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ORIGINAL ARTICLE

PREVALENCE OF MULTIDRUG RESISTANT *SALMONELLA TYPHI* FROM NAIL SCRAPINGS OF RESTAURANT WORKERS

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ABSTRACT

Background: *Salmonella typhi* forms many multidrug resistant strains causing typhoid fever, which creates a global threat in many developing countries where the hygienic standards are rather poorer. The study aimed to isolate *Salmonella typhi* from the nail scrapings of restaurant workers and to establish the multi-drug resistance and to determine the plasmid profiling of the isolates with various procedures and protocols.

Materials & Methods: This cross sectional study which was designed during the period of February 16, 2021 and March 1, 2022 to analyse 100 nail scrapping samples from healthy and asymptomatic restaurant workers for the presence of *Salmonella Typhi.* The isolates were subjected to the antibiotic sensitivity testing against commercial antibiotics like Amikacin, Cephalothin, Ampicillin, Ciprofloxacin, Erythromycin, Gentamycin, Ofloxacin minocycline, Cefaclor, Penicillin G, Rifampicin, Novobiocin, Netillin, Teicoplanin and Bacitracin. Multiple antibiotic indices were calculated for the isolates. The plasmid Deoxyribonucleic acid (DNA) was isolated and the data were statistically analysed.

Results: Out of 100 specimens, only six showed the presence of *Salmonella typhi* and all were multidrug resistant, as they were resistant to the seven of the selected antibiotics. All the isolates were harboured the plasmids with comparatively higher molecular weight justifying the resistance.

Conclusion: The study underlines the importance of awareness creation on hygiene practices and continuous monitoring of restaurant workers as they are in very close contact with customers.

KEY WORDS: *Salmonella typhi;* Multidrug resistant strains; Antibiogram; Nail scrapings; Plasmid isolation.

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1. INTRODUCTION

1.1 Background: Typhoid fever is one of the major health issues across the globe with an approximate death rate of 200,000 per year in countries with 'low and middle income' where the hygiene is poor, access to protected water is insufficient and the antimicrobial therapy is limited.[1,2] The incidents of such outbreaks of the disease from restaurant workers due to poor hygiene have been reported even in developed countries like Japan,[3] the situation in developing countries is worse. Before the antibiotic therapy, the mortality of typhoid was 10-30%[4] but after therapy begins, it was estimated that 1%.[5] Typhoid is caused by *Salmonella typhi* - a human restricted bacterial pathogen - which is transmitted through water and food contaminated with faecal matters from infected people.[6,7] Reports say that, typhoid fever is an endemic disease in Latin America as well as Asian and African counties.[8,9]

The treatment of typhoid fever primarily involves antibiotics therapy which includes first line antibiotic drugs such as beta lactams combined with chloramphenicol and co-trimoxazole, unfortunately resistant to first line antibiotic strains (called as multidrug resistant strains) were first noted in 1970s.[10] Various reports say that, resistant to first line antibiotics lead to global spread of *Salmonella typhi*.[11,12] which is constantly undergoing the gene mutation in the plasmid, transposon or chromosome to make them

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resistant to first line antibiotics.[13,14] Consequently, the fluoroquinolones have been used for the management of typhoid fever as second-line antibiotics, but increasing resistance was observed against fluoroquinolone[15] Though, cephalosporins are used for the treatment, the resistant strains were isolated in the regions where outbreaks and sporadic cases are reported.[16,17] It was in this scenario that this investigation was designed for the isolation of *Salmonella typhi* and their susceptibility pattern from the nail scrapings of restaurant workers.

1.2 Research Objectives (ROs)

RO 1: To isolate and identify *Salmonella typhi* from the collected samples.

RO 2: To determine the antibiotic sensitivity, multiple antibiotic resistances (MAR) indexing of the isolate and to understand its antibiogram.

RO 3: To determine the plasmid profiling by Isolation plasmid DNA and Gel electrophoresis.

2. MATERIALS & METHODS

2.1 Design, Duration & Setting: This study was designed as in field (sample collection and transportation) and in lab, and was performed during a period of February 16, 2021 and March 1, 2022 at KSRCAS and PSAU, Saudi Arabia.

