ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 02 | February - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A MATTER OF CAUTION: A SPURT IN SALMONELLA CASES DURING WINTER INDICATING A CHANGING TREND IN SEASONALITY IN HUMAN ENTERIC FEVER CASES

Microbiology

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ABSTRACT

Introduction: Salmonella outbreaks from several sources are increasingly reported. Among the different pathogens found in blood, Salmonella enterica serovar Typhi and Paratyphi A are the predominant types and these pathogens are mostly found during the summer and monsoon. The present study was conducted to point out the unusual spurt of Salmonella serotypes during the cooler months of the year, where we found four cases within twenty days gap in January 2020. **Materials and methods:** Samples were collected from suspected patients and after incubation , routine subculture was done on MacConkey agar and Blood agar media. Gram's stain, motility and relevant biochemical tests were carried out. Final confirmation and sensitivity done by Vitek2 Compact system. **Results** During this study period we found four cases of Salmonella serotypes (three Salmonella enterica serovar Typhi and one Salmonella enterica serovar Paratyphi A) . All of them were Nalidixic acid Resistant [NARST strain], thus excluding the Fluoroquinolones group of antibiotic. **Conclusion** Due to global warming and other factors, Salmonellae has extended its seasonal pattern beyond summer and monsoon, thus causing a sudden spurt of Salmonella serotypes (Typhi and Pratyphi A) during the cooler months. Also, indiscriminate use of antibiotics has caused a resistance to Fluoquinolone group. We must be cautious whenever we get Gram negative non-lactose fermenting bacilli from relevant specimen particularly from blood as it is evident from our study, there is no such specific environmental pattern of these food borne pathogens in recent times. So continued screening of isolates is very much important along with the general preventive measures.

KEYWORDS

Blood culture, Enteric fever, Salmonella Typhi, Paratyphi A, Vitek 2 Compact system , COVID PANDEMIC YEAR.

BACKGROUND

Blood culture is the most significant laboratory investigation used for the diagnosis of Blood stream infections (BSIs). Bacteriological culture is done to isolate the offending pathogen and also to know about the sensitivity pattern of the isolates [1]. Blood cultures also provide essential information for the evaluation of variety of diseases like endocarditis, pneumonia, puerperal sepsis, osteomylitis, unexplained leucocytosis or leucopenia, pyrexia of unknown origin and enteric fever.

Many infectious agents, vectors , nonhuman reservoir species and rate of pathogen replication are sensitive to climatic conditions. Salmonella species proliferate at higher temperature, growth of Salmonella is greatly reduced at $<15^0$C, also highest outbreaks occur during the summer to early rainfall [2] . Precipitation or storm may mobilize the pathogens and transport them into the aquatic environment. In regions where low temperature , low rainfall or absence of vector habitat restrict transmission of Vector borne diseases, climatic changes could break the ecological balance and trigger epidemics. We all know, 2020 is an unique year, the COVID Pandemic has left an all pervasive effect on the ecolological system thus altering the normal trend of agent, host and environment. Global warming has also extended the summer months disrupting the nature of many pathogens.

Each year a seasonal trend of Salmonella infection is observed in our bacteriology laboratory, with the highest outbreaks during the summer to monsoon. We generally receive two or three cases of Enteric fever. But this year we faced a different situation. Within twenty days gap (starting from 6th January to 25th January ,2020) we isolated four Salmonella species from blood samples. All the samples were from different socioeconomic backgrounds, residing in different parts of the city .This sudden spurt of Salmonella infection during cooler month of the year violates the normal seasonal trend of the Salmonella infection. Out of these four samples, three samples were diagnosed as Salmonella Typhi and one turned out to be Salmonella Paratyphi A. These cases were confirmed by Vitek2 Compact System.

Salmonella enterica serovar Typhi and serovar Paratyphi A belongs to the family Enterobacteriaceae and Genus Salmonella. They cause Typhoid and Paratyphoid A fever respectively and also gastroenteritis. The infection is usually food-borne and water borne. To produce infection in case of Salmonella species, about 200 to 106 colony forming units are required whereas only 100 organisms can produce infection in case of Shigella [3]. Alteration of bowel flora, neutralization of acidity in stomach, alteration of the integrity of bowel mucosa facilitates Salmonella infection.

Most Salmonella species have no animal reservoir. Different modes of transmission include- feco-oral (main route) and sexual among homosexual males. Healthcare workers may get the infection from patients and during handing the specimens due to unhygienic practices.

The study was conducted to notify a sudden spurt of Salmonella infection during an unusual climatic condition when temperature was between 12-15 0 c, which had not been observed in our laboratory before.

MATERIALS AND METHODS

This is an observational study. We received four blood samples of Salmonella at the beginning of 2020,i.e two samples were received between 6th January to 8th January, both of them reported as Salmonella Typhi and other two came on the same day, 25 th January. One of them was reported as Salmonella Paratyphi A and the other as Salmonella Typhi.

