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# A STUDY ON BIOTYPING, BACTERIOCIN TYPING AND DRUG RESISTOGRAM OF *Salmonella* Paratyphi B ISOLATES FROM ANIMALS, THEIR PRODUCTS AND ENVIRONMENT

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**In this study 39 isolates of *Salmonella* Paratyphi B from animals/environment and one of human origin were characterized to understand epidemiological distribution of this pathogen. Of the 40 isolates under the study, 24 belonged to *S.* Paratyphi B var java (utilizing d-tartrate) and 16 were *S.* Paratyphi B (not utilizing d-tartrate). Fermentation of xylose and sorbitol and production of DNase were able to further subtype the two groups of *S.* Paratyphi B (classical-SPB, and var Java-SPBJ). Among 24 isolates of SPBJ, 6 biovars namely, biovar I (xylose+, sorbitol+, DNase+) 15 isolates, biovar II (xylose+, sorbitol+, DNase-) 2 isolates, biovar III (xylose-, sorbitol+, DNase+) 2 isolates, biovar IV (xylose-, sorbitol+, DNase-) 2 isolates, biovar V (xylose+, sorbitol-, DNase+) 2 isolates and biovar VI (xylose-, sorbitol-, DNase+) one isolate, could be identified. Isolates of biovar II to VI were isolated only from Mumbai. Of the 16 SPB isolates, 11 (10 from fish and 1 from khoa) belonged to biotype I while remaining 5 (1 pork, 2 sewage, 1 fish and 1 human) to biotype II. Multiple drug resistance (MDR) pattern of *S.* Paratyphi B isolates revealed their resistance to dicloxacillin, cefazolin, colistin and ceftazidime and sensitiveness to augmentin, ciprofloxacin, nalidixic acid, enrofloxacin, tetracycline, lomefloxacin, crystal violet, acriflavin and mercuric chloride. Other frequently resisted drugs were nitrofurantoin (17 SPBJ and 9 SPB), streptomycin (2 SPB and 9 SPBJ) and cephalexin (1 SPBJ and 7 SPB). No correlation could be found out between biovar and antibiogram of different isolates. All the SPB strains were of same bacteriocin type while 24 SPBJ isolates could be classified into 5 bacteriocin types. Bacteriocin type I was the predominant (19 isolates) and prevalent all over India while other four bacteriocin types (II, III, IV and V) were prevalent only in Mumbai. The study revealed the significance of bacteriocin typing, biotyping and resistogram in understanding the epidemiology of paratyphoid infection in India.**

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Salmonellosis has been reported to outnumber all other bacterial food poisoning outbreaks all over the globe, irrespective of development in economy and hygiene. In India, however, owing to inadequate disease reporting system and lack of efforts to determine the etiology in clinical cases of food poisoning, it is difficult to assess the magnitude of food- borne salmonellosis. Among the emerging zoonotic serotypes, *Salmonella* Paratyphi B, primarily thought to be a human adapted serotype has also been isolated from diseased as well as healthy cattle (Nath *et al.,* 1970), pig (Gupta *et al.,* 1984), poultry (Saxena *et al.,* 1983), dog (Gupta *et al.,* 1984), fish (Singh, 1989: Yadav, 2001), guinea pigs and house rats (Gupta *et al.,* 1981) in India. *Salmonella* Paratyphi B has been seen to persist up to 10 days even in cockroaches, which may act as mechanical carrier for this pathogen (Singh *et al.,* 1995). Ever growing animal husbandry and persistence of *Salmonella* in animals and their environment may be the cause of re-emergence of paratyphoid (Lassen e*t al.,* 1999). In many of those who become carriers, it may enhance the risk of cancer of gall bladder, pancreas, colorectum and lungs (Caygill *et al*., 1994). Besides, it may also cause abortion in cattle (Thomas, 1978), gastroenteritis in dogs and pigs (Gupta *et al.,* 1984). Because of recent emergence of *S.* Paratyphi B as a zoonotic pathogen, a number of typing methods have been tried to determine the relation between epidemic/ endemic strains isolated from different sources. These include phage typing, biotyping, colicin typing, antibiotic sensitivity pattern and reactivity to specific monoclonal antibodies etc. (Barker *et al.,* 1988). Differentiation of strains to sub-serovar level is essential during investigation of outbreaks of salmonellosis. Therefore, this study was undertaken to standardize some assays for phenotypic diversity in Indian strains of *Salmonella* Paratyphi B.

