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Research article

INCIDENCE OF *SALMONELLA* IN AGE AND ANTIBIOTIC SENSITIVITY PATTERN OF THE ISOLATES

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ABSTRACT: *Salmonella* is responsible for the majority of invasive AGE. Antibiotic treatment is essential to reduce invasive infections. This study was undertaken to study the incidence of Salmonella among AGE and antibiotic sensitivity pattern of the salmonella isolates. Standard methods were adopted to identify *Salmonella sp,*. Disc diffusion method was used to assay antibiotic sensitivity pattern of the isolates. Results revealed that 15% of AGE is associated with Salmonella sp., and all the isolates were resistant to any one of the antibiotics tested & fall within 24 different antibiotics resistance pattern, morethan 80% of the strains were resistant to Ampicillin. Among antibiotics group amyloglycosides & cephalosporin group of antibiotics showed moderately less resistant to strains tested. We must develop a specific antibiotic usage policy for the reduction of antibiotic resistance.

Key words: *Salmonella*, Antibiotic sensitivity, AGE

INTRODUCTION

Salmonella that causing gastroenteritis are called nontyphoidal salmonellae. Typhoidal salmonella also responsible for gastroenteritis in children and adults. Salmonellosis is a broad term to describe gastroenteritis caused by the members of the genus Salmonella. Salmonella is responsible for approximately 93.8 million illnesses and 155000 deaths annually around the world (Majowicz *et al.,* 2010). Though salmonellosis showed higher incidences, there is a lack of reporting of gastroenteritis due to typhoidal salmonellae (Salmonella enterica serovar typhi and paratyphi). Gastroenteritis due to these groups of microorganisms is self limited, but may lead to serious complications (Millemann *et al.,* 2010). Antimicrobial treatment is highly essential for invasive infection resulted from these bacteria and several other host factors. Now a day, microorganisms not responds to the prescribed antibiotics and patients need special attention. Numerous studies conducted throughout the world found mortality associated with these diseases. Hence this study reported the incidence of typhoidal as well as nontyphoidal salmonella along with the resistance pattern of the salmonella species isolated from patients with AGE.

MATERIALS AND METHODS

Sample collection and processing

A total of 831 stool samples were collected from clinically diagnosed cases of acute gastroenterit.is (AGE) admitted in Meenakshi Mission Hospital and Research center, Madurai. Stool samples were collected from children and adults. The age ranges from 0-65 years. The samples were categorized based on age and sex. Standard methods were adopted to analyse the epidemiology of acute gastroenteritis (Hendriksen, 2003). The samples were not refrigerated, as some of the enteric pathogens are highly sensitive to temperature. Culturing the organism in enriched, selective cum differential medium helps to isolate and identify the bacterial etiology of stool samples

Isolation and identification of *Salmonella sp.*

A loop full of stool sample was taken and inoculated on Selenite F broth and was incubated at 37°C for 8 to12 hours and the broth was looked for visible growth. A loop full of culture from enrichment broth was inoculated on selective and differential media like Hektoein enteric agar, XLD agar, Bismuth Sulphite agar and Rajhans medium. Selected colonies from selective and differential media were subjected to macroscopy, microscopy and biochemical tests for identification (Hendriksen, 2003).

Antibiotic sensitivity Assay

One hundred and sixty nine *Salmonella sp.* (typhoidal and non typhoidal) were subjected for antibiotic sensitivity assay. Both broad spectrum and narrow spectrum antibiotics were used to assess sensitivity pattern of the clinical isolates. Antibiotics like A-Ampicillin, AM-Amoxicillin, AC-Amoxyclav, I-Imipenum, CO-Cotrimoxzole, G-Gentamycin, AMI-Amikacin, TO-Tobramycin, CIP-Ciproflaxcin, NO-Norfloxcin, NA-Nalidixic acid, CEF-Ceftizoxime, CEFO-Cefotaxime, CEFT-Ceftriaxone, CEZ-Ceftazidime were used to see the sensitivity pattern of *Salmonella sp.* Disc diffusion method was followed to look for anti - bacterial activity. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4 hours fresh culture of clinical isolate. By making use of template drawn commercial antibiotic discs were dispensed on the solidified Mueller Hinton agar. This was incubated at 37^{o}C for 24 hours in an incubator (Rands SBC) and were looked for the development of inhibition zones around the antibiotic disc. The zone of inhibition was measured by making use of antibiotic zone scale (Hi-Media) and the results were recorded (Anonymous, 2006).

