Salmonella enterica serovar senftenberg infection in the neonatal intensive care unit

CP-6

R. Chaudhry[1], N. Sharma[1], S. Bhushan[2], V.K. Paul[2], D.S. Chandel[1], B. Mishra[1], M. Singh[2], P. Panigrahi[3]

Abstrak

Salmonella senftenberg *(Salmonella grup E) pertama kali diisolasi di India pada tahun 1963. Serovar ini mulai dikuatirkan setelah tahun 1985 ketika menimbulkan suatu kejadian luar biasa pada bangsal anak dan neonatus di India. Studi ini memperlihatkan kolonisasi* Salmonella sp *grup E pada usus bayi prematur di unit perawatan intensif neonatus AIIMS, New Delhi. Pada 58 bayi prematur yang dirawat di NICU telah dilakukan pemeriksaan mikroba dalam sampel feses pada hari ke-3, 7, 14, 21 dan 28. Mikroba diidentifikasi berdasarkan metode kultur, biokimia konvensional dan kit identifikasi cepat (BioMerieux). Teknik reaksi berantai polimerase (PCR) untuk mendeteksi gen flagelin untuk semua* Salmonella sp *dengan menggunakan primer yang sebelumnya telah distandarisasi telah diaplikasikan untuk mendeteksi adanya* Salmonella sp *dalam feses. Lima (8,6%) dari 58 sampel bayi tumbuh kuman* Salmonella sp *grup E, dan juga PCR positif. Kuman ini lebih lanjut diidentifikasi sebagai* S. senftenberg *yang mempunyai profil resistensi antibiotik AGKT (ampisilin, gentamisin, kanamisin dan tetrasiklin).* S. senftenberg *tidak ditemukan pada sampel dari lingkungan, staf medik dan paramedik.*

Abstract

Salmonella senftenberg *(Group E* Salmonella*) was first isolated in India in 1963. It became a serovar of concern after 1985 when it caused outbreak in paediatric wards and neonates in India. This study shows gut colonisation of preterm neonates by group E* Salmonella sp *in Neonatal Intensive Care Unit (NICU) at AIIMS, N. Delhi. A total of 58 preterm babies admitted to the NICU were examined for microbial flora in stool samples on day 3rd , 7th, 14th, 21st and 28th. Organisms were identified by methods based on culture, conventional biochemical and rapid identification kits (BioMerieux). Polymerase chain reaction (PCR) technique for detecting flagellin gene for all* Salmonella sp *using previously standardised primers detect presence of* Salmonella sp *in stool. Five (8.6%) out of 58 babies grew group E* Salmonella sp *and were also PCR positive. These were further identified as* S. senftenberg *having antibiotic resistant profile AGKT (Ampicillin, Gentamicin, Kanamycin & Tetracyclin).* S. senftenberg *could not be identified in any sample from the environment, medical and paramedical staff.*

INTRODUCTION

Salmonella serotypes are one of the frequent etiologic agents incriminated in nosocomial epidemics in neonatal intensive care units. Infections in the newborn due to these organisms carry special significance as they are associated with higher attack rate, morbidity and mortality. *S. typhimurium* is the commonest causative agent of nursery outbreaks amongst the non-typhoidal *Salmonella* serotypes, the others being *S. anatum*, *S. newport*, *S. oranienberg*, *S. weltevreden*, and *S. bareilly*[1].

S. senftenberg is a rare serotypes of *Salmonella* and was first isolated from India in 1963[2]. It became a serovar of concern after 1985 when it caused outbreaks in paediatric wards [3-5] and sporadic infections in neonates, infants, children and adults[6,7]. Human carriers have also been reported[8]. *S. senftenberg* causes diarrhoea, septicaemia, meningitis, sepsis and urinary tract infections[3-8]. The recent reports regarding the prevalence of *Salmonella* in the country described this serovar as the third most common serovar among human isolates[9]. Besides India, it has also been reported in more than 30 countries[9]. In 1976, it caused a major outbreak in UK where 3500 school children, teachers and others were at risk and several developed gastroenteritis[11].