## MATERIALS AND METHODS

**Bacterial strains**: A total of 16 classical *Salmonella* Paratyphi B and 24 *S.* Paratyphi B var Java strains, isolated during 1977 to 2001 from various sources in different regions of India (Table 1 and Table 2) were revived from the stocks available at National *Salmonella* Centre (Veterinary), Indian Veterinary Research Institute, Izatnagar and confirmed serologically (Edwards and Ewing, 1986) by slide agglutination test using somatic (4, 5, 12) and flagelllar (b) group specific antisera available at the Centre. The strains were maintained in semisolid phosphate buffered agar and on nutrient agar (HiMedia, Mumbai) slants at 4ºC.

**Biotyping:** The 18 h cultures in 2% peptone water were inoculated to study the acid and gas production in glucose, acid production in xylose, sorbitol, dulcitol, adonitol, mannitol, mannose, maltose, cellobiose, glycerol (using 1% peptone water

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with 0.5% of respective sugars), utilization of D-tartrate and mucate (using organic acid media) and decarboxylation of lysine, arginine and dehydrolation of ornithine (using decarboxylase test media).

Strains were stabbed on egg yolk agar plates, incubated at 37ºC for 48 h and examined after every 12 h to observe opacity/ clearance zone around lecithinase/ phospholipase A producing colonies (Joshi and Sharma, 1991).

For DNase test, DNase agar (Hi-Media, Mumbai) was prepared as per producer's instructions and plates were made for spot inoculation of the test strains. After incubation at 37ºC for 48 h, plate was exposed to ultra violet rays (150 to 3900 Aº) for half an hour and re-incubated at 37ºC for 12 h. Colonies showing development of violet zone were considered positive for DNase activity.

For caseinase production, milk agar (made by adding 10 ml of tyndallized milk to 90 ml of molten yeast extract agar, Hi-Media, Mumbai) plates were spot inoculated with test cultures and incubated at 37ºC for 24 h. Clearance zone around colonies was taken as the indication of positive test (Cruickshank *et al.,* 1975).

Stab cultures made in gelatin agar tubes were incubated upto 7 days to observe gelatin liquefaction. Before reading the results, the cultures were held at 4°C for half an hour (Cruickshank *et al.,* 1975).

**Bacteriocin typing:** Agar overlay method of Barker and Old (1979) with little modifications was followed to detect bacteriocin production. Briefly, single colony of each of the strains was picked up from Hektoen enteric agar plates and spots inoculated onto a single trypticase soy agar (TSA) plate and incubated for 48 h at 37ºC. The macrocolonies in the plate were killed by exposure to ultra violet rays for one hour.

To check the bacteriocin sensitivity of individual strain, the strain was grown in Luria Bertani (LB) broth for 18 h. 0.2 ml of culture was mixed aseptically with 10 ml of molten (at 50ºC) trypticase soy broth (Hi-Media, Mumbai) containing 1% agar (Hi-Media, Mumbai) and poured over ultra violet rays exposed TSA plate and allowed to solidify. The plate was then incubated at 37ºC for 18 h. A clear zone surrounding the site of any of the spotted strains on TSA plate was read as positive and zone was measured. Similarly, bacteriocin sensitivity of all the 40 isolates used in this study was determined.

