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Original Article

Microbiology Section

Identification and Characterisation of *Salmonella* spp. Isolated from Stool Samples of a Food Poisoning Outbreak in Chite, Mizoram, India due to Consumption of Bekang-Um (Fermented Soybean)

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ABSTRACT

Introduction: Traditional fermented soybean ("bekang-um") is widely consumed by the indigenous Mizo tribe in Mizoram and plays a significant part of the local staple food. They are commonly prepared with herbs like culantro and often with just locally dried chilli flakes, implying it is mostly consumed raw without further cooking. Though it plays a staple part of the diet because of its unique taste, awareness on proper and regulated hygienic conditions during its preparation and fermentation processes is quite negligent. The fermentation process often warrants for the growth of not only commensal lactic acid fermenter bacteria like *Acinetobacter* spp., *Lactobacillus* spp., but also provides ample growth conditions for harmful bacteria like *Salmonella* spp., *Clostridium* spp., Intestinal Pathogenic *E.Coli* (IPEC) and *Bacillus cereus*.

Aim: Phenotypic and molecular characterisation of *Salmonella* spp. isolated from the fermented food product bekang-um.

Materials and Methods: The cross-sectional study was conducted within three months (October to December, 2020) in Multidisciplinary Research Unit (MRU), Zoram Medical College in collaboration with the Department of Veterinary Microbiology, CVSc and AH, Central Agricultural University, Selesih, Mizoram. A total of 18 stool samples were collected from patients (living in Chite, a small locality in Aizawl) who were still exhibiting symptoms of food poisoning (diarrhoea, vomiting and nausea) after consumption of fermented soybean all bought from their local neighbourhood fermenter and seller of the food item. The data was tabulated and statistically analysed using Microsoft excel and sensitivity/resistance patterns were expressed in percentages.

Results: *Salmonella* spp. was isolated from 17 out of 18 stool samples collected. Out of 17 samples, 15 exhibited Multi-Drug Resistance (MDR) phenotypically to clinically relevant and widely used antibiotics like cefotaxime, ceftriaxone, ciprofloxacin, amoxicillin-clavulanic acid. Alarmingly, resistance genes like *bla*CTX-M1 (70.6%), *bla*CTX-M2 (35.3%), *bla*SHV (70.6%), *bla*TEM (52.9%), *bla*NDM (5.8%), *bla*AmpC (100%), *bla*KPC (29.4%), *bla*VIM (23.5%), *bla*IMP (11.8%) were commonly detected. Moreover, *Salmonella* virulence determinants like *invA* (41.2%), *stn* (41.2%), and biofilm-associated virulence genes like *csgA* (11.8%), *csgD* (70.6%) and *adrA* (5.9%) were also identified.

Conclusion: The identification and characterisation of *Salmonella* spp. isolated from a widely consumed food product "bekang-um" (fermented soybean) with co-occurring crucial virulence, biofilm-producing and resistance gene determinants is quite alarming. The findings suggest the urgent need of implementing food safety conditions and proper preparation standards of the exotic food item, and the seriousness of the potential threat it can cause in cases of unhygienic fermentation processes.

Keywords: Contaminated food, Drug resistance, Drug resistance gene, Fermented products

INTRODUCTION

The consumption of 'bekang-um', an indigenous fermented soybean food of the Mizo tribe in Mizoram, India is highly popular. The consumption and preparation of this food item plays a crucial part of the food culture and is well-loved by the people of the region and generally consumed as a side dish prepared often as chutney with other herbs like culantro, spring onion and chilli. Although bekang-um has been part of the tribes' diet for generations, awareness in hygienic fermentation process, the actual food content (especially microorganisms) and nutritional benefits of the food item is still highly under-studied. The presence of Lactic Acid Bacteria (LAB) as the main fermentation by-product is predictable, but also, the existence of foodborne pathogens like *Salmonella* spp., *Clostridium* spp., IPEC is also suspected to be often inevitable [1]. Hence, several food-poisoning episodes have occurred in the State after the common consumption of the fermented food product often bought from a single source in a neighbourhood. Such food-poisoning incidents have recurrently happened in the community but often go unreported. This further highlights the urgent need of following standard hygienic food safety guidelines, awareness in the proper production and fermentation process of this valuable food item, and surveillance studies of foodborne outbreaks to eliminate and avoid further food-poisoning occurrences to happen in the future.

