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Research Article
Isolation and Identification of *Salmonella paratyphi* from Enteric Fever Patients at Different Hospitals of Quetta City

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Abstract

Background and Objective: *Salmonella paratyphi* cause enteric fever which is an important public health problem worldwide. In Pakistan incidence is increasing and affect all age groups. Therefore, the present research was designed to study the different microbiological aspects of *Salmonella paratyphi*. **Materials and Methods:** The study was conducted to identify the *Salmonella paratyphi* from blood samples in Quetta. Total 480 blood samples were collected from different hospital of Quetta. Specific colony characters, microscopic examination, biochemical tests and PCR were used for identification of *Salmonella paratyphi*. **Results:** Total 55% samples were positive and 45% were negative for *Salmonella paratyphi*. Results showed that males (34%) were more affected with *Salmonella paratyphi* as compare to female (20%). Age wise distribution revealed that *Salmonella paratyphi* was high in 20-30 years (38%) followed by 10-20 years (9.16%) and 1-10 years (7.5%) age group patients. Paratyphoid fever cases were significantly high (25.41%) in Pashtoon population as compare to other population of Balochistan. The 40% paratyphoid fever was observed in the patients with low socioeconomic status, 9.16% in middle socioeconomic status and 5.83% in the patients belonged to high socioeconomic status. The *Salmonella paratyphi* were sensitive to Chloramphenicol (23 mm), Amikacin (24 mm), Gentamicin (12 mm), Quinolones (23) and Polypeptide (13 mm) classes. The PCR based identification of *Salmonella paratyphi* showed clear bands of 329 bp of *flic*-a gene. **Conclusion:** To control paratyphoid fever strong initiatives must be taken to improve water sanitation, hygiene level, supply of save drinking water and vaccination is recommended in order to eradicate the disease.

Key words: Blood samples, paratyphoid fever, *Salmonella paratyphi*, *flic*-a gene, socioeconomic status

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella paratyphi is Gram-negative, rod shaped, facultative anaerobe, non-encapsulated, non-spore forming, flagellated and motile bacteria[1]. Worldwide three serotypes of *Salmonella paratyphi* are described that are *Salmonella paratyphi*[2] A, B and C. *Salmonella paratyphi* transmission is through fecal-oral route or via consumption of contaminated food/water as well as interaction with chronic asymptomatic carriers[3]. The *S. paratyphi* cause enteric fever which is an important health issue in many developing countries[4]. The incidence is increasing globally[2], particularly in endemic regions such as certain provinces in China and Pakistan[3]. The disease mortality rate[5,6] is up to 30 and 90% of deaths is due to enteric fever occur in Asia[7].

Humans are the only reservoir and natural host for *S. paratyphi*[8]. The *S. paratyphi* can be isolated from paratyphoid fever patients' blood for diagnosis[9]. Paratyphoid fever is highest in teenagers and young adults as compared to typhoid fever that is common in children[10].

The *S. paratyphi* antibiotic resistance seems to be an emerging problem and disease occur due to poor sanitary conditions and lack of facilities[11]. The *S. paratyphi* and *S. typhi* infections usually treated with Ciprofloxacin. In 1990s, increasing multidrug resistant strains of *S. paratyphi* and decreasing Ciprofloxacin susceptibility have been reported *S. paratyphi* with decreased susceptibility to Fluoroquinolones and resistance to Nalidixic acid are commonly reported in India, Pakistan, Japan, China and Southeast Asia[12]. The *S. paratyphi* was more resistant to Nalidixic acid and Ciprofloxacin instead of *S. typhi*[13] and reported high mutation rate of gyrA gene *S. paratyphi* and such strains are resistant to Nalidixic acid[14]. The knowledge of predominate microbial patterns, however, provide at least as important basis or initial decision about empirical therapy of *Salmonella paratyphi* infection. The issues mentioned highlight the need for continuing the research at national and local level to attain effective therapy of *Salmonella paratyphi* infection as well as to promote rational prescribing of anti-microbials with consequent slowing of the development of resistance to both existing and new agents. In this light, to do away with the lack of authentic data on the etiology of *Salmonella paratyphi* infection in Quetta Balochistan. Therefore, the present research was designed to study the different microbiological aspects of *S. paratyphi*.

MATERIALS AND METHODS

Research study was conducted in (CASVAB) Center for Advanced Studies in Vaccinology and Biotechnology University of Balochistan, Quetta from 1 January, 2017 to 1 March, 2018.

Collection of blood samples: A total 480 blood samples were aseptically collected from different hospital of Quetta. The samples were collected by venipuncture victimization sterile technique. The samples were taken to laboratory in cold chain condition for further process. All information about patient history, age, gender was collected through predesign proforma.