**Drug resistance and antibiogram:** All the isolates were examined for drug resistance using 23 antimicrobial agents through disk-diffusion method of Bauer *et al*. (1966). The antibiotic disks of dicloxacin (1µg), cefazolin (30µg), ceftazidine (30µg), colistin sulphate (100µg), augmentin (30µg), ciprofloxacin (5µg), nalidixic acid (30µg), enrofloxacin (10µg), tetracycline (100µg), lomefloxacin (30µg), ampicillin (25µg), nitrofurantoin (50µg), trimethoprim (25µg), cephalexin (30µg), chloramphenicol (50µg), kanamycin (30µg), gentamicin (10µg), streptomycin (25µg), sulphafurazole (300µg), ceftriaxone (30µg) (all from Hi-Media, Mumbai), crystal violet (100 µg), acriflavin (100 µg) and mercuric-chloride (100 µg) were used. Diameter of inhibition zone surrounding the disks (after 24 h of incubation at 37ºC) was measured and matched with respective standard zone diameter to interpret the test culture as resistant or sensitive.

## RESULTS

**Biotyping:** Of the 40 isolates under the study 24 were d-tartrate positive belonging to biovar *S.* Paratyphi B var Java and other 16, not utilizing d-tartrate, belonged to classical *S.* Paratyphi B group. All the 40 isolates fermented glucose, maltose, mannose, mannitol, dulcitol but could not ferment adonitol, cellobiose and glycerol, however variable results were observed with xylose and

**Table 1. *Salmonella* Paratyphi B var Java strains under the study.**

| Sl. No. | Strain No. | Place of Isolation | Source of Isolation |
|---|---|---|---|
| 1. | 2601 | Mumbai | Knife of poultry shop |
| 2. | 2602 | Mumbai | Meat shop |
| 3. | 2603 | Mumbai | Wash water poultry shop |
| 4. | 2604 | Mumbai | Meat shop |
| 5. | 2605 | Mumbai | Poultry shop |
| 6. | 2606 | Mumbai | Poultry shop |
| 7. | 2607 | Mumbai | Poultry shop |
| 8. | 2608 | Mumbai | Meat shop |
| 9. | 2609 | Mumbai | Poultry shop |
| 10. | 2610 | Mumbai | Meat shop |
| 11. | 3259 | Ranchi | Fish |
| 12. | 3261 | Ranchi | Fish |
| 13. | 3264 | Ranchi | Fish |
| 14. | 3265 | Ranchi | Fish |
| 15. | 3266 | Ranchi | Fish |
| 16. | 3268 | Ranchi | Fish |
| 17. | 3274 | Ranchi | Fish |
| 18. | 723 | Bareilly | Food product |
| 19. | 801 | Agartala | Calf faeces |
| 20. | 885 | Kolkata | Dog stool |
| 21. | 1986 | Kolkata | Fish |
| 22. | 1991 | Kolkata | Fish |
| 23. | 2598 | Mumbai | Meat shop |
| 24. | 2599 | Mumbai | Poultry shop |

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sorbitol (Table 3). All strains decarboxylated lysine and arginine and dehydrolated ornithine and utilized sodium mucate. None of the 40 strains produced gelatinase, lecithinase, phospholipase A or caseinase. However, with DNase test they could be classified into DNase producer and non-producer.

On the basis of fermentation of xylose and sorbitol and production of DNase, 24 isolates of SPBJ could be classified in 6 biovars (Table 3), while 16 SPB isolates belonged to only two biovars (Table 3). All 6 biovars of SPBJ were detected among isolates from Mumbai.