RESULTS

Salmonella sp., produce circular, raised, entire, colourless and translucent colonies on nutrient agar and it is a rod shaped gram negative & motile bacterium. Common biochemical properties of *Salmonella sp.*, are indole -, MR -, VP-Citrate utilization test +, Urease -, Nitrate +, catalse +, oxidase -. Salmonella sp., grown on Hektoein enteric agar and produce greenish colonies with or without black center, similarly on XLD agar it produce pinkish colonies with or without black center. On Bismuth Sulphite agar, it produce jet black colonies. Reddish colonies were noted on Rajhans medium. Eight hundred and thirty two stool samples were subjected for assessing salmonellosis among the inpatients of Meenakshi Mission Hospital and Research center, Madurai. Totally 1136 number of isolates was recovered from the stool. Among these 832 isolates belong to *Escherichia coli* (i. e., all the samples showed the presence of *E. coli*), followed by 135 number each of *Salmonella enteritidis* & *Klebsiella sp.*, 10 number each of *Salmonella paratyphi A* & *Salmonella typhimurium. Salmonella typhi* was isolated from 14 samples (Table 1).

Table 1: Recovery rate of bacterial isolates from AGE

S. No	Test Organism	Number of isolates
1	*Klebsiella sp*	135
2	*Salmonella enteritidis*	135
3	*Salmonella paratyphi A*	10
4	*Salmonella typhi*	14
5	*Salmonella typhimurium*	10
6	*Escherichia coli*	832

Overall 15% of organisms belong to the genus *Salmonella*. Very low numbers of typhoidal *Salmonella* (*Salmonella typhi*, *Salmonella paratyphi*) was isolated out of 832 stool samples (n=24; 3%) whereas 12% of the strains belongs to non typhoidal *Salmonella* (*Salmonella enteritidis*, *Salmonella typhimurium*) (Figure 1).

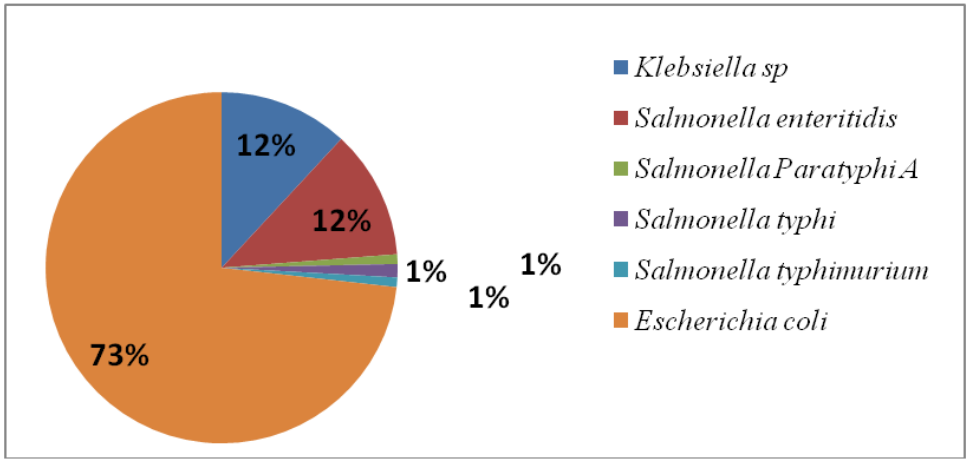

Figure-1: Incidence of Different pathogens in AGE

Age wise categorization of samples revealed that maximum incidences were noted among 21-40 age group followed by 0-20 years of age group. Very low microbial incidence was noted among the age group patients 41-85 age group. Microbial recovery was high in 21-40 age group patients (Table 2).