Processing of samples: Collected samples from hospital were inoculated directly into blood culture bottles containing 50 mL brain heart infusion broth and incubated at 37°C for 24 h. The samples were streaked on selective and differential media plates and incubated at 37°C for 24 h. All isolates were triple cloned to get pure growth for gram staining, different biochemical tests (Indole, Methyle red, Voges-proskeuer, Simmon Citrate, Sugar Fermentation test, Oxidase and Catalase) and PCR.

Anti-microbial susceptibility test: Anti-microbial susceptibility was checked by disc diffusion method according to clinical and laboratory standards institute guidelines[15] (CLSI, 2006). Suspension of bacterial cells (0.5 McFarland) was prepared and dispersed on the surface of Mueller-Hinton agar (Oxoid, UK) and incubated at 37°C for 24 h. The isolates were considered as sensitive and resistant to particular anti-microbial agent on the basis of inhibitory zone.

Polymerase chain reaction for *Salmonella paratyphi*: The DNA was extracted from culture through DNA purification kit (Hiper® Bacterial Genomic DNA Extraction Teaching Kit). The template DNA was stored at -20°C for further use. Primer of following sequence F: (AATCAACAACAACCTGCAGCG) R: (TAGTGCTTAATGTAGCCGAAGG) were design to allow amplification of 329 bp fragment of *fliC*-a gene. For PCR amplification 25 µL volume reaction mixture was used which contain 12 µL master mix (2x AmpMaster™ *Taq*), 9 µL grade water, 1 µL of each primer (forward, reverse) and 2 µL template DNA. The PCR cycling for reaction mixture were: Initial melting 95°C for 5 min, denaturing 95°C 30 sec, annealing 48°C for 1 min, extension 72°C for 45 sec, final

extension 72°C for 10 min. The final PCR product was run on 1.5% agarose gel and observed under UV light.

RESULTS

Total 480 blood samples were collected from different hospitals of Quetta. Out of which 55% were positive and 45% negative for *S. paratyphi* as shown in Fig. 1.

Gender wise distribution result showed that the male (34%) were more affected with paratyphoid fever as compare to female (20%) as shown in Fig. 2.

Age wise distribution revealed that *Salmonella paratyphi* was high in 20-30 years (38%) followed by 10-20 years (9.16%) and 1-10 years (7.5%) age group patients as shown in Fig. 3.

Race wise distribution results shown that paratyphoid fever cases were significantly high (25.41%) in Pashtoon followed by 15.83% in Baloch, 8.33% in Punjabi and 5.41% in Hazara as shown in Fig. 4.

The 40% paratyphoid fever were observed in patients with low socioeconomic status, 9.16% in middle socioeconomic status and 5.83% in the patients belonged to high socioeconomic status as shown in Fig. 5.

The *S. paratyphi* was identified through differential media, selective media, gram staining and different

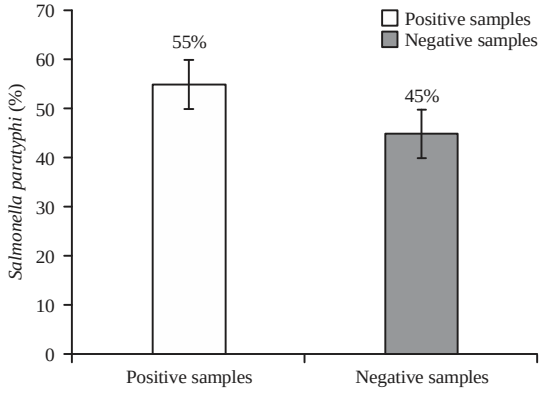

Fig. 1: Positive and negative samples of *S. paratyphi* isolated from blood samples