2.2 Sample collection, isolation and identification of *Salmonella typhi* stains: From a selected hundred healthy, asymptomatic restaurant workers, nail scraping samples were collected in a sterile test tube containing Rappaport Vassiliadis (RV) broth. Without any further delay, the tubes with the samples were transported to the laboratory where the work has been carried out. In the laboratory, to enrich it, the samples were incubated for a period of 24 h at 37^{0}C. To isolate *Salmonella typhi*, after the period of incubation, a loopful of the culture was streaked onto Wilson and Blair agar and Bismuth sulphite agar plates. The colonies isolated were subjected for various biochemical tests (like methyl red, indole, citrate utilization, Voges-Proskauer, urease and carbohydrate fermentation tests), morphological analyse and were further confirmed with triple sugar iron agar test.[18] The isolated and identified *Salmonella typhi* was used for further analysis.

2.3 *In vitro* determination of antibacterial sensitivity - disc diffusion method: The isolated *Salmonella typhi* strains were adjusted to a cell suspension concentration of 0.5 McFarland units by dilution. This suspension was used for further analysis. To understand the sensitivity pattern of the isolated *Salmonella typhi* strains against antibiotics which are commercially available, the disc diffusion assay using Mueller Hinton agar plates the as explained by Beshiru *et al*.[19] was used. The antibiotics screened were all purchased from Hi Media and were: Amikacin (30 mg), Cephalothin (30 mg), Ampicillin (10 mg), Ciprofloxacin (5 mg), Erythromycine (15 mg), Gentamycin (10 mg), Ofloxacin-minocycline (30 mg), Cefaclor (10 mg), Penicillin G (10 mg), Rifampicin (5 mg), Novobiocin (30 mg) Netillin (30 mg), Teicoplanin (30 mg) and Bacitracin (10 mg). Briefly, the prepared sterile Mueller Hinton agar plates were uniformly swabbed with the prepared *Salmonella typhi* inoculum and were left for about five min. then the desired antibiotic discs were aseptically placed on the plates and were incubated at a temperature of 37^{0}C for a period of 24 h. After the period of incubation, the plates were analysed for the formation of growth inhibition zones around the antibiotics and the results were recorded.

2.4 Determination of multiple antibiotic resistances (MAR) indexing: MAR index of the isolates were calculated for resistant pattern of six isolates of *Salmonella typhi* as described by Singh *et al*.[20] using the formula, where MAR index is equal to number of antibiotics resistant (a) divided by the number of total antibiotics used for the study (b).

2.5 Plasmid profiling

2.5.1 Isolation plasmid DNA: Phenol chloroform extraction method was used[21] for the isolation of plasmid DNA from *Salmonella typhi*. In brief, in 2 ml of Luria Bertani (LB) broth, one loopful of multidrug resistant *Salmonella typhi* was inoculated and was incubated at a temperature of 37^{0}C for overnight in a shaker (150 – 200 rpm). After incubation, the bacterial culture (1.5 ml) was centrifuged for 5 min at 10,000 rpm in a fresh Eppendorf tube, and then the supernatant was avoided. Then, with 100 ml of ice-cold suspension buffer (25 mM Tris – HCl (pH 8.0), 50 mM glucose and 10 mM EDTA at pH 8), the pellets of the bacteria were suspended again and it was incubated for on ice for a period 5 min. Subsequently, 200 ml of lysis buffer (0.2 N NaOH with 1% sodium dodecyl sulphate) which was prepared freshly, was added, mixed and the tubes were incubated for a period of 5 min on ice. Thereafter, 150 ml ice-cold solution of 3M potassium acetate was added and mixed thoroughly. It was then incubated for a period of 5 min on ice and was then centrifuged at 12,000 rpm for a period of 5 min. Then, the supernatant was collected in fresh Eppendorf tube. After that, 0.5 ml of chloroform: phenol (1:1) was added and thoroughly mixed followed by addition of ice-cold absolute ethanol (twice the volume) and incubated for 30 min at 70^{0}C. Centrifugation was done for 5 min at 12000 rpm and discards the supernatant and once again, the pellet was again suspended in 70% ethanol (1 ml) and was again centrifuged. Finally, discarded the supernatant and the pellet which contains plasmid DNA was air dried.

