

# Emergence' of Multi-drug Resistance Among Beta-Lactamase Producing Salmonella

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## Abstract

Multi-drug resistant strains of Salmonella isolated from blood and bone marrow cultures of pyrexial patients received from physicians, hospitals and different clinics were studied from May to November, 1993. Of 2143 samples collected, 424(20%) cases yielded the growth of different organisms. Out of these 266(63%) were positive for Salmonella strains. The strains isolated were Salmonella typhi 239(90%) and Salmonella paratyphi A 27(10%), Two hundred twenty(82%) strains of Salmonella showed increased beta- lactamase activity and an alarming increase in resistance against commonly used antibiotics for enteric fever (JPMA 44:253,1994).

## Introduction

Enteric fever is a generalized infection of the reticuloendothelial system causing septicaemia and prolonged pyrexia<sup>1</sup>. It is endemic in many developing countries and is mainly caused by Salmonella typhi and paratyphi A and B. There are marked differences in the antibacterial pattern of Salmonella strains isolated from cultures. These strains were morphologically, biochemically and serologically alike. Therefore, beta-lactamase test on these strains was carried out. Beta-lactamase is an extracellular enzyme produced by many strains of gram negative bacteria and is chromosomally mediated. This study was planned to assess the increase in beta-lactamase activity amongst Salmonella associated with emergence of multidrug resistance. The antibiotics tested for this purpose were semisynthetic penicillins, chloramphenicol, cotrimoxazole, fosfomycin, cephalosporins and quinolone-carboxylic acid derivatives.

## Materials and Methods

Blood and bone marrow cultures were done on 2143 samples collected from pyrexial patients referred from various hospitals and clinics. Approximately 3-5ml of blood was collected from each pyrexial patient and inoculated into 50 ml Brain heart infusion broth (Oxoid). Blood culture bottles were incubated at 37°C. Gram stain and plating was done after 24,48,72 hours and on day 8 before discarding the bottle as negative for Salmonella. The plating was done on blood agar and MacConkey's agar (Oxoid). Positive growth was identified by standard biochemical and serological methods<sup>2</sup>. The antisera of Bio Merieux (France) were used for serological identification. All Salmonella strains were further tested for beta-lactamase activity by beta-lactamase disc (discs were provided by SmithKline Beecham). The disc is about 12 mm in diameter and is of light yellow in colour and contains nitrocefin as the active ingredient. The colonies of Salmonella were picked up by a straight wire and then touched over the surface of the disc. The immediate development of red colour over that area was considered positive for beta-lactamase. All strains were then studied for antibacterial pattern on sensitest agar (Oxoid) by Kirby - Bauer single disc diffusion method<sup>3</sup>. Following antibiotic discs used were supplied by Oxoid and Difco: amoxicillin (25ug), anipidillin 25ug, augmentin (30ug) cotrimoxazole (25ug), ceftizoxime(30jxg), Ceftriaxone (30ug), Cefuroxime (30gg), cephradine (30ug), quinolone- carboxylic

acid derivatives [norfloxacin (10j.tg), ofloxacin (5ug), enoxacin (10ug), ciprofloxacin (5grn, pefloxacin (5ug) lomefloxacin (10ug)] chloramphenicol (30ug) and fosfomycin (30ug).

## Results

Out of 2143 specimens studied, 2067(96%) were blood and 76(4%) bone marrows. Of these 1603(75%) were from cases under 15 years of age, 299 (14%) and 241 (11%) from adult males and females respectively. Four hundred and twenty-four (20%) cultures were positive. Of these 377(89%) were blood and 47(11%) bone marrows. Forty-five of 47 (96%) bone marrows and 221 (59%) of 377 blood specimens were positive for Salmonella species while 2 (4%) bone marrows and 156 (41%) blood yielded growth of other pathogens. Among the Salmonella isolates, S.typhi was predominantly isolated both from blood and bone marrow specimens. Of 266 isolates, 239 (90%) were S.typhi and 27 (10%) S. paratyphi A. S. paratyphi B was not isolated from either specimen. (Table I).