Salmonella spp. was first identified and isolated from the pig intestine suffering from classical swine fever in the year 1855 by Theobad Smith [2]. It is considered as one of the most significant pathogens correlated with food-borne diseases and outbreaks in the world that causes the highest number of gastroenteritis and deaths, also attributed to frequent epidemics or outbreaks [1] and also predicted to cause roughly 93.8 million human infections and 155,000 mortality per annum worldwide. *Salmonella* genus consists of more than 2500 serological variants and is predominantly grouped into *S. enterica* and *S. bongori* [3,4]. Food poisoning outbreaks among humans caused by *Salmonella* spp. are often due to *S.enterica, S.typhimurium, S.choleraesuis, S.enteritidis* and many other similar species which are recently often MDR [4].

The prevalence in isolation of *Salmonella* strains with resistance to one or more antimicrobial agents has increased in different countries [5] and the superbugs are often resistant to distinct classes of antibiotics, therefore lead to an immense threat of using antibiotics [6]. Often, antimicrobial agents such as chloramphenicol, trimethoprime-sulfamethoxazole and ampicillin are used in the medication of *Salmonella* spp. infections and the resistance against these antibiotics are indicated as MDR [7]. The requirement of new antimicrobials to *Salmonella* spp. serovars resistant to fluoroquinones, quinolones, carbapenems and 3rd and 4th generation cephalosporins, which are medically important remedies, has crucially elevated [8].

A well-known *Salmonella* spp. enterotoxin (*stn*) has been proven to serve as a virulence factor in foodborne diseases with clinical manifestations like diarrhoea, acute gastroenteritis and typhoid fever [9-11]. Furthermore, it has also been demonstrated that the *stn* gene is precisely distributed in *Salmonella* spp. irrespective of their serotypes [12,13]. The invasion A (invA) virulence gene is also used as a biomarker for detecting *Salmonella* spp. as it consists of sequences that are unique only to the genus Salmonella. Further, invA is present on the outer membrane of *Salmonella* spp. and is therefore known to be the gene responsible for entering the host epithelial cells in the intestines thus prompting infection, hence it plays a crucial role in displaying virulence in the intestine [14]. *Salmonella* spp. are generally known to form biofilms, which aids in the organisms' resistance to adverse conditions [15] and can form biofilms on a variety of surfaces and cellulose and curli fibres are dominant components of their biofilm matrix [16]. The regulation of biofilm formation in *Salmonella* spp. is associated with biofilm-producing genes like *csgA, csgD, adrA* and many others. The gene *csgA* produces curli protein, *csgD* functions as an activator structural gene responsible for the production of curli fimbriae by transcriptional activation [17]. Biofilm-producing gene *adrA* is known to activate cellulose production [18] and often aid in the virulence or pathogenicity of the organism.

This study has reported the first in-depth characterisation of *Salmonella* spp. identified and isolated from one of the many foodborne outbreaks caused by consumption of the fermented soybean product, bekang-um in Aizawl, Mizoram. Further, the molecular characterisation of important resistance and virulence gene determinants present in the isolated *Salmonella* spp. was also performed.

MATERIALS AND METHODS

This is a cross-sectional study conducted in the department of Multidisciplinary Research Unit (MRU), Zoram Medical College in collaboration with Department of Veterinary Microbiology, CVSc and AH, CAU, Mizoram from October, 2020 to December, 2020.

Inclusion criteria: Patients exhibiting symptoms such as diarrhoea, stomach uneasiness (slight cramps) and mild fever after five days of consuming bekang-um were included in the study.

Exclusion criteria: On the other hand, the patients who were under antibiotic and asymptomatic during sample collection were excluded.

Study Procedure

Sample collection: The food poisoning incident in this particular region occurred on 23rd September, 2020 in a small locality in Aizawl East, Chite. The state Government immediately delegated medical experts to the site where the affected individuals exhibiting food poisoning symptoms were accommodated in a Community Hall. In total 52 patients were affected who were from 17 families all hailing from the same locality. They were closely monitored under medical supervision and provided appropriate treatment. The source of the particular bekang-um consumed by the patients was identified and located to be from the same neighbourhood's single fermenter. Stool samples were collected in sterile vials with enrichment media from 18 out of 52 food poisoning affected persons after obtaining informed consent. Samples collection was done five days after the outbreak, by which most of the affected individuals were already on antibiotics treatment. Stool samples were collected and transported through cold chain to Microbiology laboratory at CVSc and AH, Selesih for further processing and characterisation.