2.5.2 Gel electrophoresis: To perform the gel electrophoresis, the pellet was dissolved in 30 ml of TE solution. Then, the samples were loaded onto the gel along with standard marker (Eco RI /Hind III digested l DNA) and at 50 volts, the electrophoresis was performed using 1 x TBE as the running buffer.

When it was noticed that the bromophenol blue dye has travelled a sufficient distance for DNA fragment separation, the electrophoresis process was stopped. Then, on an UV trans-illuminator, the gel was observed for plasmid bands.

3. RESULTS

3.1 Isolation and identification: Hundred nail scraping samples were aseptically collected from healthy and asymptomatic restaurant workers. Among them, only six isolates namely 11, 20, 23, 50, 53 and 57 were isolated as shown in figure 1. Based on the bacteriological analysis, the bacteria isolated were identified as *Salmonella typhi*. All the isolated *Salmonella typhi* showed methyl red positive, Voges Proskauer negative, indole negative, urease negative, citrate utilization positive, the results are presented in figure 2. All the isolates were fermented the tested carbohydrates with the production of gas and acid but not the lactose and the triple sugar iron test confirmed *Salmonella typhi*.

3.2 Antibiotic sensitivity patterns of isolated strains: The sensitivity pattern of all of the isolated *S. typhi* strains against the tested antibiotic were studied against above mentioned commercially available antibiotics and the findings are presented in table 1. It shows that, all the six isolates of *Salmonella typhi* shows resistance to seven antibiotics such as Cefaclor, Ofloxacin, Ciprofloxacin, Erythromycin, Novobiocin, Penicillin - G, Rifampicin and Telcoplamin. Isolates 23, 53 and 57 showed resistances to Ampicillin and isolates 11, 20 and 50 showed sensitivities to Ampicillin. Isolates 20, 23, 50 and 57 showed resistances to Amikacin and sensitivity were showed by isolates 11 and 53. Isolates 20, 23, 53 and 57 showed resistances to gentamycin and sensitivity and intermittence were reported for 11 and 50 respectively. Isolates 11,23,50,53 and 57 showed resistances to Bacitracin and sensitivity was noted for isolate 20. Isolates 11, 20, 50, 53 and 57 showed resistances and sensitivity were observed for isolate 23 to Minocycline as well as isolates 11, 20, 53 and 57 showed resistances to Netillin and sensitivity was noted for isolates 23 and 50.

Based on the resistant patterns of the isolates, the MAR index was calculated and interpreted. Accordingly, the MAR indexes for isolates 11, 20, 23, 50, 53, and 57 were 0.85, 0.78, 0.78, 0.71, 0.92 and 0.92 respectively.

3.3 Plasmid profiling: The plasmid profiling of all the six isolates of *Salmonella typhi* were observed and the results are presented in figure 3. The results show that, all the isolates had the plasmid and displayed a high molecular weight as 23130 bp and also the plasmids isolated from *Salmonella typhi* showed 100% resistant to Cefaclor, Ofloxacin, Ciprofloxacin, Erythromycin, Novobiocin, Penicillin - G, Rifampicin and Telcoplamin.