**Table 2. Classical *Salmonella* Paratyphi B strains under the study.**

| Sl. No. | Strain No. | Place of Isolation | Source of Isolation |
|---|---|---|---|
| 1. | 3256 | Ranchi | Fish |
| 2. | 3257 | Ranchi | Fish |
| 3. | 3258 | Ranchi | Fish |
| 4. | 3260 | Ranchi | Fish |
| 5. | 3267 | Ranchi | Fish |
| 6. | 3269 | Ranchi | Fish |
| 7. | 3271 | Ranchi | Fish |
| 8. | 3273 | Ranchi | Fish |
| 9. | 3275 | Ranchi | Fish |
| 10. | 651 | Kolkata | Rat |
| 11. | 663 | Kolkata | Pig |
| 12. | 896 | Hyderabad | Pig |
| 13. | 923 | Mathura | Sewage |
| 14. | 1294 | Chennai | Khoa |
| 15. | 1984 | Kolkata | Fish |
| 16. | 2403 | Bareilly | Human |

**Bacteriocin typing:** Among the 24 strains of SPBJ only 5 could produce any detectable bacteriocin activity inducing 3-8 mm zone of growth inhibition. None of the SPB strain produced bacteriocin. Based on the bacteriocin sensitivity pattern SPBJ isolates could be divided into 5 bacteriocin types (Table 4). Two SPB isolates were also found to be sensitive to bacteriocin produced by SPBJ. One non-pathogenic strain from Kolkata fish (E1984) was sensitive to bacteriocin produced by 4 strains.

**Drug resistance and antibiogram:** Antimicrobial drug resistance studies on 24 SPBJ and 16 SPB strains revealed 10 and 8 resistance pattern (Table 5), respectively. All the strains were resistant to dicloxacin, cefazolin, ceftazidime and colistin sulphate and sensitive to augmentin, ciprofloxacin, nalidixic acid, enrofloxacin, tetracycline, lomeflaxacin, crystal violet, mercuric chloride, acriflavin. Additionally, all the SPBJ strains were sensitive to gentamicin and SPB strains to ampicillin, trimethoprim, choramphenicol, sulfafurazole and ceftriaxone.

**Table 4. Bacteriocin types in *Salmonella* Paratyphi B var Java.**

| Type | Bacteriocin sensitivity pattern | No. of isolates |
|---|---|---|
| I | Not inhibiting any susceptible strain | 19 |
| II | Inhibiting strain No. 2604, 885, 2598, 1984 | 2 |
| III | Inhibiting strain No. 2598 | 1 |
| IV | Inhibiting strain No. 3264 and 1984 | 1 |
| V | Inhibiting strain No. 3275 and 1984 | 1 |

**Table 3. Biotypes of *Salmonella* Paratyphi B var Java (SPVJ) and classical *Salmonella* Paratyphi B (CSPB) biovars prevalent in India**

| Biotype | Characteristics | Biovar (numbers) | Sources of isolation |
|---|---|---|---|
| I | Xylose+, Sorbitol+, DNase+ | SPVJ (15) | 7 Ranchi fish (3259, 3261, 3264, 3265, 3266, 3268, 3274), 2 Kolkata fish (1986, 1991), 3 Mumbai meat/broiler shops (2607, 2598, 2599), 1 Agartala calf (801), 1 Kolkata dog (885), 1 Bareilly food product (723) |
| | | CSPB (11) | All 9 Ranchi fish , 1 Chennai khoa (1294), 1 Kolkata pig (663) |
| II | Xylose +, Sorbitol +, | SPVJ (2) | 1 Mumbai poultry shop (2609), 1 Mumbai meat shop (2610) |
| | DNase - | CSPB (5) | 1 Kolkata fish (1984), 1 Hyderabad pig (896), 1 Mathura sewage(923), 1 Kolkata rat(651), 1 Bareilly human(2403) |
| III | Xylose -, Sorbitol+, DNase+ | SPVJ (2) | 2 Mumbai poultry shop (2601, 2603) |
| IV | Xylose -, Sorbitol+, DNase - | SPVJ (2) | Mumbai poultry shop (2606), Meat shop (2608) |
| V | Xylose+, Sorbitol -, DNase+ | SPVJ (2) | Mumbai meat shop (2402, 2404) |
| VI | Xylose -, Sorbitol -, DNase+ | SPVJ (1) | Mumbai poultry shop (2605) |