Blood samples were taken from suspected patients and inoculated in conventional blood culture bottle. Bottles were incubated at 37^0 and after 24 hours subculture was done in MacConkey agar and Blood agar media. From the growth, we performed Gram stain and Hanging Drop preparation for motility detection. For identification of the organism, relevant biochemical tests were done. Further identification antibiotic sensitivity were done by VITEK2 Compact machine taking GN 280 card for ID and AST

RESULTS

Inoculated MacConkey agar and Blood agar plates were incubated at 370C for 24 hours and colony morphology was noted from the growth obtained. **On MacConkey's agar-** nonlactose fermenting, flat colonies noted

Fig.1 Growth on MacConkey's agar

Blood agar showed- non haemolytic, transluscent, convex, moist , greyish colonies.

Fig.2 Growth on Blood agar

Gram stain showed -Gram negative, non-sporing bacilli arranged haphazardly.

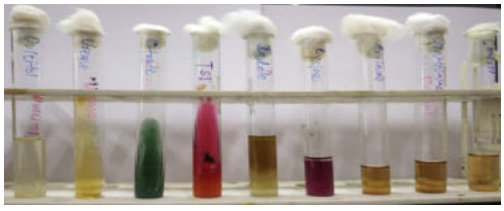
Hanging Drop Preparation showed motile organism.

Biochemical tests

- **For confirmation of Salmonella Typhi-**

Catalase- Positive, Oxidase – Negative, Indole – Negative , Simmon's Citrate – Negative, Urease – Negative, TSI – K/A with H2S production(with typical Moustache sign) ,Lysine – Positive, Ornithine – Negative, Arginine- Negative, Dulcitol- Negative.

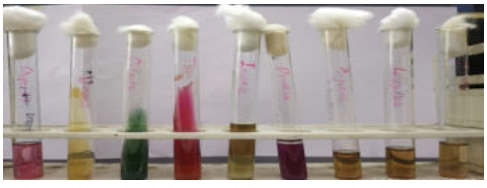
Fig.3 From right side sequence - Control tube for Decarboxylase test, Ornithine, Arginine, Lysine, Indole, TSI, Simmon's Citrate , Urease and Dulcitol.



- **For Confirmation Of Salmonella Paratyphi A**

Catalase- Positive, Oxidase- Negative, Indole- Negative, Simmon's Citrate - Negative, Urease – Negative, TSI- K/A without production of H2S, Ornithine- Positive, Arginine-Negative, Lysine- Negative, Dulcitol- fermented with production of gas.

Fig. 4 From Right to left - Control tube for Decarboxylase test, Lysine, Arginine, Ornithine , Indole, TSI, Simmon's Citrate, Urease and Dulcitol

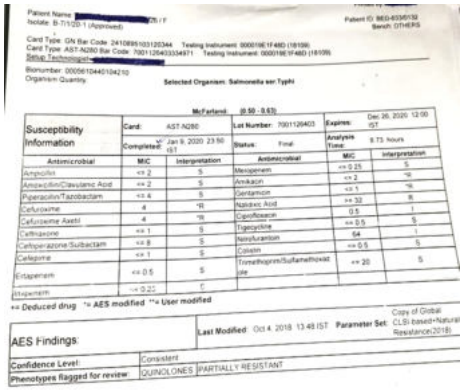


Identification and Antibiotic susceptibility pattern of the organisms by VITEK 2

Fig.5 First Case came on 6th January,2020. A 26 year old female patient came from Howrah, with history of fever for 8 days along with diarrhoea, blood samples was collected ,inoculated into the conventional blood culture media, followed by subculture on solid media, colonies appeared after 24 hrs incubation of solid media ,after routine identification procedure, growth was put into automated system VITEK 2 for confirmation of the organism and antibiotic susceptibility. It was detected as Salmonella Typhi on 9th January . So the whole procedure took 72hours to report a culture positive Salmonella.

The organism was resistant to Nalidixic acid and partially resistant to Ciprofloxacin.

- **Note 1: First and Second Generation(Cefuroxime) Cephalosporins and Amynoglycosides (Amikacin and Gentamycin) are clinically ineffective to Salmonella species. That's why updated VITEK system automatically skipped them and showed resistant with highlights.**



Selected Organism: Salmonella ser Typhi

Susceptibility Information	Card: AST-N280	Lot Number: 7001126403	Expires: Dec 26, 2020 12:00 IST
	Completed: Jan 9, 2020 23:50 IST	Status: Final	Analysis Time: 8.73 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	<= 2	S	Meropenem	<= 0.25	S
Amoxicillin/Clavulanic Acid	<= 2	S	Amikacin	<= 2	*R
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	*R
Cefuroxime	4	*R	Nalidixic Acid	>= 32	R
Cefuroxime Axetil	4	*R	Ciprofloxacin	0.5	I
Ceftriaxone	<= 1	S	Tigecycline	<= 0.5	S
Cefoperazone/Sulbactam	<= 8	S	Nitrofurantoin	64	I
Cefepime	<= 1	S	Colistin	<= 0.5	S
Ertapenem	<= 0.5	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Imipenem	<= 0.25	S			