Phenotypic characterisation: All the collected stool specimens were enriched in Luria Bertani or LB broth (HiMedia Laboratories Pvt., Ltd.,). After 18 hours incubation, the bacterial culture growing on LB broth was subcultured onto Rappapport-Vassilidis or R-V broth (HiMedia Laboratories Pvt., Ltd.,) and incubated in shaking condition at 37°C for 24 hours. The broth was observed for colour turbidity and cultures which turned slight opaque greyish from the normal transparent blue medium were presumed as preliminary positives [5].

Antimicrobial Susceptibility Test (AST): AST was performed on all *Salmonella* spp. isolates using Kirby-Bauer disc diffusion method following standard Clinical and Laboratory Standards Institute (CLSI) guidelines and breakpoints [19]. A single colony of the isolated bacterial cultures from selective media like XLD (black colonies), SS (black colonies) were inoculated onto LB broth and used to perform AST further. Clinically, relevant antibiotics belonging to different classes like Beta-lactams-ampicillin (AMP, 10 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), Cephalosporins-ceftriaxone (CTR, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), aztreonam (AT, 30 µg), cephalexin (CN, 30 µg), cefixime (CFM, 5 µg), cefuroxime (CXM, 30 µg), Carbapenems-imipenem (IPM, 10 µg), meropenem (MRP, 10 µg), Aminoglycosides-amikacin (AK, 30 µg), gentamycin (GEN, 10 µg), streptomycin (S, 10 µg), azithromycin (AZM, 15 µg), Tetracyclines-tetracycline (TE, 10 µg), Fluoroquinolones-ciprofloxacin (CIP, 5 µg), enrofloxacin (EX, 5 µg), Quinolones-nalidixic acid (NA, 30 µg), Folate pathway inhibitors-co-trimoxazole (COT, 1.25/23.75 µg), Phenicols-chloramphenicol (C, 30 µg), Nitrofurans-nitrofurantoin (NIT, 300 µg), Polymyxin-polymyxin-B (PB, 300 units), colistin methane sulphonate (CL, 10 µg) (HiMedia Laboratories Pvt., Ltd.,) were used for the susceptibility testing.

Molecular characterisation: DNA extraction was carried out on all 17 *Salmonella* spp. isolates using standard lysis method (boiling and snap chilling). A total of 16S rRNA typing was performed on all isolates using the 16S primer set: F: 5'-TATCTGGCTATCGCTGGCAGTG-3', R: 5'-TCCGCTAATCTTTTGGCAACC-3' with 480 bp amplicon size [20].

Antimicrobial resistance genes characterisation: The *Salmonella* spp. isolates were screened for the presence of resistance gene determinants to different antimicrobial classes including Extended Spectrum Beta-Lactams (ESBLs) like blaCTX-M1, CTX-M2, CTX-M9, *bla*SHV, *bla*TEM, AmpC type β-Lactamase (ACBL) like *bla*AmpC, and carbapenems like *bla*NDM, *bla*VIM, *bla*IMP, *bla*KPC. The primers details and amplicon size are listed in [Table/Fig-1].

Oligonucleotide primers	Sequence (5'-3')	Amplicon size
TEM-1	F-CATTTCCGTGTCGCCCTTATTC R-CGTTCATCCATAGTTGCCTGAC	800 bp
SHV-1	F-AGCCGCTTGAGCAAATTAAAC R-ATCCCGCAGATAAATCACCAC	713 bp
OXA-1, 4, 30	F-GGCACCAGATTCAACTTTCAAG R-GACCCCAAGTTTCCTGTAAGTG	564 bp
CTX-M Gp 1	F-TTAGGAARTGTGCCGCTGYA R-CGATATCGTTGGTGGTRCCAT	688 bp
CTX-M Gp 2	F-CGTTAACGGCACGATGAC R-CGATATCGTTGGTGGTRCCAT	404 bp
CTX-M Gp 9	F-TCAAGCCTGCCGATCTGGT R-TGATTCTCGCCGCTGAAG	561 bp
IMP	F-TTGACACTCCATTTACDG R-GATYGAGAATTAAGCCACYCT	139 bp
VIM	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCACCAG	390 bp