MATERIALS AND METHODS

A total of 58 pre-term babies admitted to neonatal intensive care unit (NICU) were examined for microbial flora in stool samples on day 3rd, 7th, 14th, 21st and 28th as a part of the study on "Normal stool flora in premature babies". The presence of *Salmonella* sp. in stool samples of these neonates prompted us to carry out this study to trace the source of acquisition of this organism.

Dept. of Microbiology[1] and Paediatrics[2] All India Institute of Medical Sciences (AIIMS) New Delhi 110029, India.
Dept. of Paediatrics[3], University of Maryland, School of Medicine, Baltimore (UMAB), Maryland 21201-1595, USA.

Table 1. Environmental samples studied for Isolation of *S. senftenberg*

	Nature of samples	Organisms
1.	Mattress (Infant incubator)	Staph coag-ve
2.	Inner wall surfaces (infant incubator)	Staph coag-ve
3.	Water sink surfaces	Staph coag-ve, *K. pneumoniae*
4.	Oxygen tube (inner surface)	Sterile
5.	Cot surfaces	Staph coag-ve
6.	Suction pump surfaces	Sterile
7.	Distilled water	Sterile
8.	Air sterility test by exposed plate	Staph coag-ve, *K. pneumoniae* (3 colonies), *Entero faecalis* - 5 colonies
9.	Formula milk	GPB, Staph coag-ve
10.	Milk powder	GPB, Staph coag-ve
11.	Cockroach	*Enterobacter cloacae*
12.	Expressed breast milk	Staph coag-ve

The stool and blood samples of the medical and paramedical staff of the NICU were done to investigate the possibility of carrier.

Environmental study was done which included culture of different samples expected to be contaminated with *S. senftenberg*. The list of samples are given in Table 1.

All the stool and blood samples were processed according to the standard methods[12]. The isolates of *Salmonella* were identified by conventional biochemical method[12] and rapid identification kit (API 20E, BioMerieux, Vitet Inc). Serotyping was done using polyvalent O and H antisera as well as with group specific O and phase specific H antisera for *Salmonella*. The antibiotic sensitivity of all the isolated strains was done by disc diffusion methods of Kirby Bauer.

Standardisation of polymerase chain reaction (PCR) for detection of flagellin gene for the genus *Salmonella* was carried out using primers as described by Frankel et al[13] with minor modifications (RC_1,& RC_2)[14,15]. This included standardisation for optimum concentrations of primer, $MgCl_2$ and Taq polymerase to amplify the specified gene (1530 bp). The resulting PCR reaction consisted of 50 pM of each primer, 200 µM each dNTP, 2 mM $MgCl_2$ and 1 U of Taq polymerase (Perkin Elmer) in a 50 µl reaction volume. It consisted of 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min 30 sec and extension at 72°C for 2 min. The amplified product (1530bp) was detected under UV after staining with ethidium bromide. Positive and negative controls were *S. typhi* S901 and PCR mix without template respectively. Sensitivity of this assay was determined by using serial dilutions of DNA from *S. typhi* S901 ranging from 5 µg to 1 pg as template and the minimum detectable limit was up to 10 pg. Specificity was checked with various gram positive and gram negative bacteria such as *E. coli*, *Klebsiella, Pseudomonas* and *Staphylococcus aureus* etc (Figure 1).