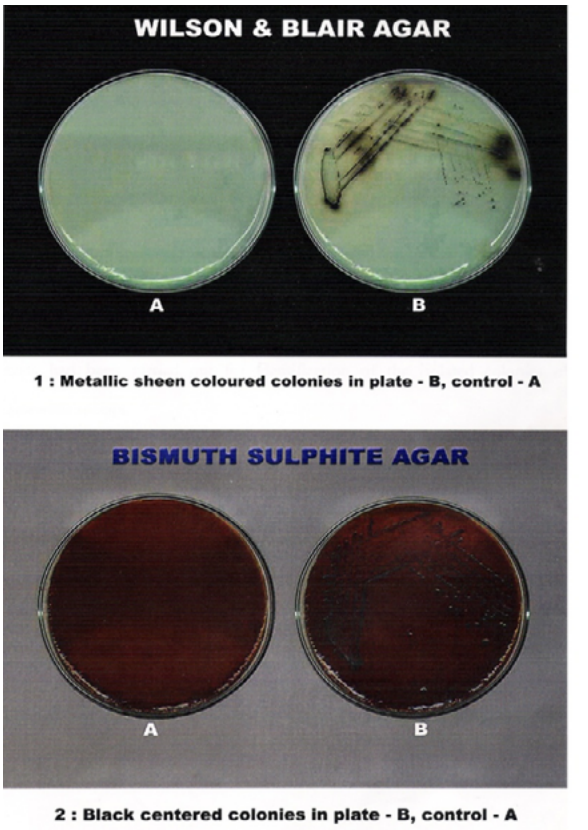

Figure 1: Colony morphology on respective selective medium.

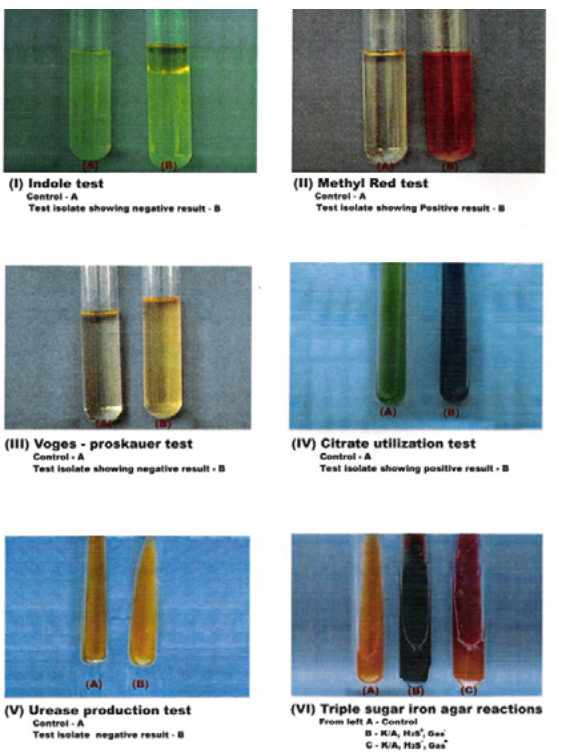

Figure 2: Identification of *Salmonella typhi* using IMViC, urease test and triple sugar iron agar.

Table 1: Antibiotic sensitivity pattern of six isolates of *Salmonella typhi*

S.No.	Name of the antibiotic Disc	Antibiotic concentration in mg	Isolates					
			11	20	23	50	53	57
1.	Ampicillin (A)	10	S	S	R	S	R	R
2.	Amikacin	30	R	S	S	S	R	S
3.	Cefaclor (Cl)	30	R	R	R	R	R	R
4.	Ofloxacin (Of)	5	R	R	R	R	S	R
5.	Ciprofloxacin (Cf)	10	R	R	R	R	R	R
6.	Erythromycin (E)	10	R	R	R	R	R	R
7.	Gentamycin (G)	30	S	R	R	I	R	R
8.	Novobiocin (No)	30	R	R	R	R	R	R
9.	Bacitracin (B)	10	R	S	R	R	R	R
10.	Minocycline (Mi)	10	R	R	S	R	R	R
11.	Penicillin – G (P)	10	R	R	R	R	R	R
12.	Rifampicin (R)	5	R	R	R	R	R	R
13.	Netillin (Nt)	30	R	R	S	S	R	R
14.	Telcoplamin (Te)	30	R	R	R	R	R	R

Note: S- Sensitive, I- Intermediate and R- Resistant

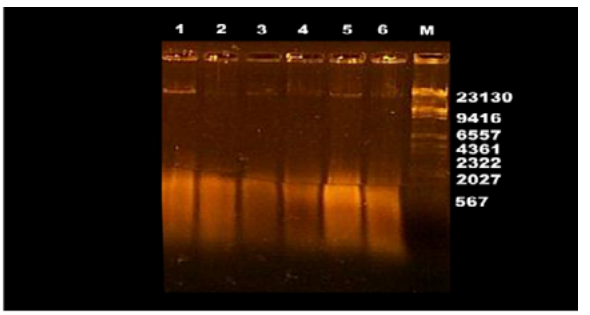

Figure 3: Lane 1-6 shows isolation of plasmids from six isolates of *Salmonella typhi* and M- Eco RI/ Hind III digested I DNA