KPC	F-CATTCAAGGGCTTTCTTGCTGC R-ACGACGGCATAGTCATTTGC	538 bp
NDM-1	F-GGGCAGTCGCTTCCAACGGT R-GTAGTGCTCAGTGTCGGCAT	475 bp
AmpC	F-CCCCGCTTATAGAGCAACAA R-TCAATGGTCGACTTCACACC	634 bp

[Table/Fig-1]: Primer details and amplicon size for detection of resistance genes.

The PCR conditions used for the primers include an initial denaturation at 94°C for five minutes, followed by 30 cycles of denaturation step at 94°C for 45 seconds, annealing at 60°C for TEM, SHV, OXA, CTX-M (all groups), NDM-1, and 55°C for IMP, VIM, KPC, AmpC, then extension step at 72°C for 45 seconds. A final extension step at 72°C for seven minutes is eventually carried out [21,22]. The reaction was performed in a thermalcycler (Eppendorf, Germany) with a total volume of 10 µL, containing 5 µL of readymade power EmeraldAmp GT PCR Mastermix (Takara), 10 pmol of each primer (0.5 µL each), and 4 µL of the extracted DNA. PCR was run along with negative control (no added DNA) and a positive control (*S.enteritidis*).

Characterisation of virulence and biofilm-associated gene determinants: Identification of virulence determinants was executed using the invasion gene (*invA*) specific primer pairs [23]. The PCR reaction conditions consisted of a primary denaturation at 94°C for 5 minute, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension at 72°C for seven minutes. In addition, the isolates were screened for enterotoxin virulence factor determinant *stn* gene at annealing temperature of 60°C [24]. The presence of biofilm-producing gene determinants like *csgA*, *csgD*, *adrA* was also screened. The annealing temperatures for *csgA* and *csgD* (duplex PCR) [25] and *adrA* were 59°C for 30 and 45 seconds, respectively [Table/Fig-2] [26]. The PCR products (10 µL) were loaded in 1.2% agarose gel prepared with 5uM ethidium bromide (Sigma Aldrich) and ran alongside 5 µL of 100 bp DNA ladder (Thermo Fischer Scientific, India). The gels were then run in 1xTAE for 35 minutes at 120 volts and then visualised using ultraviolet transilluminator (Gel Documentation System, Bio-Rad).

Oligonucleotide primers	Sequence (5'-3')	Amplicon size
invA	F-GTGAAATTATCGCCACGTTCGGGGCAA R-TCATCGCACCGTCAAAGGAACC	284 bp
Stn	F-TTGTGTCGCTATCACTGGCAACC R-ATTCGTAACCCGCTCTCGTCC	617 bp
csgA	F-ATTGCAGCAATCGTAGTTTCTGG R-ATWGAYCTGTCATCAGAGCCCTGG	245 bp
csgD	F-TGAAARYTGGCCGCATATCAATG R-ACGCCTGAGGTTATCGTTGCC	355 bp
adrA	F-GGCCATTAAATTAGCGGAAC R-AATAAAATTTCCCAGTGGCG	99 bp

[Table/Fig-2]: Primer details and amplicon size for detection of virulence gene determinants.

STATISTICAL ANALYSIS

The data was tabulated and statistically analysed using Microsoft excel and sensitivity/resistance patterns were expressed in percentages.