**Table I Distribution of Salmonella species (n=266).**

Specimen	S.typhi		S.paratyphi A	
	No	(%)	No.	(%)
Blood (221)	197	(89)	24	(11)
Bone marrow (45)	42	(93)	3	(7)

Majority of S.typhi strains (85%) were beta-lactarnase producers, while no significant difference was seen among beta lactaniase producers (59%) and non-producers (41%) of S.paratyphi A. (Table II).

**Table II. Frequency of Beta-lactamase producing Salmonella species.**

Isolates	Positive for $\beta$ -lactamase		Negative for $\beta$ -lactamase	
	No.	(%)	No.	(%)
Salmonella (266)	220	(83)	46	(17)
S.typhi (239)	204	(85)	35	(15)
S.paratyphi A (27)	16	(59)	11	(41)

Antibiotic resistant pattern of Salmonella strains indicated that beta-lactamase producing S.typhi were highly resistant to amoxycillin, ampicillin, chloramphenicol and cotrimoxazole, while augmentin showed less than 50% resistance (Table III).

Table III. Resistance pattern of Salmonella strains in percentage.

Antibiotics	S.typhi β-lactamase positive n=204	S.typhi β-lactamase negative n=35	S.paratyphi A β-lactamase positive n=16	S.paratyphi A β-lactamase negative n=11
Amoxycillin (25μg)	87	6	31	0
Ampicillin (25μg)	87	6	31	0
Augmentin (30μg)	48	3	6	0
Chloramphenicol (30μg)	82	0	6	0
Cotrimoxazole (25μg)	76	6	12	0
Fosfomycin (30μg)	14	0	25	0
Cephradin (30μg)	1	0	0	0
Ceftriaxone (30μg)	0.5	0	0	0
Ceftizoxime (30μg)	1	0	0	0
Cefuroxime (30μg)	2	0	12	0
Quinolone group	0	0	0	0

Note: Figures in the columns are the percentages.

However, only 6% resistance against these antibiotics were seen in S.typhi strains unable to produce beta-lactamase. Cephalosporins showed negligible resistance to S.typhi and no resistance to quinolone. Beta lactamase producing S.paratyphi A have also shown some degree of resistance, while non producers were not resistant to these antibiotics (Table III).

## Discussion

Enteric fever, a public health problem, has a high morbidity and mortality rate. During the period of

study increase in beta- lactamase activity of Salmonella typhi and Salmonella paratyphi A (Table II) and the emergence of high degree of resistance (Table III) was noted. Multi-drug resistant strains of Salmonella continue to occur as is reported in many countries. In a study (1987-1991) done in Northern Pakistan, 186 of 540 Salmonella strains isolated were found to be resistant and majority of these were isolated in 1991<sup>4</sup>. In a The mysterious typhoid fever not responding to chloramphenicol, penicillin or cotrimoxazole was reported from all parts of India in 1990. During surveillance of Salmonella in India in the year 1984-85, multi-drugresistance was observed in 73% diarrhoeal and non-diarrhoeal isolates. Antibiotic markers were transferable in almost 50% of these isolates<sup>5</sup>. In the year 1985 and 1988 the invitro susceptibility of gastroenteric Salmonella and 36 strains of S. typhi were examined in Hong Kong<sup>6</sup>. Only one isolate of S.typhi was found resistant to chloramphenicol and another to cortimoxazole but the gastroenteric Salmonella showed multi-drug resistance in these cases accompanied with the increased production of beta- lactamase. Recent study has shown the presence of plasmids and the production of beta-lactamase in the resistant strains of S.typhi. Out of 114 strains isolated during 1985-1991 in Dakar, 27 were identified as Salmonella and S. typhi predominated with 45%<sup>7</sup>, Only 28% of the strains were susceptible to all antibiotics and 17% were found multiresistant. The production of beta-lactamase and presence of plasmids in the resistant strains were found. This study provides evidence of marked resistance to amoxycillin, ampicillin, augmentin, chloramphenicol and cotrimoxazole among beta-lactamase producing Salmonella strains especially Salmonella typhi. Some resistance to cephalosporins and fosfomycin was also found, although quinolone carboxylic acid derivatives have not shown any resistance for both groups.

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