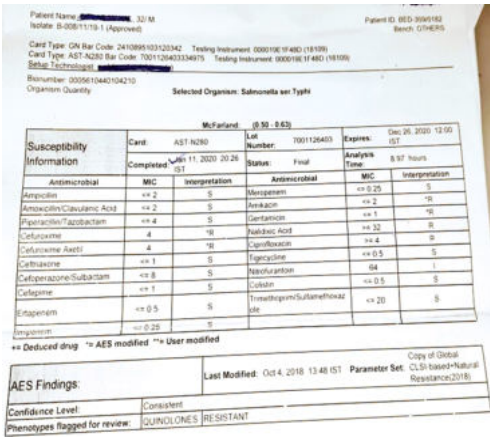
** Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Oct 4, 2018 13:48 IST	Parameter Set: Copy of Global: CLSI based+Natural Resistance(2018)
Confidence Level:	Consistent	
Phenotypes flagged for review:	QUINOLONES	PARTIALLY RESISTANT

Fig6. Second Case came on 8 th January 2020, where a 32 year old male patient came with fever, semisolid stool for 7 days. He also gave a history of recent travel . Blood sample was collected, same protocol was followed , causative organism was Salmonella Typhi, reported on 11th January.

The organism was totally resistant to Nalidixic acid and Ciprofloxacin.

- **NOTE 1**



Selected Organism: Salmonella ser Typhi

Susceptibility Information	Card: AST-N280	Lot Number: 7001126403	Expires: Dec 26, 2020 12:00 IST
	Completed: Jan 11, 2020 20:26 IST	Status: Final	Analysis Time: 8.97 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	<= 2	S	Meropenem	<= 0.25	S
Amoxicillin/Clavulanic Acid	<= 2	S	Amikacin	<= 2	*R
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	*R
Cefuroxime	4	*R	Nalidixic Acid	>= 32	R
Cefuroxime Axetil	4	*R	Ciprofloxacin	>= 4	R
Ceftriaxone	<= 1	S	Tigecycline	<= 0.5	S
Cefoperazone/Sulbactam	<= 8	S	Nitrofurantoin	64	I
Cefepime	<= 1	S	Colistin	<= 0.5	S
Ertapenem	<= 0.5	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Imipenem	<= 0.25	S			

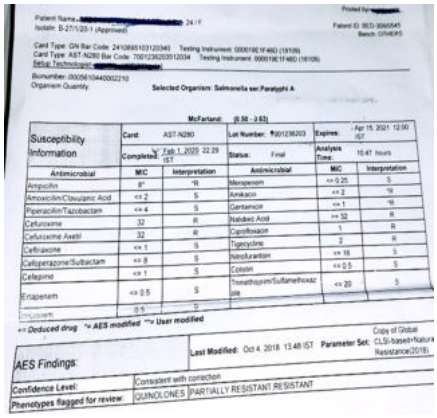
** Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Oct 4, 2018 13:48 IST	Parameter Set: Copy of Global: CLSI based+Natural Resistance(2018)
Confidence Level:	Consistent	
Phenotypes flagged for review:	QUINOLONES	RESISTANT

Fig. 6 Third case came on 25th January, 2020. A 24 year old female from a slum of Dumdum, presented with fever, abdominal discomfort, loss of appetite for several weeks. Blood sample was collected, following same protocol we isolated Salmonella Parartyphi A on 1st February.

The organism was resistant to Nalidixic acid and Ciprofloxacin.

NOTE 1



Selected Organism: Salmonella ser Paratyphi A

Susceptibility Information	Card: AST-N280	Lot Number: 7001126203	Expires: Apr 15, 2021 12:00 IST
	Completed: Feb 1, 2020 22:29 IST	Status: Final	Analysis Time: 15.47 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	8*	*R	Meropenem	<= 0.25	S
Amoxicillin/Clavulanic Acid	<= 2	S	Amikacin	<= 2	*R
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	*R
Cefuroxime	32	R	Nalidixic Acid	>= 32	R
Cefuroxime Axetil	32	R	Ciprofloxacin	1	R
Ceftriaxone	<= 1	S	Tigecycline	2	R
Cefoperazone/Sulbactam	<= 8	S	Nitrofurantoin	<= 16	S
Cefepime	<= 1	S	Colistin	<= 0.5	S
Ertapenem	<= 0.5	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Imipenem	0.5	I			