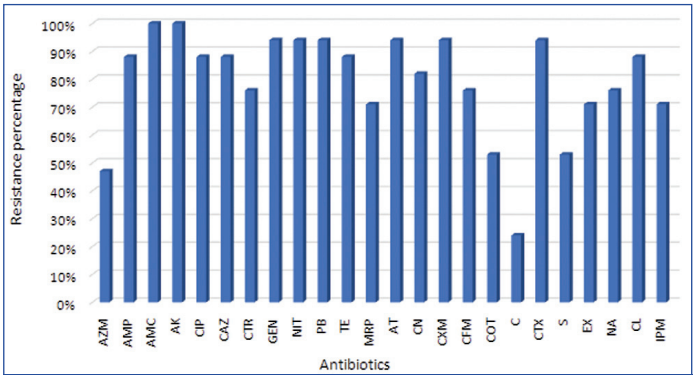
RESULTS

The cultures from R-V broth were then plated onto different selective medium like MacConkey agar (non lactose fermenting colourless colonies), Brilliant Green Agar (BGA) (red to pink-white colonies), Xylose Lysine Deoxycholate (XLD) Agar (black colonies), and Salmonella-Shigella Agar (SS) Agar (black colonies) [Table/Fig-3] (all HiMedia Laboratories Pvt., Ltd.,) for further confirmation of *Salmonella* spp.

Phenotypic characterisation: Out of 18 stool samples collected from patients with common food-poisoning symptoms like diarrhoea, nausea, vomiting, 94.4% (N=17) harboured *Salmonella* spp. confirmed phenotypically using different selective media.

[Table/Fig-3]: *Salmonella* spp. (black colonies) isolated in Salmonella-Shigella (SS) agar and Xylose Lysine Deoxycholate (XLD) agar.

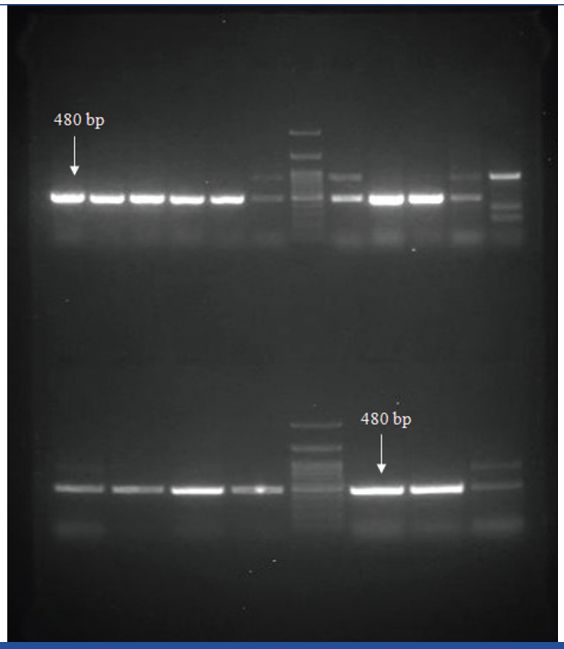
Antimicrobial resistance of all the *Salmonella* spp. isolates were tested against different antimicrobial agents using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) following the standard procedure of the CLSI [19]. Phenotypic resistance was found highest to antimicrobials like amoxicillin-clavulanic acid, amikacin (100%) followed by gentamycin, nitrofurantoin, polymyxin-B, aztreonam, cefuroxime, cefotaxime (94% each), then followed closely by ampicillin, ciprofloxacin, ceftazidime, tetracycline, colistin (88% each). Cephalexin resistance was found in 82% isolates, followed by high resistance observed against ceftriaxone, cefixime and nalidixic acid (76% each). Resistance to last resort antimicrobial belonging to class carbapenem like meropenem and imipenem were found in 71% of the samples and also to the widely used antibiotic enrofloxacin. Resistance was found to be lower in antibiotics like co-trimoxazole (53%), streptomycin (53%), azithromycin (47%) and lowest resistance was observed in chloramphenicol (24%) {[Table/Fig-3,4], [Supplemental Table/Fig-1]}.



[Table/Fig-4]: Antimicrobial resistance percentage of *Salmonella* spp. against 24 clinically relevant antibiotics.

Molecular characterisation: Out of 18 samples tested, 17 samples (94.4%) were confirmed to be *Salmonella* spp. by molecular characterisation of 16S rRNA [Table/Fig-5]. Amplification of the 480 bp 16S rRNA gene confirmed the identity of *Salmonella* spp. in the isolates screened.

After further characterisation, the isolates were found to harbour antimicrobial resistance genes such as *bla*SHV, *bla*TEM, *bla*NDM, *bla*AmpC, *bla*KPC, *bla*VIM and *bla*CTX-M1, *bla*CTX-M2 [Table/Fig-6] and virulence genes including *stn*, *invA*, and also biofilm-associated genes like *csgA, csgD* and *adrA* were also identified [Table/Fig-7].