Table -2: Incidence of microbial etiology with reference to different age groups in AGE

S. No	Isolate	0-20		21-40		41-60		61-80	
		Male	Female	Male	Female	Male	Female	Male	Female
1	*Klebsiella sp*	25	16	59	28	3	4	0	0
2	*Salmonella enteritidis*	25	16	59	28	3	4	0	0
3	*Salmonella Paratyphi A*	2	5	1	2	0	0	0	0
4	*Salmonella typhi*	2	2	8	2	0	0	0	0
5	*Salmonella typhimurium*	2	3	5	0	0	0	0	0
6	*Escherichia coli*	138	108	381	157	15	25	5	3

Figure 2 indicated the recovery rate of Salmonella in different age groups. Among these 145 isolates were called as nontyphidal *Salmonella* and 24 strains belong to typhoidal *Salmonella*. Overall 304 isolates other than *Escherichia coli* were recovered from the stool samples.

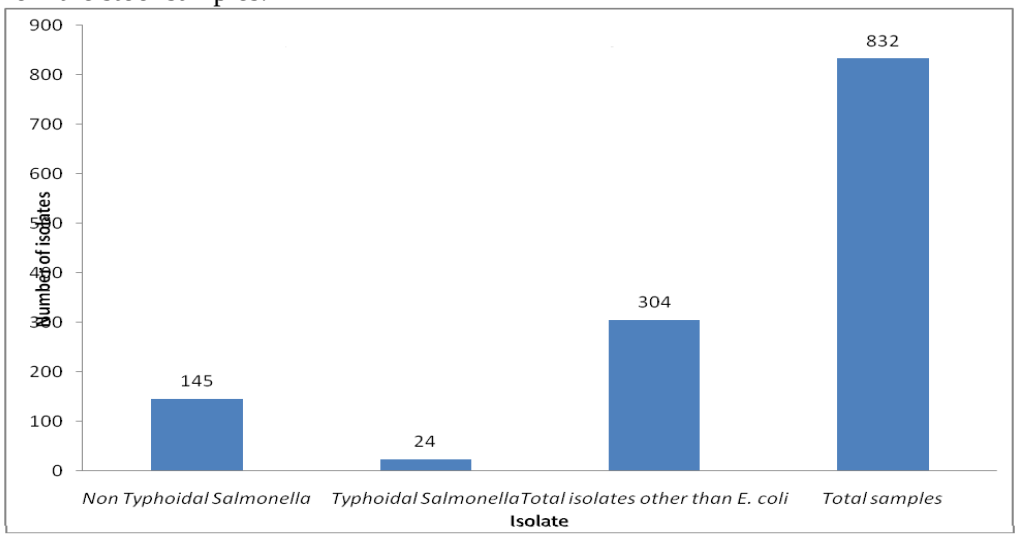

Figure-2: Recovery rate of salmonella among different isolates

In this study only Salmonella members were selected and screened for assessing antibiotic sensitivity pattern. One hundred and sixty nine Salmonella isolates were subjected for antibiotic sensitivity assay using fifteen clinically available antibiotics. Results revealed that all the test pathogens were resistant to any one of the antibiotics. Tobramycin and amikacin are the only moderately best antibiotics which are evidenced in antibiotic sensitivity assay (Figure 3).

Table 3 revealed the antibiotic resistance pattern of the Salmonella isolates. Twenty four different pattern was expressed among 169 isolates. Ampicillin, Amoxicillin and Amoxyclav resistance pattern was expressed in 23.5% of the isolates. Sixteen percentage of resistance pattern was expressed with I, AMI, TO, NO, NA.