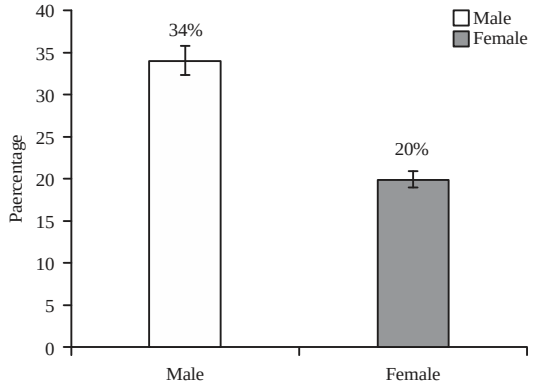

Fig. 2: Gender wise distribution of patients affected by *S. paratyphi*

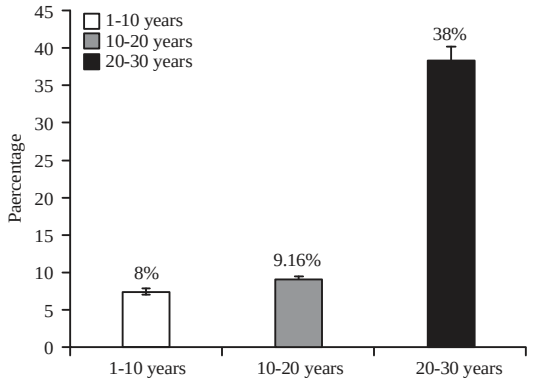

Fig. 3: Age wise distribution of patients affected by *S. paratyphi*

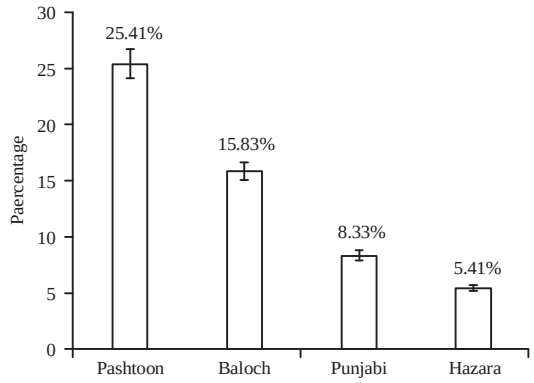

Fig. 4: Race wise ratio of paratyphoid infection in patients positive admitted in different hospitals of Quetta city

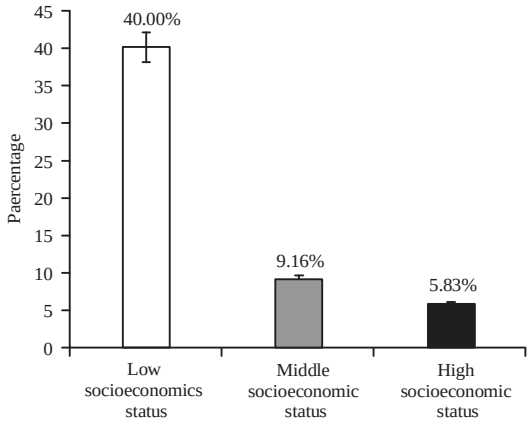

Fig. 5: Distribution of positive patients based on socioeconomic status

Table 1: Biochemical characterization for *S. paratyphi* isolated from blood samples

Biochemical characterization	Different media								
	MacConkey agar			Xylose lysine deoxycholate			Salmonella shigella agar		
Salmonella paratyphi	Non-lactose fermenting, cloudy, non-hemolytic, humid colonies			Salmonella colonies were red with black centers,3-5 mm in diameter			Salmonella colonies appear colorless with black centers		
Gram's staining	Gram staining			Shape			Size		
	-			Rods			1-3 µm in length 0.5-0.7 µm in wide		
Biochemical tests	Motility	Indole	Citrate utilization	MR	VP	Urease	Catalase	Oxidase	
	+	-	+	+	-	+	+	-	
Sugar fermentation tests	Glucose	Sucrose	Sorbitol	Rhamnose	Lactose	Mannitol	Dulcitol	Xylose	Inositol
	+	-	+	+	-	+	+	+	-

+: Positive, -: Negative

Table 2: Antibiotic susceptible pattern of *Salmonella paratyphi* isolated from blood samples

Classes	Antibiotics	Abbreviations	Zone of inhibition (mm)	Remarks
Chloramphenicol	Chloramphenicol	C	23	Sensitive
Cephalosporines	Cefotaxime	CTX	0	Resistance
Penicillin	Amoxicillin	AML	0	Resistance
Aminoglycoside	Gentamicin	GEM	12	Sensitive
	Streptomycin	STR	0	Resistance
	Neomycin	K	0	Resistance
	Kanamycin	K	0	Resistance
	Amikacin	AMK	24	Sensitive
Polypeptide	Colistin sulphate	CT	13	Sensitive
Quinolones	Ciprofloxacin	CIP	23	Sensitive
Glycopeptides	Oxalinic acid	OXA	0	Resistance
	Vancomycin	VA	0	Resistance
Flagyl	Metronidazole	MTZ	0	Resistance
Lincosamides	Lincomycin	L	0	Resistance
Macrolides	Erythromycin	E	0	Resistance
Sulfonamides	Trimethoprim	W	0	Resistance

biochemical test. *Salmonella paratyphi* produce non-lactose fermenting colonies on MacConkey agar while on selective media *Salmonella paratyphi* produce red colonies with black center on Xylose lysine Deoxycholate and colorless colonies with black on Salmonella Shigella agar. The *S. paratyphi* have methyl red, motility, simmon citrate, urease and catalase positive tests. The *Salmonella paratyphi* ferment following sugar such as glucose, sorbitol, rhamnose, mannitol, dulcitol and xylose as shown in Table 1.