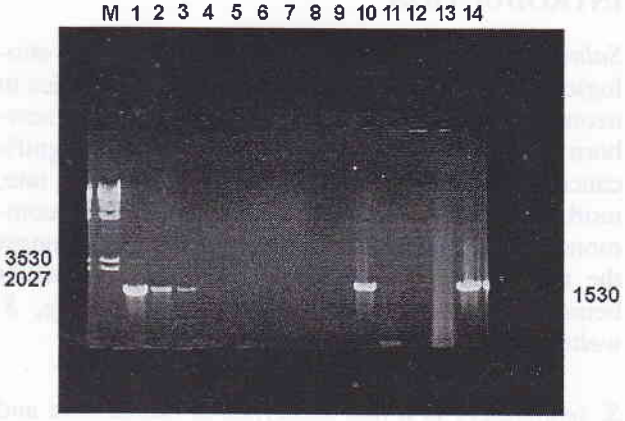

Figure 1. *Stool sample positive for Salmonella sp by PCR*

1. B.Babli.....Positive
2. B.Kamlesh.....Positive
3. *Sanno d*.....Positive
4. B.Simple.....Negative
5. B.Seema .....Negative
6. B.Sanno b.....Negative
7. B.Sanno c.....Negative
8. B.Pushpinder .....Negative
9. B.Poonam .....Negative
10. B.Rani .....Positive
11. *E.Coli* (NCTC) ...Negative
12. Staph (NCTC) ...Negative
13. Pseudomonas (NCTC).....Negative
14. *S. paratyphi A* 1220.....Positive

RESULTS

The stool samples of five out of 58 babies were found to be positive for *Salmonella* species by conventional and API 20E kit. They were further confirmed by PCR using RC_1 and RC_2 primer specific for flagellin gene common to the genus salmonella. All the isolated salmonella strains were group as "E" by serotyping using polyvalent O and H and group specific O antisera. They were finally confirmed *as S. senftenberg* by serotyping with specific g,s,t phase 1 H antisera.

All the five neonates who were positive for *S. senftenberg* did not present with classical gastroenteritis or septicaemia. However, four of these neonates had necrotising enterocolitis (NEC), one had acute gastroenteritis and one of these also developed infective diarrhoea in the later period. Three babies had severe birth asphyxia and respiratory distress syndrome which required endotracheal intubation. The details regarding the clinical features and organisms isolated from their stool samples is given in Table 2. The blood cultures of these five babies however were found to be sterile.

The stool and blood samples of the nursery staff were negative for *S. senftenberg* by culture technique and there was no band for *Salmonella* species in PCR as well. *S. senftenberg* could not be identified by culture or PCR from any of the environmental samples tested including the expressed breast milk and formula milk (Table 1). All the isolated strains of *S. senftenberg* were uniformly resistant to ampicillin, gentamicin, kanamycin and tetracyclin (AGKT).

DISCUSSION

Salmonella senftenberg was a rare serovar of Salmonella associated with human infection[16]. However in the last decade there are many reports of nursery outbreak caused by this serovar[3,8,16]. It causes diarrhoea, septicaemia, meningitis, sepsis and urinary tract infections[3-8]. However, the isolation of *S. senftenberg* from two asymptomatic cases have been reported by Chaturvedi et al[17]. The weight of these two babies were appropriate for gestation and they had normal apgar score at birth in comparison to seven other babies, who were symptomatic small for gestation age and apgar score. In our study though four babies had NEC only one had acute gastroenteritis and septicaemia.

As hypothesised by Chaturvedi et al[17], we also presume that the endotracheal intubation in 3 babies due to asphyxia might have predisposed them to a low dose of infection. The isolation of *S. senftenberg* from five cases within a period of 4 months suggests a common source of infection. This was further confirmed when no more cases of *S. senftenberg* was re-

Table-2. Clinical picture of neonates with *S. senftenberg* isolates

S.N.	Name	Sex	DoB	B.Wt. (gm)	Ges. (wk)	F. diagnosis	isolated organism
1.	B. Anita	M	29/12/96	1.249	31	NEC D7, S/PT/M/AFD/Em LSCS Resp. depression due to pethidine D1, hyperbil. PDA, NEC, Perforation of bowel, Fecal fistula.	*S. senftenberg*
2.	B. Babli	F	05/02/97	1.299	31	S.PT/AFD/Em LSCS, RDS due to Anemia at birth, NEC, Hyperbil, Apnea of prematurity.	*Micrococcus sp.* *S. senftenberg*
3.	B. Kamlesh	F	23/02/97	737	28	S/PT/F/AFD. Severe birth Asphyxia (AS-1,4,7). D4-IVH. D5- stage I A NEC. D43-Septicemia (Culture-negative). ROP Stage II	*E. faecium* S. senftenberg
4.	B. Shanno	F	26/04/97	1.316	32	Trilet- III/PT/F/AFD/Em LSCS for eclampsia, D3-suspect NEC hyper billirubin (PT for 98 hrs.).	*Clostridium sp.* *S. senftenberg*
5.	B. Rani	M	05/06/97	1.512	39	Acute gastroenteritis of one day oxymia.	*S. senftenberg*

