during 1999 while in previous study carried out by Sheikh *et al.,* (1997), it was found that 92% of the total isolates were sensitive to Chloramphenicol during 1992-1993. Similar results were reported by Nitsure *et al.,* (1997) while Khan *et al.,* (1989) also found 100% sensitivity of isolates against Chloramphenicol.

In previous reports 100% isolates of *Vibrio cholera* were resistant to Trimethoprim/Sulfamethoxazole which is commonly being used against gastroenteritis (Sheikh *et al.,* 1997). All the Similar results have been previously reported by different researchers favor the outcomes of present study (Sheikh *et al.,* 1997 ; Khan *et al.,* 1988), The complete resistance against this group of drugs signifies the fast and appropriate diagnosis between cholera and other gastroenteritis, which otherwise unnecessary delay may result in the death of the patients.

The Quinalones group has been widely used against wide range of gram negative bacterial infections. From this group of antibiotics, Nalidixic acid, Norfloxicin and Ciprofloxacin were tested against isolates of *Vibrio cholera*. Almost all the isolates (96.15%) were sensitive to Nalidixic acid and Norfloxicin during 1998. These findings coincide with the previous studies carried out by Sheikh *et al.,* (1979).

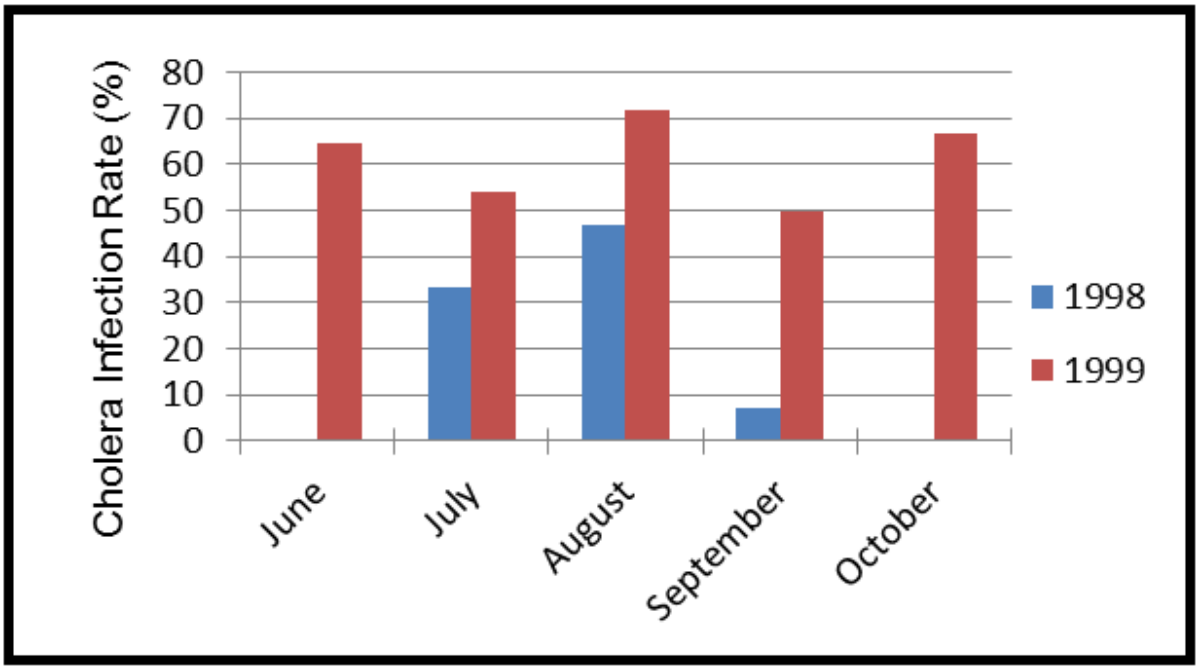

Fig. 1. Effects of Seasonal variations on Infection rate of *Vibrio cholerae*.

Erythromycin, a macrolides, was noted to be resistant to *Vibrio cholerae* during the present study. All isolated strains of *Vibrio cholera* were intermediately resistant during 1998 while in the next year some of the strains were totally resistant to it. Tetracycline is demonstrated to be an effective drug against *Vibrio cholera*, 76.34% of isolates were sensitive to it. These results showed similarities with the previous reports demonstrated by Khan *et al.,* (1989).

Among the Antibiomimetics group, Nitrofurantion was investigated and all the isolates showed resistance against it. Antibiotics from Cephalosporin group including Cefamendol, Cefotaxime, Ceftriazone and were tested against *Vibrio cholerae* and sensitivity rate was found to be 40.74%, 86.68% and 94.34% respectively.

V*ibrio cholerae from* Aquatic environment were studied because several epidemiological studies reveal that it is a best source for the sporadic and epidemic diseases (Islam *et al.,* 1993).For that purpose water samples were also studies. Out of 76 water samples two were positive for 0139 Ogawa and no-01 Inaba. Recently large number of epidemics are reported by these strains in Pakistan (Sheikh *et al.,* 1997), Bangladesh (Albert, 1996) and India(Ramamurthy *et al.,* 1993). The serogroup 0139 survive better than 01 strain in the aquatic environment under stress conditions (Khan *et al.,* 1989). The 0139 strain was found sensitive to Chloramphenicol, Tobramycin, Enoxicin, Norfloxicin, Nalidixic acid and Cotrimoxazole while sensitive to Streptomycin and Kanamycin and intermediately resistant to Erythromycin.

The second isolate from water sample was non-01 Inaba not agglutinated with 01 polyvalent antiserum. It was also sensitive to Chloramphenicol, Tobramycin, Enoxicin, Norfloxicin, Nalidixic acid, Cotrimoxazole and Kanamycin while resistant to Streptomycin and intermediately resistant to Erythromycin.

The population size for the isolation of *Vibrio cholerae* and their susceptibility pattern were studied from relatively large geographical area including Rawalpindi (adjacent areas), Gilgit and Afghanistan. Throughout the study, same pattern of antibiograms was observed in all these regions except few variations e.g. Chloramphenicol; in 1998 the isolates selected from Afghanistan were resistant to it in contrast to isolates of Rawalpindi region with intermediate sensitivity. Same variations were also observed during 1999 in susceptibility pattern of *Vibrio cholerae* to Tetracycline and Chloramphenicol. Isolates obtained from Rawalpindi and Gilgit were most sensitive to the respective antibiotics while the isolates of Afghanistan were most sensitive to Nalidixic acid and Cefamandole as compared to those of Rawalpindi and Gilgit region (Okeke *et al.,* 2005).

Conclusions

The findings obtained from this study will help to understand the distribution of antibiotic resistant strains, define suitable monitoring programs, and to provide evidence for assessing the exposure of this pathogen at the consumption sites. Ofloxicin, Norfloxicin and Nalidixic acid were found to be most effective drug against *Vibrio cholerae*, with highest susceptibility rate and effectiveness.

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