[Table/Fig-5]: Gel electrophoresis image representing 16S rRNA *Salmonella* spp. (480 bp amplicon size) positive isolates.

samples (70.58%) were positive for *bla*SHV gene in 9 (52.94%) isolates were confirmed to be positive for the resistance gene *bla*TEM, while *bla*NDM was identified in only 1 (5.8%) isolate, *bla*IMP was detected in 2 isolates (11.76%), *bla*AmpC in 17 isolates (100%), KPC in 5 (29.41%), *bla*VIM in 4 (23.52%), *bla*CTX-M1 in 12 (70.58%) and *bla*CTX-M2 in 6 isolates (35.29%). Seven kinds of resistance genes were identified in the *Salmonella* spp. isolates characterised. The maximum rate of resistance was observed to be in *bla*AmpC gene (100%), followed by *bla*SHV (70.58%), *bla*CTX-M1 (70.58%), *bla*TEM (52.94%), *bla*CTX-M2 (35.29%), *bla*KPC (29.41%). *bla*VIM (23.52%) was detected at low rates and the resistance gene *bla*NDM presented the lowest rate (5.8%). The outcome discloses that 4 out of the 17 isolates were found to exhibit resistance against three or four groups of resistance genes, thus classifying them as MDR. Our results highlighted that antimicrobial resistance genes are extensively identified in *Salmonella* spp. isolates. In a study reported by Ghazaei C, it was found that 85% of the isolates harboured *bla*TEM which was higher than the current results, 60% and 35% for *bla*SHV and *bla*CTX-M genes which was lower than our present findings [37]. In another study reported by Qiao J et al., the resistance gene *bla*TEM was found to be 57.3% of the isolated *Salmonella* spp., comparatively higher than the current data [38].

Number of isolates (%)	*bla*CTX-M1	*bla*CTX-M2	*bla*SHV	*bla*TEM	*bla*NDM	*bla*VIM	*bla*IMP	*bla*KPC	*bla*AmpC
17 (%)	12 (70.58%)	6 (35.29%)	12 (70.58%)	9 (52.94%)	1 (5.8%)	4 (23.52%)	2 (11.76%)	5 (29.41%)	17 (100%)

[Table/Fig-6]: Antimicrobial resistance genes representation to Extended Spectrum Beta-Lactamases (ESBLs), carbapenems, AmpC-type beta-lactamase (ACBL) on *Salmonella* spp. Isolates.

Number of isolates (%)	*csgA*	*csgD*	*adrA*	*invA*	*stn*
17 (%)	2 (11.76%)	12 (70.58%)	1 (5.8%)	5 (41.2%)	5 (41.2%)

[Table/Fig-7]: Biofilm-associated genes and virulence determinants representation on *Salmonella* spp. Isolates.

DISCUSSION

Salmonella spp. is one of the most recurrent bacterial causes of food poisoning and a prominent foodborne pathogen in human that causes the highest number of mortality [1]. In the present study, an outbreak of food poisoning was investigated and was found to be caused by *S.enterica,* the most analysed causative agents implicated in foodborne salmonellosis [3]. Our results were consistent with the study reported by Di Giannatale E et al., and CDC (1991, 1992), where an outbreak of food poisoning caused by *S.enterica* was described [27-29]. Similar findings were studied where the causative agent of the food borne outbreak was found to be *Salmonella* spp. [30]. In another study by Antony B et al., the significant role of *S. enterica* in food poisoning was also documented [31]. Singh BR et al., studied the safety and microbial aspect of fermented soybean food [32]. They found out that one of the important bacteria capable of causing foodborne illness includes strains of *Salmonella* spp.

In comparison Choudhary A et al., studied the antimicrobial susceptibility pattern among the *Salmonella* spp. isolates [33]. Their findings revealed that the isolates were resistant to ciproflaxacin with 44% which was lower than the present results and nalidixic acid resistance was found to be 91% comparatively higher than the current data. The present study concurred with the previous findings that *Salmonella* spp. isolates were resistance to different antibiotics such as streptomycin, nalidixic acid, tetracycline and chloramphenicol [34]. Correlative findings were reported by Ammar AM et al., where the isolated *Salmonella* strains exhibited 100% resistance to amoxicillin-clavulanic acid [35].