Abbreviations:

S = Single; PT = Preterm; F = Female; M = Male; AFD = Appropriate-for-dates; RDS = Respiratory distress syndrome; PDA = Patent ductus arteriosus; HMD = Hyaline membrane disease; LSCS = Lower segment caesarean section; NEC = Necrotizing enterocolitis

ported for a period of more than 6 months when all possible precautions were taken in the nursery to stop the spread of infection.

Investigation of staff of NICU, environmental samples, formula milk & expressed breast milk for *S. senftenberg* could not establish the source of the acquisition. This report emphasises the careful monitoring of all preterm babies for the isolation of any pathogen which has the potency to lead to a serious outbreak, so that they can be detected in earliest possible time and the morbidity and mortality in preterm babies can be checked. As reported earlier, this organism is difficult to eradicate completely as it resists temperatures up to 70°C[18].

All the isolates have antimicrobial resistance pattern of AGKT. Prêsence of this multi resistant organism is a potential threat for another outbreak, which if not restricted may spread in neonates. Application of molecular tools such as rapid detection of *Salmonella sp.* isolates may help in early diagnosis and effective prevention and containment of such infections in NICU.

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Table 3. Microbiological profile of Neonates

Sno.	Name	Date	Aerobic Org. CFU/gm		Anaerobic Org.
1.	B.Anita	06/01/97	1. *E.coli*	1.9×10^5	No anaerobes
			2. *S.gallinarum*	7×10^8	
			3. *Aerococci viridans*	4×10^5	
		16/01/97	1. *Staph. Coag-ve*	8×10^4	No anaerobes
			2. *S.typhi*	6.1×10^5	
		07/02/97	1. *S.senftenberg*	2.7×10^5	No anaerobes
2.	B.Babli	07/02/97	1. *Staph. xylosus*	2×10^5	No anaerobes
		14/02/97	1. *E.faecalis*	7×10^7	No anaerobes
			2. *Staph. xylosus*	2.2×10^5	
		19/02/97	1. *Micrococcus sp.*	22.5×10^6	No anaerobes
			2. *S.senftenberg*	1.1×10^6	
3.	B.Kamlesh	25/02/97	No growth		No growth
		03/03/97	1. *C.frundii*	1×10^8	No anaerobes
			2. *E.faecium*	3.1×10^7	
		10/03/97	1. *S.senftenberg*	2×10^6	No anaerobes
			2. *E.faecium*	5×10^6	
		17/03/97	1. *S.senftenberg*	8×10^5	No anaerobes
			2. *E.faecium*	7×10^6	
		24/03/97	1. *E.coli*	5×10^7	No anaerobes
			2. *Proteus vulgaris*		
4.	B.Sanno	28/04/97	1. *K.pneumoniae*	5×10^7	No anaerobes
			2. *Enterococcus sp.*	7×10^7	
		05/05/97	1. *E.coli*	3.2×10^9	*Clostridium sp.*
			2. *Micrococcus sp.*	5×10^8	
		10/05/97	1. *Micrococcus sp.*	13×10^4	No anaerobes
		17/05/97	1. *S.senftenberg*	2.9×10^4	1. *Clostridium sp.*
					2. *Clostridium sp.*
5.	B.Rani	09/06/97	1. *S.senftenberg*	5×10^4	No anaerobes
			2. *Micrococcus sp.*	5×10^6	

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