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Table 5. **Multiple drug resistance patterns among *Salmonella* Paratyphi B var Java (SPVJ) and classical *Salmonella* Paratyphi B (CSPB) biovar strains prevalent in India**

| Resistance to No. of drugs | Biovar (No. of resistant isolates) | Resistance pattern (No. of strains) | Source of isolates |
|---|---|---|---|
| 10 | SPVJ (1) | Dc, Cz, Ca, Cl, A, Nf, Tr, C, Sf, Ci (1) | Mumbai meat shop (2604) |
| 9 | CSPB (1) | Dc, Cz, Ca, Cl, Nf, Cp, K, G, S (1) | Kolkata pig (663) |
| 8 | SPVJ (2) | Dc, Cz, Ca, Cl, Nf, Tr, S, Sf (1) | Mumbai meat shop (2602) |
| | | Dc, Cz, Ca, Cl, Tp,Tr, S, Sf (1) | Mumbai poultry shop (2603) |
| | CSPB (1) | Dc, Cz, Ca, Cl, Nf, Cp, K, S (1) | Mathura sewage (923) |
| 7 | SPVJ (2) | Dc, Cz, Ca, Cl, Nf, K, S (2) | Mumbai poultry shop (2605, 2606) |
| | CSPB (4) | Dc, Cz, Ca, Cl, Nf, Cp, S (4) | Ranchi fish (3256, 3269, 3275), Chennai khoa (1294) |
| 6 | SPVJ (10) | Dc, Cz, Ca, Cl, Nf, S (8) | Mumbai Meat/broiler shop (2601, 2608), Kolkata fish (1986, 1991), Bareilly food product (723), Ranchi fish (3261, 3264, 3266) |
| | | Dc, Cz, Ca, Cl, S, Sf (1) | Ranchi fish (3265) |
| | | Dc, Cz, Ca, Cl, Nf, K (1) | Mumbai poultry shop (2599) |
| | CSPB (3) | Dc, Cz, Ca, Cl, Nf, S (2) | Ranchi fish (3258), Kolkata fish (1984) |
| | | Dc, Cz, Ca, Cl, Cp, S (1) | Ranchi fish (3273) |
| 5 | SPVJ (6) | Dc, Cz, Ca, Cl, Nf (4) | Mumbai meat/broiler shop (2607, 2609, 2610, 2598) |
| | | Dc, Cz, Ca, Cl, S (2) | Ranchi fish (3268, 3274) |
| | CSPB (6) | Dc, Cz, Ca, Cl, S (5) | Ranchi fish (3257, 3260, 3267, 3271) |
| | | Dc, Cz, Ca, Cl, Nf (1) | Man at Bareilly (2403) |
| 4 | SPVJ (3) | Dc, Cz, Ca, Cl (3) | Ranchi fish (3259), Kolkata dog (885), Agartala calf (801) |
| | CSPB (1) | Dc, Cz, Ca, Cl (1) | Hyderabad pig (896) |

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Many of the SPBJ isolates were resistant to nitrofurantoin and streptomycin. Strain No.E 2604 of SPBJ was resistant to 10 drugs and was the only strain resistant to ampicillin, chloramphenicol and ceftriaxone, while strain No. 2603 resistant to 8 drugs was the only strain resistant to cephalexin. Resistance to kanamycin was exhibited only by 3 strains (E2605, E2606, E2599). SPBJ strains, isolated in Mumbai, were in general resistant to many different types of drugs.

Among SPB isolates resistance to nitrofurantoin, cephalexin and streptomycin was common. A strain isolated from Kolkata pig was resistant to 9 drugs and was the only strain resistant to gentamicin. In contrast, human isolate was the only strain sensitive to streptomycin. Only 2 strains isolated from Kolkata pig and Mathura sewage showed resistance to kanamycin.

