

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Microbiology Research

Journal homepage: www.ipinnovative.com

Original Research Article

Isolation of enteric bacteria from asymptomatic food handlers

Musab Umar Abubakar^{1,*}, Felix Oluwasegun Alao², Shehu Hadi Abubakar¹, Mukhtar Aminu Garba¹, Abba Umar Yakub¹

¹Dept. of Life Sciences, Mewar University, Chittorgarh, Rajasthan, India



ARTICLE INFO

Article history: Received 11-06-2020 Accepted 30-06-2020 Available online 28-10-2020

Keywords: Antibiotic sensitivity Asymptomatic Food contamination Food handlers

ABSTRACT

The study was carried out to determine the prevalence and antimicrobial sensitivity pattern of enteric bacteria isolated from food handlers within Bells University of Technology, Ota, Ogun State. Stool samples from thirty-five food handlers were collected. The samples were cultured on Salmonella-Shigella agar and MacConkey agar and growth was observed in all samples. The organisms isolated were Salmonella species (8.6%), Escherichia coli (8.6%), Proteus vulgaris (51.4%), Citrobacter freundii (8.6%), Staphylococcus saprophyticus (8.6%), Providencia sp. (2.9%), and Enterococcus sp. (17.1%). The most prevalent of these organisms is Proteus vulgaris and the least prevalent of these organisms is Proteus vulgaris and the least prevalent of these organisms is Providencia sp. Antibiotic sensitivity test showed that majority of the isolates in this study were sensitive to ofloxacin, augmentin, nitrofurantoin, ciprofloxacin, ceftazidime and gentamicin, and showed various degree of sensitivity to cefuroxime. While most of the isolates were resistant to cefixime. Based on the result antibiotics such as gentamicin, augmentin, and ofloxacin are most efficient for the diseases that can be caused by these isolated enteric bacteria while previously use antibiotic such as cefixime was poorly effective against majority of the isolates in this study. Health education along with continuous food safety training should be given to food handlers so as to adhere to effective hygienic practices which can help in preventing transmission of these enteric bacteria to the consumers.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/)

1. Introduction

Enteric bacteria are aerobic or facultative anaerobic, Gram negative, non-spore forming, rod shaped bacteria, that reside in the guts of animals and humans, the human gut therefore serve as the natural habitat for various bacterial species and majority of them partake in metabolic activities that recover energy and absorbable nutrients thereby protecting the colonized host against invasion by foreign microbes. The gastrointestinal tract contains vast number of aerobic and anaerobic bacteria which may be in symbiotic relationship with the host but can have adverse effects in causing food borne gastroenteritis in humans becoming an important health problems worldwide, resulting in morbidity, mortality and socioeconomic

E-mail address: masoyinbabaa@gmail.com (M. U. Abubakar).

impacts.² One of the major reasons for morbidity as well as mortality associated with gastrointestinal infections is the increasing resistance of the organisms to available antimicrobial agent.³ Food handlers who harbor enteric bacteria asymptomatically and who are not adhered with good hygienic practices and inadequate knowledge on food safety are likely to contaminate the food with enteric bacterial pathogens and could also be the source of food borne infections.4 However, the consequences of food contamination vary among countries and regions of the world depending on climate, geography and degree of social and economic development.⁵ Since food handlers are engaged in food preparation, transport and provision, they are implicated for the transmission of enteric bacterial pathogens to the community if appropriate hygienic practices are not ensured.⁶

²Bells University of Technology Ota, Ogun State, Nigeria

^{*} Corresponding author.

Indeed a prerequisite for the prevention and control of diseases due to enteric bacterial pathogens is a clear understanding of their epidemiology. Therefore, an effective means of prevention of their transmission from food handler is associated to good personal hygiene and hygienic food handling practices.⁷

Several studies have been conducted In Nigeria, giving reports regarding enteric bacterial infections and risk factors among food handlers, though the study area is limited. Moreover, emergence of drug resistant enteric bacterial pathogens is now a major public health concern that need to be addressed. ⁸ Hence, this study is aimed at determining the prevalence and antimicrobial sensitivity pattern of enteric bacteria associated with food.

2. Materials and Methods

2.1. Sterilization of glass wares

All the glass wares such as McCartney bottles, conical flask and test tubes used in this study were washed with detergent, rinsed in clean water, dried in drying cabinet and sterilized in the hot air oven at 160°C for 2 hours.