The extracted genomic DNA of *Salmonella* spp. was used to identify antimicrobial resistance genes, biofilm-producing genes and virulence determinants. The virulence of bacteria is made graver both by the existence of antibiotic resistance and virulence genes [36]. Twelve

Different virulence factors and biofilm-producing genes play extensive aspects in *Salmonella* spp. infection [36]. The isolated *Salmonella* spp. collected in the study were tested for the presence of virulence genes with their respective primers. It was found that *csgD* revealed the highest presence rate with 70.58%. Out of 17 isolates, 2 (11.76%) were confirmed to be positive for the *csgA* gene. Subsequently, 41.17% of the *Salmonella* spp. isolates were found to be amplified by *stn* and *invA* genes respectively. All the 17 isolated colonies were positive for 16S rRNA primer. Only one isolate carried the *adrA* gene accounting for 5.88%. The results revealed that three out of the 17 isolates were found to carry two or three virulence genes. In this study, all the isolates were investigated for the presence of 5 virulence genes to evaluate the predictable factors that may enrich to the capability of *Salmonella* spp. to cause an infection. The detection rate for virulence genes was lower than for antimicrobial resistance genes in the present study. The results retrieved from the study indicated that the identified *Salmonella* strains harboured both virulence and antimicrobial resistance genes. The most common virulence gene i.e., *invA* gene present in *Salmonella* spp. which was also detected in the present study were also identified by Thung TY et al., [39]. Another study conducted by Hai D et al., examined the virulence genes and prevalence of antimicrobial resistance with variable percentage [36]. The variability rate of virulence genes was observed for *Salmonella* strains isolated from food products [40].

Limitation(s)

Our study was conducted five days after the actual outbreak occurred when most of the patients were already under treatment therefore most of them were already asymptomatic. Due to delay in the information about the outbreak there was delay in sample collection and thus led to reduction in the sample size.

CONCLUSION(S)

The present study indicate that the organism *Salmonella* spp. identified is highly resistant and virulent. The findings can be viewed as quite alarming considering the fact that most of the *Salmonella* spp. isolates were highly resistant to broad-spectrum antibiotics

commonly used as therapeutic agents in such outbreak cases (like amoxicillin-clavulanic acid, ciprofloxacin, cefotaxime, ceftriaxone, to name a few). Our study further adds information and confirms the rapid rise of antimicrobial resistance in harmful organisms. Consequently, forthcoming study should target on how to constrain contamination by *Salmonella* spp., moderate the rapid rise of antibiotic resistance of *Salmonella* spp. and repress outbreaks of foodborne diseases.

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ID	AMP	AMC	AK	AZM	GEN	CIP	EX	CAZ	CTR	CXM	CFM	CTX	AT	CN	NIT	TE	COT	C	S	NA	PB	CL	MRP	IPM
ZMC1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R
ZMC2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ZMC3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	I	I	R	R	R	R	R
ZMC4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
ZMC5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ZMC6	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R
ZMC7	I	R	R	S	R	I	S	R	R	R	R	R	R	R	R	S	S	I	I	R	R	R	S	S
ZMC8	R	R	R	S	R	R	S	R	R	R	I	R	I	S	R	R	S	S	I	I	R	R	R	S
ZMC9	R	R	R	S	R	I	R	I	S	R	I	I	R	R	R	R	I	R	I	I	R	R	S	I
ZMC10	R	R	R	S	R	R	R	I	I	I	S	R	R	S	R	R	R	S	R	R	R	R	I	R
ZMC11	S	R	R	S	I	R	R	R	I	R	R	R	R	R	I	R	I	I	R	R	R	R	I	R
ZMC12	R	R	R	S	R	R	S	R	R	R	I	R	R	R	R	S	S	S	I	I	R	S	R	I
ZMC13	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	I	S	R	R	R	S	R	R
ZMC14	R	R	R	S	R	R	I	R	S	R	R	R	R	R	R	R	S	S	I	I	R	R	I	I
ZMC15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
ZMC16	R	R	R	R	R	R	I	R	R	R	R	R	R	R	R	R	R	I	I	R	R	R	R	R
ZMC18	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R	R	R	R	R

Supplemental [Table/Fig-1]: Antimicrobial sensitivity worksheet of all 17 *Salmonella* spp. Isolates.