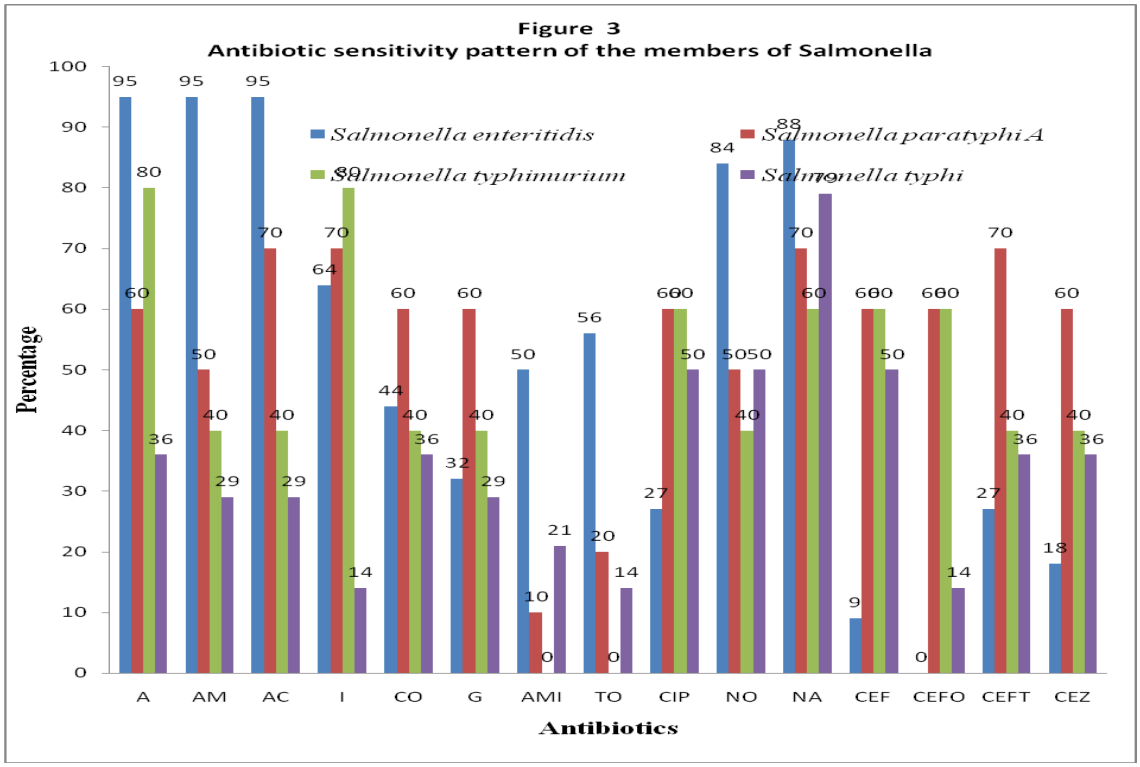

Figure-3: Antibiotic sensitivity pattern of the members of salmonella

A-Ampicillin, AM-Amoxicillin, AC-Amoxyclav, I-Imipenum, CO-Cotrimoxzole, G-Gentamycin, AMI-Amikacin, TO-Tobramycin, CIP-Ciproflaxcin, NO-Norfloxcin, NA-Nalidixic acid, CEF-Ceftizoxime, CEFO-Cefotaxime, CEFT-Ceftriaxone, CEZ-Ceftazidime

Table 3: Resistant pattern of Salmonella isolates

S. No	Resistance patterns	No. of isolates	% of resistance
1	A	12	7
2	A, NA, CEZ	3	1.5
3	A, AC, CO, G, CIP, NO, NA, CEF, CEFO, CEFT, CEZ	2	1
4	A, AM, AC	38	23.5
5	A, AM, AC, CO, CIP, NO, NA	4	2
6	A, AM, AC, CO, G, CIP, NO, NA, CEF, CEFO, CEFT, CEZ	5	3
7	A, AM, AC, CO, G, CIP, NO, NA, CEF, CEFT, CEZ	15	9
8	A, AM, AC, G, CIP, NO, NA, CEF, CEFO, CEFT, CEZ	2	1
9	A, AM, AC, I, CO, G, AMI, TO, CIP, NO, NA, CEF, CEF	1	0.5
10	A, AM, AMI, TO, CIP, NO, NA, CEF, CEZ	1	0.5
11	A, AM, AC, CO, CIP, NO, NA	12	7.5
12	A, AMI, TO, CIP, CEFT, CEZ	5	3
13	A, CO, CIP,	2	1
14	A, NA, CEF, CEFO,	4	2
15	AC	1	0.5
16	AMI,TO,NO,NA	8	5
17	CO,CIP,NO,NA,	5	3
18	I	1	0.5
19	I, AMI, TO, NO, NA	27	16
20	I, AMI, TO, NO, NA, CEZ	7	4
21	I, CIP, NO, NA, CEFO, CEFT, CEZ	1	0.5
22	I, NA	2	1
23	I, NA, CEZ	5	3
24	NA	6	4

Table-4: Different groups of antibiotic resistance against Salmonella spp

Group of antibiotics	*S.typhi*	*S.Paratyphi B*	*S.paratyphi A*	*S.typhimurium*	% of Resistance
Betalactamase	42.3	62.4	40	25.7	42.6
Aminoglycosides	33	33.2	20	11.8	24.5
Quinolone	39	63.8	46.6	59.5	52.2
Cepholosporines	8.6	43.7	30	23.1	26.3

All the fifteen antibiotics were categorized as four groups as betalactam antibiotics, Aminoglycosides, Quinolone and Cepholosporines. *S.paratyphi B* was 68.4% resistance to Quinolone group of antibiotics followed by betalactam antibiotics (62.4%). Overall 52.2% organisms were resistance to quinolone group of antibiotics followed by betalactam antibiotics (42.6%) (Table 4).