2.2. Media and reagent used

Salmonella-Shigella agar, Nutrient agar, MacConkey agar, Sulphide Indole Motility (SIM) agar, Triple Sugar Iron agar (TSI), Simmon citrate agar (SCA), Selenite broth, Peptone water, Gram stain reagent (Gram Lugol's iodine, Crystal violet, 95% Ethanol, Safranin), Kovac's reagent, Catalase test, Oxidase test.

2.3. Preparation of media

Solid culture media such as *Salmonella-Shigella* agar, Nutrient Agar, MacConkey Agar, Sulphide Indole Motility (SIM) Agar, Simmon Citrate Agar (SCA) and Triple Sugar agar (TSI) used in this study were sterilized in the boil at 121°C for 15 minutes. After preparation, MacConkey Agar, *Salmonella-Shigella* Agar was boiled and Nutrient Agar were allowed to cool to about 45°C and about 20ml was poured into the Petri-dish and was left at room temperature to solidify. Sulphide Indole Motility (SIM) Agar, Simmon citrate Agar (SCA), Triple Sugar Iron agar (TSI) were dispensed into test tubes and allowed to solidify at room temperature.

2.4. Study population

The study population was drawn from food handlers from various canteen within Bells University of Technology, Ota, Ogun State in Nigeria between 15th -20th of March, 2018. Thirty Five food vendors were sampled in this study, which included males and females.

2.5. Stool sample collection

Thirty-five Stool samples were collected using clean, dry and leak proof Universal bottles. The specimens were then transported to Bells University of Technology Microbiology Laboratory for bacterial culture and identification.

2.6. Sample processing

Prior to culturing, samples were preserved in the refrigerator at 4°C and then processed. The samples were examined macroscopically. A sterile inoculating loop which is to deliver loop full of the stool sample onto Selenite broth and peptone water was used. These were incubated at 37°C aerobically for 24 hours. The inoculum from overnight Selenite broth was subcultured onto SSA(*Salmonella Shigella* Agar). While inoculum from Peptone water was subcultured onto other media. All plates were incubated at 37°C aerobically for 24 hours. The plates were then examined for bacterial growth and isolates were characterized biochemically as described by Cowan and Steel (1974).

2.7. Identification of bacteria

After 24 hours of incubation at 37°C, isolates from nutrient agar were identified following the standard procedures using biochemical tests hydrogen sulphide (H₂S) production, indole production and motility in Sulphide Indole Motility (SIM) medium, citrate utilization, in Simmon's Citrate agar, Triple Sugar Iron agar.

2.8. Purification of isolates

The nutrient agar plate were dried in a hot air oven at 45°C; this was done to get rid of moisture on the cover of the plates and on the agar itself. Suspected colonies of *Escherichia coli, Salmonella spp, Shigella, Citrobacter spp, Serratia spp* and *Klebsiella spp* etc. were purified by streaking on Nutrient agar plates and were subjected to Gram staining and other Biochemical tests.

2.9. Colonial characteristics of the Salmonella spp and Shigella on Salmonella-Shigella Agar

Salmonella spp- colourless with black centres.

Shigella spp- colorless.

Escherichia coli spp- They appear as pink color. Klebsiella- larger than E. coli pale, opaque cream to pink.

2.10. Morphology and biochemical tests carried out to identify the isolates

The following tests were carried out:

- 1. Gram staining
- 2. Triple Sugar Iron Agar

- 3. Catalase Testt
- 4. Oxidase Testt
- 5. Coagulase Test
- 6. Sulphide Indole Motility Test
- 7. Citrate Test

2.11. Gram staining reaction

The procedure was carried out as follows:

A thin smear film of the organism (a 24-hour old bacterial culture) was prepared on a sterile clean glass slide, air-dried and heat-fixed by passing it horizontally over the Bunsen flame. The dried smear was stained with Crystal violet stain for 60 seconds after which it was rinsed with tap water. The resulting smear was then stained with Lugol's iodine for 60 seconds and was rinsed with tap water.

The smear was decolorized with 95% ethanol until the slide appears free of the crystal violet stain. The slide was rinsed under tap water, counter–stained with safranin for 1minute and was finally rinsed with tap water. The prepared slide was allowed to dry and it was examined under the microscope using the x40 objective lens and x100 (oil immersion objective lens). The organisms that retained the crystal violet stain(purple in colour), indicated Gram positive organisms, while the organisms that appeared pinkish or reddish indicated Gram negative organisms.