** Deduced drug *= AES modified **= User modified

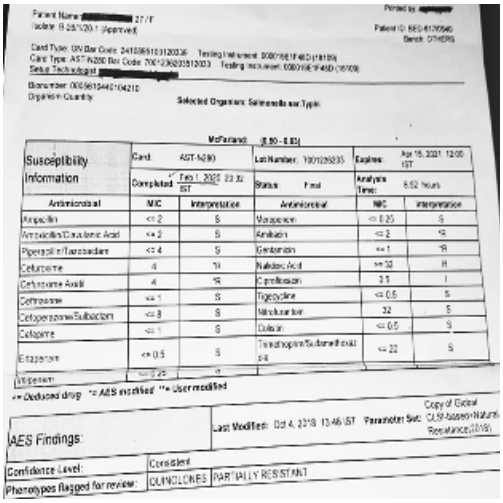
AES Findings:	Last Modified: Oct 4, 2018 13:48 IST	Parameter Set: Copy of Global: CLSI based+Natural Resistance(2018)
Confidence Level:	Consistent with correction	
Phenotypes flagged for review:	QUINOLONES	PARTIALLY RESISTANT,RESISTANT

Fig. 7 Fourth Case came again on 25th January , 2020. A 27year old female patient presented with rash,fever, diarhhoea for 5 days, blood samples were collected and tested for Dengue, Scrub typhus, Malaria

and Enteric fever. The sample was turned out to be positive for Salmonella Typhi on 1st February.

The organism was resistant to Nalidixic acid and partially resistant to Ciprofloxacin.

- **NOTE 1**



Susceptibility Information	Card: AST-N280		Lot Number: 190126835		Expires: Apr 15, 2021 12:00 IST	
	Completed: Feb 1, 2020 23:30 IST		Status: Final		Analysis Time: 8.62 hours	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation	
Ampicillin	<= 2	S	Meropenem	<= 0.25	S	
Ampicillin/Sulbactam	<= 2	S	Amikacin	<= 2	*R	
Piperacillin/Tazobactam	<= 4	S	Gentamicin	<= 1	*R	
Cefuroxime	4	*R	Nalidixic Acid	>= 32	R	
Cefuroxime Axetil	4	*R	Ciprofloxacin	2.5	I	
Ceftriaxone	<= 1	S	Tigecycline	<= 0.5	S	
Cefoperazone/Sulbactam	<= 8	S	Nitrofurantoin	32	S	
Cefepime	<= 1	S	Colistin	<= 0.5	S	
Ertapenem	<= 0.5	S	Trimethoprim/Sulfamethoxazole	<= 20	S	
Imipenem	<= 0.25	S				

** = Deduced drug * = AES modified ^ = User modified

AES Findings: Last Modified: Oct 4, 2018 13:46 IST Parameter Set: Copy of Global CLSI-based+Natural Resistance (2018)

Confidence Level: Consistent

Phenotypes flagged for review: QUINOLONES PARTIALLY RESISTANT

DISCUSSION

Apart from the actual data we have obtained and analyzed, there is an angle that has opened up in this study. According to our textbook knowledge, we know already that organisms producing faeco-oral infections through food and water, normally raged during the summer and monsoon months. The abundant rainfall and hot temperature at this time is suitable for their multiplication and transmission, particularly in a country like India where unhygienic conditions, lack of health education and population explosion are present in combination. In our study, we find that, the Enteric Fever causing organisms have spread their wings beyond the typical summer and monsoon months. This can be explained by the fact that global warming has actually caused an extension of the summer months and almost every alternate year, we have heavy monsoons. This extended summer and heavy rainfall indulges the Salmonellae that we have studied. Over and above this, we find that as case number increases antibiotic use obviously has to increase. In certain cases, empirical and unnecessary antibiotic use without confirmation of disease diagnosis, becomes more harmful and ultimately results in drug resistant strains. In this way, we have practically lost the use of a very good class of antibiotic – Fluoroquinolones. Nalidixic acid is practically useless at present time [NARST- Nalidixic Acid Resistant Salmonella Typhi] and Ciprofloxacin also has almost lost its momentum. This is clear from our study.

Previous studies show varying results - A study in The USA in 2010 showed high incidence of Salmonella cases from May to July.

Another study in Odisha in 2013 showed high Salmonella cases from summer to monsoon.

CONCLUSION

Such frequent studies with organisms in their endemic zones help us to monitor their status regarding their epidemiology. Physician also mandates the use of such studies to form a well calculated antibiotic policy and update from time to time by reducing the use of drugs which starts showing resistance. As the antibiotic industry is not as active as it was previously for reasons we all know, their rational use as stated above will help us to keep our heads above water. Apart from this, we also have to be cautious regarding control measures throughout the year, like , widespread health education , cleanliness and proper waste disposal practices .

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