DISCUSSION

This study evaluated the incidence and prevalence of typhoidal and nontyphoidal Salmonella isolated from AGE cases admitted in Meenakshi Mission Hospital and Research center, Madurai. Biochemical features, colony morphology and microscopic features play a vital role in the characterization of enteric pathogens (Ogbu et al., 2008 and Atkins & Santiago, 2005). Our results also confirmed that all the isolates were identified using growth characteristics, microscopy and biochemical tests. An incidence report of this study was entirely different from the report given by Daniel *et al.*, (2005) Brazil. Malla *et al.*, (2006) also reported variable results of Salmonella incidence. Overall 15% of microbial incidence was due to Salmonella sp, Age plays a vital role in the outcome of microbial infection (Rajan and Selvichristy, 2014). Active immune cells in healthy individuals prevent microbial entry and thereby avoid infection. Li et al., (2014) from China reported the incidence of nontyphoidal Salmonella. They reported that 38.9% *Salmonella enteritidis* and 29.7% *Salmonella typhimurium* were the common serotypes, which was very high when compared to our report. Nasreldin *et al.*, (2013) from Saudi Arabia showed that very low incidence (9%) of non typhoidal Salmonella was reported whereas our study revealed 12% of nontyphodal Salmonella incidence.

Ifeanyi *et al.*, (2014) from Nigeria reported that all isolates were susceptible to Nalidixic acid whereas our report showed 60-88% of *Salmonella sp.*, were resistance to more than two antibiotics tested, which is an alarming situation in India like developing countries. We should follow standard antibiotic use policy to reduce antibiotic resistance pattern.

Casmir et al (2014) reported the resistance nature of *Salmonella* isolates to commonly used antimicrobials. They showed that 77.8% of the *Salmonella* isolates tested for antimicrobial susceptibility were resistant to two or more antibiotics, which was similar to other results.

Nasreldin *et al.*, (2013) from Saudi Arabia reported that NTs showed resistance to 31.3% ampicillin, 29.9% amoxicillin/ clavulonic acid, 20.9% trimethoprim and 14.93% cefotoxime. Similar kind of resistance pattern was reported in this study. Ampicillin, Amoxicillin and Amoxiclave combined drug resistance was reported in 23.5% strains. .

The emergence of antimicrobial resistance within Salmonella species has been reported worldwide (Fewtrell *et al.*, 2005). In the present study, a strong prevalence of antibiotic resistant Salmonella was observed (95%). *S. paratyphi* was the most frequent resistant serotype. *S. enteritidis* also reported resistance to antibiotics.

Different version of the antibiotic resistance report was given by Sofia and Ioannis (2014) from Greece. They indicated that <15% resistance ampicillin, amoxicillin/clavulanic acid, chloramphenicol, tetracycline, and cotrimoxazole. They also stated that *S. enteritidis* remains the predominant serotype. Resistance to antibiotics is becoming more severe problem in underdeveloped countries and cause severe invasive illness and death brought about by resistance (Okeke *et al.*, 2007).

In our study, all the *Salmonella* isolates were resistant to Nalidixic acid, it is different from the studies in the Republic of Ireland. They reported resistance in only 2.6% of strains (Gorman and Adley, 2003; Ryan *et al.*, 2011).

Ceftriaxone is commonly used to treat children with *Salmonella* infection. Ceftriaxone showed good antimicrobial activity against the *Salmonella* isolates. Ciprofloxacin showed good antimicrobial activity against the isolates. This is also comparable with the result reported by Akinyemi *et al.*, (2005) and Ifeanyi et al., (2013) from Lagos, Nigeria.

CONCLUSION

Our results indicated that 15% of AGE was due to Salmonella sp. All the strains were resistance to multiple numbers of antibiotics. All the hospitals in India must develop a specific antibiotic treatment policy to overcome the problem of antibiotic resistance.

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