2.12. Sulphide indole motility test

This test was carried out to detect the motility, sulphide and indole production of each isolate. The medium, sulphide-indole-motility (SIM) medium is a semi-solid medium. The isolates were stab-inoculated aseptically and were incubated at 37^{o} C for 24 hours.

Motility is indicated by the spreading of the organism outside the line of stab, indole production is by the presence of a red-pink ring at the interphase after Kovac's reagent has been added; sulphide production is by the presence of a black colour in the medium.

2.13. Citrate test

The citrate test was carried out in order to determine the ability of the isolates to utilize citrate as their sole source of carbon and ammonia as the only source of nitrogen. Simmon citrate agar was used for this test; the agar was prepared in test tubes and was inoculated with a 24-hours old culture of each of the isolates aseptically. This was then inoculated at 37°C for 24-hours. A colour change from green to deep blue indicates positive citrate utilization while the absence of a colour change indicates negative citrate utilization.

2.14. Triple sugar iron test

This test was used to detect the fermentation of lactose (slope) and glucose (butt) due to the production of acid, the production of gas (CO_2) and the release of H_2S (hydrogen suphide) which is a four in one test. The Triple Sugar Iron was inoculated with each isolate from the pure cultures on Nutrient agar using a straight wire to stab the butt and then streaking the slope in zig-zag pattern and it was incubated at $37^{o}C$ for 24 hours.

A yellow butt (acid production) and red-pink slope indicates the fermentation of glucose only; cracks and bubbles in the medium indicate gas production from glucose fermentation; a yellow slope and a yellow butt indicates the fermentation of lactose; a red-pink slope and butt indicates no fermentation of glucose or lactose; blackening along the stab line or throughout the medium indicates hydrogen sulphide production.

The reaction is: Fe₂ + 2H₂S \rightarrow 2FeS (Black) + 2H₂

2.15. Catalase test

Most aerobic microorganisms are capable of producing the enzyme catalase although of different extents. The principle of this is that when organisms containing catalase enzyme are mixed, Hydrogen peroxide (H_2O_2) and gaseous oxygen is released. A suspension of 18-24 hours old culture of the test organisms was placed on a clean glass microscope slide. A few drops of H_2O_2 were added using a syringe.

The evolution of gas bubbles caused by the liberation of free oxygen indicated the presence catalase enzyme which shows that the reaction is positive; the absence of bubbles indicates a negative reaction.

2.16. Coagulase test

This test is used to identify *Staphylococcus aureus* which produces the enzyme coagulase. A drop of distilled water is placed on each end of a slide or on two separate slide. The colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of freshly collected plasma was added to one of the suspensions and was gently mixed. Observe for clumping of the organisms within 10 seconds. If there is clumping within 10 seconds, it is Staphylococcus aureus; if there is no clumping within 10 seconds, it is coagulase negative.

2.17. Antibiotic susceptibility test

This test was carried out to determine the antibiotic susceptibility pattern of the different isolates. Nutrient agar plates were inoculated with isolates from stock cultures. The Kirby-Bauer disc-diffusion test which conforms to the recommended standard of the Clinical and Laboratory Standards Institute.

Turbidity of the inoculum of various isolates of enteric bacteria is compared with 0.5 McFarland standard and each of the isolates was inoculated onto the surface of Mueller Hinton Agar plates using a sterile swab in order to ensure even distribution of the inoculum, the plates were allowed to dry for not more than 15 minutes and the antibiotic discs with different concentration were placed on the surface of the agar plates. After 30 minutes of applying the discs, the plates were inverted and incubated for 24 hours at 30°C. The clear zone that developed around each disc were measured as the zone of inhibition from underneath each plate with the aid of a ruler in centimeter (cm) and converted to millimeter (mm). The antimicrobial discs used include the following: Ofloxacin $(5\mu g)$, Ciprofloxacin $(5\mu g)$, Augmentin $(30\mu g)$, Cefuroxime $(30\mu g)$, Gentamicin $(10\mu g)$, Nitrofurantoin $(300\mu g)$, Cefixime $(5\mu g)$, and Ceftazidime $(30\mu g)$.