## DISCUSSION

*Salmonella* Paratyphi B, a pathogen of zoonotic significance, has emerged in last few decades as major foodborne pathogen in India. Particularly fish (Singh, 1989; Yadav, 2001), meat (Verma *et al*., 2001) and poultry products (Saxena *et al*., 1983) has been reported to be the major sources of it. Besides, *S*. Paratyphi B has also been isolated from milk products (Gupta *et al*., 1981), articles used in meat and poultry shops (Verma and Gupta, 1995), humans (Agarwal, 1963) and sewage (Nath *et al*., 1970) indicating that this pathogen is not uncommon in India. Considering the zoonotic significance and foodborne nature of *S*. Paratyphi B, it is pertinent to study the different profiles of *S*. Paratyphi B isolates available from different sources to understand the epidemiology of the disease. In general, serotyping alone can not be used as epidemiological marker, so some more methods e.g. biotyping, phage typing, sensitivity to bacteriocins and antibiograms etc. (Barker *et al*., 1988) are to be used as epidemiological tools. Kapperud *et al.* (1989) have revealed that even simpler techniques as plasmid profiling, phage typing and biotyping may work as better epidemiological marker than the more complex ones. In view of the earlier reports it appears that serotyping followed by biotyping, bacteriocin typing and antibiogram studies may be right tools for epidemiological identification of *Salmonella* strains.

Though many different tests including sugar fermentation, amino acid utilization DNase production, lecithinase production,

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caseinase production, gelatin liquefaction were tried to employ for biotyping, variability could be observed only for fermentation of xylose and sorbitol and production of DNase. On the basis of xylose and sorbitol fermentation and DNase production, 24 SPBJ strains could be divided in 6 biotypes and 16 SPB strains in 2 biotypes. Strains belonging to biotypes I and II have been isolated from various parts of India but rest of the 4 biotypes could only be isolated from Mumbai city. This much variability in biotypes of *S.* Paratyphi B strains of a city may be due to its metropolitan culture, food habits and population shift, all are common in Mumbai, the economic capital of India.

Biotyping is one of the important conventional typing methods used to differentiate strains within a serotype. Subdividing common *Salmonella* serotypes according to their biochemical characters is often used for their value in epidemiological investigation. Many attempts had been made earlier for biotyping of *S.* Paratyphi B using different tests, in one of such attempt to biotype *S.* Paratyphi B, Barker *et al.* (1988) using 13 characters could further classify 123 strains of *S.* Paratyphi B of two major biovars i.e. SPBJ and SPB. Further subdivision of Barker *et al.* (1988) failed to find the characters consistent to any one of biovars. Electrophoretic typing by Selander *et al.* (1990) revealed that different biovar may belong to same electrophoretic type and vice versa was also true. Furthermore, extensive polymorphic variation in biotype character not only failed to establish any phylogenetic clone among *S.* Paratyphi B strains but many of the biotypes also matched with those of *S.* Typhimurium.

Biotype I and II had no separate identity for SPBJ and SPB i.e. similar biotypes were evident in both of the groups as reported in earlier studies (Barker *et al*., 1988; Selander *et al*., 1990). DNase, an important determinant of biotype, has been reported to be an important marker of virulence in many other bacteria (Cunningham *et al*., 1956 and Bachhil, 1989), however, there appears to be only few reports on DNase production by *Salmonella* (Chandra, 2002) but none on the strains of *S.* Paratyphi B, hence comparison can not be made.

Association of a specific biotype with source and place of isolation can be important criteria to identify the origin of outbreak. The present investigation revealed that all Ranchi fish isolates belonged to biotype I irrespective of biovar (SPBJ or SPB) and of the 3, 2 isolates from Kolkata fish also belonged to biotype I, indicating that in fish prevalence of biotype I was predominant. Besides, biotype I of SPBJ was the only biotype distributed widely in all parts of the country. While biotype II of SPB was more widely distributed but less common in occurrence. Therefore, from biotyping it seems to be quite possible to determine the prevalence of *S.* Paratyphi B strains of a particular biotype and the technique may be useful as an epidemiological tool in future studies.