Antimicrobial susceptibility test: The *Salmonella paratyphi* were sensitive to Chloramphenicol, Gentamicin, Polypeptide, Quinolones and Amikacin while resistance to Penicillin, Amoxicillin, Cephalosporines, Cefotaxime, Glycopeptides, Aminoglycosides, Flagyl, Lincosamides, Macrolides and Sulfonamides as shown in Table 2.

Molecular detection of *Salmonella paratyphi* : In present study PCR based identification of specific gene was used to detect *Salmonella Paratyphi.* All isolates of *Salmonella paratyphi* was used to produce the specific size of 329 base pair fragment of *flic*-a gene as shown in Fig. 6.

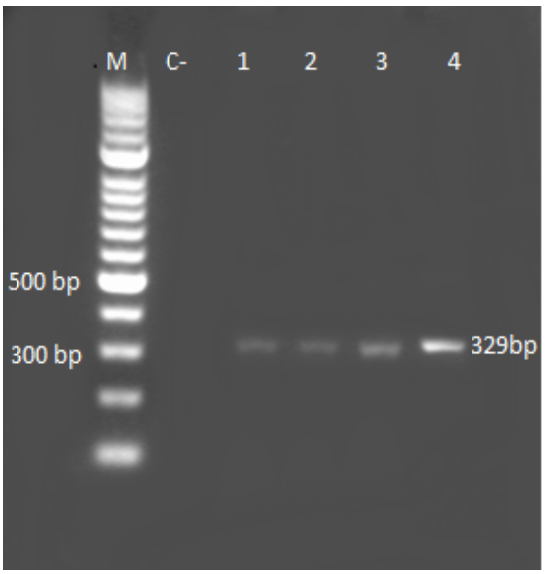

Fig. 6: Molecular identification of *Salmonella paratyphi* in blood samples by direct using *flic*-a genes specific primer
Lane M:100 plus DNA ladder, Lane 1 negative control, Lane 2-4 positive samples

DISCUSSION

Paratyphoid fever caused by *Salmonella paratyphi* is rising in the country due to the non-availability of safe drinking water or ingestion of food contaminated with pathogen. In our study 480 blood samples were collected in which 55% were positive and 45% were negative for *Salmonella paratyphi*. Amongst them male (34%) were more affected with *Salmonella paratyphi* as compare to female (20%). Present finding was similar with the finding of Khosla[16] and Sood and Taneja[17]. This study showed that *Salmonella paratyphi* affected all age group usually 20-30 years (38%) followed by 10-20 years (9.16%) and 1-10 years (7.5%).

In current study *Salmonella paratyphi* was identified through differential medium, gram staining and different biochemical tests like IMVIC, catalase, oxidase, sugar fermentation, citrate utilization and urease test similar finding were reported by Wanjiru[18].

Salmonella paratyphi were sensitive to Chloramphenicol (23 mm), Gentamicin (12 mm), Amikacin (24 mm), Polypeptide (13 mm), Quinolones (23 mm) while resistance to Cephalosporines, Penicillin, Streptomycin, Neomycin, Kanamycin, Glycopeptides, Flagyl, Lincosamides, Macrolides and Sulfonamides, similar results were found by Afroze *et al*.[19].

In present study PCR based identification of specific gene was used to detect *Salmonella paratyphi.* All isolates of *Salmonella Paratyphi* was used to produce the specific size of 329 base pair fragment of *flic*-a gene, similar results were found by Ali *et al*.[20] and Kulkarni *et al*.[21].

This study was largely restricted to the research setting but in future, it will provide large scale surveillance data regarding the organisms that cause paratyphoid fever, in particular changes in Salmonella Paratyphi carriage and disease in the context of vaccination.

CONCLUSION

Paratyphoid fever is endemic in Quetta, Balochistan. *Salmonella paratyphi* show resistance against number of antibiotics so there is need to find that anti-microbial drug which is sensitive to this organism. In order to control disease strong initiatives must be taken to improve water sanitation, hygiene level, supply of save drinking water and vaccination is recommended in order to eradicate the disease.

SIGNIFICANCE STATEMENT

Salmonella paratyphi isolates was found to be increasing than previously reported and no study on the isolation and identification of *Salmonella paratyphi* in blood has been reported in the patients of Balochistan. The increasing drug resistant *Salmonella paratyphi* must be controlled to prevent epidemics of enteric fever within community. For paratyphoid fever, food and water precautions are the only prevention method as no vaccines are available. There is a strong need for determining the role of *Salmonella paratyphi* and also make effective procedure that help to prevent spread of infection.

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