4. DISCUSSION

Ability to resist the commercially available antibiotics, *Salmonella typhi* causes typhoid fever which is one of the global threats in human beings in developing countries where hygiene is not up to the level.[22,23] Keeping this in mind, in the present investigation, hundred nail scraping samples were obtained from healthy and asymptomatic restaurant workers and the samples were analysed to isolate and identify the bacterium *Salmonella typhi*. Out of them, six *Salmonella typhi* strains were isolated and identified using various conventional experiments. Our findings were correlated with Siala *et al.*[24] wherein they have screened and detected 94 *Salmonella* strains from 500 natural food matrices and identified using conventional as well as molecular techniques. In the same way, Sanjeep *et al*.[25] recorded the presence of multidrug drug resistant, ESBL producing *Salmonella* spp. and *E. coli* from the vegetable salads which is served in the restaurant located in Nepal and their antibiotic sensitivity patterns shows, the isolated strains were multidrug resistant.

During the emergency period, antibiotic resistance is a significant tool for antibiotic therapy management. Hence, the isolated strains were analysed for their antibiotic resistant pattern against commercially available antibiotics as well as the MAR index values of six isolates were calculated. Antibiotic sensitivity and MAR index value are crucial in the investigation to determine the resistant pattern of the isolated *Salmonella typhi*. If, MAR value is greater than 0.2 indicates the high risk for them to treat the pathogen with limited antimicrobials. Here, all the six *Salmonella typhi* isolates showed 100% resistant to seven antibiotics and showed high MAR index values ranging from 0.78- 0.91 indicating high risk of multidrug resistance. This could be due to the consequence of antibiotics overuse in healthcare system. Our findings were correlated with recent report[26], wherein they have isolated *Salmonella* strains from poultry and other food products and analysed for their sensitivity patterns against twenty-three commercially available antibiotics with varied groups and found that all the isolates were multidrug resistant exhibiting high MAR index values ranging from 0.62-0.91. In contrast, a study reported that isolated *Salmonella* strains showed only resistant to oxacillin.[27] Another study from Egypt showed that 100% resistant was developed by isolated *Salmonella* strains against erythromycin, penicillin, and amoxicillin.[28]

Consequently, our study reported the presence of plasmids in all the six isolates of *Salmonella typhi*. Plasmids are very important in medical field which can encode the genes which is responsible for drug resistant. From our study, the plasmids isolated from *Salmonella typhi* harbours the resistant gene that may exhibit the 100% resistant to Cefaclor, Ofloxacin, Ciprofloxacin, Erythromycin, Novobiocin, Penicillin-G, Rifampicin and Telcoplamin. Our findings were correlated with earlier report from Kenya[29] wherein they have isolated 98 isolates of *Salmonella typhi* and isolated the plasmids using alkaline lysis method and found that, the isolated plasmid harbour the

resistance genes for chloramphenicol, ampicillin and tetracycline. Other reports say, IncHI1 of *Salmonella typhi* was encoding the resistant gene for chloramphenicol, sulphonamides, streptomycin, ampicillin, trimethoprim that are important in outbreaks.[30-32]

5. CONCLUSION

Hundred nail scraping samples were collected and six of the isolated strains were identified as *Salmonella typhi*. Isolates were analysed for antibiotic sensitivity pattern against fifteen commercially available antibiotics. All of the isolated *Salmonella typhi* were found to be resistant to seven selected antibiotics such as Cefaclor, Ofloxacin, Ciprofloxacin, Erythromycin, Novobiocin, Penicillin – G, Rifampicin and Telcoplamin and the MAR index values were calculated for all the six isolates indicating high risk for them to treat the pathogen. Moreover, the plasmids harbouring resistant genes were isolated from six isolates of *Salmonella typhi*.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	PK, EHAO
Acquisition, Analysis or Interpretation of Data:	PK, EHAO, MMP
Manuscript Writing & Approval:	PK, EHAO, MMP

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.