Bacteriocin typing has been used as an important epidemiological tool to identify the outbreak strain of different pathogens. Five isolates of SPBJ from Mumbai meat/broiler shop produced bacteriocin. None of the SPB strain produced bacteriocin. So bacteriocin production by only SPBJ strains may be one reason for the wide spread occurrence of SPBJ strains in all parts of the country. However, this hypothesis may not be holding true, as SPBJ strains from different parts of India (except those from Mumbai) could not produce detectable bacteriocin. Failure to detect bacteriocin production may be due to small number of bacteriocin sensitive strains used in the study. Every possibility exist that other strains those failed to show the bacteriocin activity might be producing one not active against the battery of test strains so further studies using more and more susceptible strains are essential. Bacteriocin production was found to be very common trait in case of *S.* Virchow (Verma and Gupta, 1989 and Roy, 1990) and plasmid of 59.2 MDa was found to be responsible for this trait (Bakshi, 1994) but attempts to find any such correlation were not made.

Of the 40 strains examined none proved lacking multiple drug resistance (MDR). About 58% of SPBJ and 60% SPB strains had resistance to 6 or more drugs. While none of the strain was resistant to <4 drugs. The clinical management of salmonellosis in man and animals is mainly based on antibiotics and fluid therapy, which is quite expensive and indiscriminate use of antibiotics may lead to multiple drug resistant strains, which are threat to human and animal population. Several workers have reported multiple drug resistance against 6-8 drugs in serotypes of *Salmonella* of animal and human origin in India (Gopinath, 1996; Bhattacharya, 1997; Bakshi, 1998 and Saxena, 2000). Emergence of MDR *Salmonella* strains has often been held responsible for frequently occurring outbreaks and hyperendemicity of salmonellosis in India (John, 1996).

A total of 10 drug resistance patterns were noticed in SPBJ and 8 in SPB strains. Out of 10, 7 pattern of antibiotic resistance in SPBJ strains were from Mumbai city only. Many strains of S. Paratyphi B were resistant to nitrofurantoin, streptomycin and cephalexin. Resistance to nitrofurantoin may be attributed to wide host range of *S.* Paratyphi B particularly persistence in animals, as nitrofurantoin is often added into feed of poultry birds and used to control infection in young animals as a cheap antibacterial agent, creating a selective pressure for development of nitrofurantoin resistant strains. However, tetracycline, another drug used as feed ingredient, was found effective against all the strains. The reason behind non-selection of tetracycline resistance is not clear. The possible cause may be inefficiency of *S.* Paratyphi B strains to acquire necessary R-plasmids needed to transfer tetracycline resistance (Hirsh *et al.*, 1983). Very few strains were found resistant against ampicillin, chloramphenicol, ceftriaxone, kanamycin, gentamicin and trimethoprim and sulfafurazole. The emergence of resistance against common drugs used for treatment of enteric fever may be due to indiscriminate use of these drugs as well as persistant selection pressure of the drugs in environment. As all the strains of *S.* Paratyphi B were sensitive to antimicrobial substances viz. acriflavin, crystal violet

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and mercuric chloride which are rarely been found in food chain or in the chain of salmonellosis, thus the findings corroborate with the hypothesis of continuous selection of MDR strains under persistent concentration of antibiotics in environment. The hypothesis of selection under antibacterial drug concentration is further supported by the fact that the multiple antibiotic resistance was more common in Mumbai strains, but no correlation of MDR could be found with any of the biotype. Prevalence of MDR strains in Mumbai may be attributed to metropolitan nature of the city as discussed earlier.

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