3. Results

Stool samples from thirty-five (35) food handlers from different Cafeterias in Bells University of Technology, Ota were collected and examined for the presence of enteric organisms. The color of the stool sample ranged from brown, black, formed, semi formed, unformed, presence or absence of blood and mucus. From the stool analysis, 25 samples (17 females and 8 males) were brown in color which indicated the normal color for the stool, while 10 samples (8 males and 2 females) were black in color that showed abnormal stool color also 19 samples (10 females and 9 males) were formed, 10 samples (7 females and 3 males) were semi formed and 6 samples(3 males and 3 females) were unformed, blood was found in 8 samples (6 males and 2 males) and mucus in 9 samples (5 females and 4 males). The results obtained are as shown in Table 1.

Tables 2 and 3 show biochemical characterization of the bacterial isolates.

Figures 1 and 2 show distribution of the bacterial isolates from the food handlers.

Antibiotic sensitivity and resistance patterns of the isolates were shown in Figure 3.

Table 4 shows the antibiotic susceptibility of the bacterial isolates.

4. Discussion

In the present study, *Salmonella* species were also isolated and they account for 8.6% corresponding to a study done in North India in which 2.5% *Salmonella* species were recorded in stools of food handlers. However, incomparable result was obtained from Sudan that found 3.8% *Salmonella* species. 9

On the other hand, studies done in Gondar and Egypt revealed that no *Salmonella* species were isolated from the stools of food handlers. ^{10,11} The reason for the difference in the existence of *Salmonella* species as well as other enteric

bacterial pathogens in the stools might be due to variation in climate, geography and study settings.

In this study, isolation rate of *Shigella* species was found to be 0%. Regarding *Escherichia coli* 8.6% strains were isolated and this is in agreement with the study conducted from Japan (8.25%). ¹¹ On the other hand, lower isolation rate was reported from Kenya which was 2.1%. ¹² Among 70 organisms isolated on *Salmonella-Shigella* Agar and MacConkey Agar, 51.4% were *Proteus vulgaris*, 8.6% were *Citrobacter freundii*, 8.6% were *Staphylococcus saprophyticus*, 2.9% *Providencia* sp., 17.1% were *Enterococcus* sp.

As far as antimicrobial sensitivity pattern of enteric bacterial pathogens is concerned; generally, the isolates showed varied sensitivities to eight tested antimicrobials. Hence, there was variation in drug sensitivity patterns among organisms isolated. Least resistance to ofloxacin, augmentin, nitrofurantion, ciprofloxacin, ceftazidime and gentamicin was observed by most isolates. All *Salmonella* species were sensitive to ceftazidime and gentamicin where as one (33.3%) was found to be multidrug resistant.

In coherent with the present findings, sensitivity to ciprofloxacin and nalidixic acid and frequent resistance to amoxicillin-clavulanic acid, ampicillin and tetracycline among *Salmonella* species was also reported from studies conducted somewhere. For instance, study done in Nigeria found that 96% and 27.6% *Salmonella* species were found to be sensitive to ciprofloxacin and chloramphenicol, respectively. However, the isolates showed complete resistance to tetracycline, ampicillin and amoxicillin. ¹³ According to the study conducted in Sudan, 93.1% *Salmonella* species were found to be resistant to the above mentioned antimicrobials and 47.1% isolates showed multidrug resistance. ¹⁴

One of the main sources of pathogen transfer such as *Salmonella* spp is through improper hand washing. Since pathogens of faecal, nose or throat and skin origin are most likely to be transmitted by the hands, there is need for the food handlers to be informed about the importance for effective hand hygiene and other barriers to pathogen contamination. In another Nigerian study by, ¹⁵ on the assessment of hygiene among food handlers in a Nigerian University, there was a very low frequency of hand hygiene and a poor knowledge and practice of food hygiene amongst the food handlers.

A report by Mohan et al., in 2006 on the carrier state of *S. typhi* and intestinal parasites and personal hygiene, showed that 0.47% of the food handlers studied harboured *S. typhi* and it was attributable to their poor personal hygiene. *Salmonella* sp. was isolated from the stool of 10% of asymptomatic food workers in Thailand. In the US, Buchwald reported an estimate of about 200,000 individuals that may be excreting *Salmonella* sp. at any one time and many of these excretors would be food workers.

Table 1: Macroscopic examination of stool samples

S/N	Consistency	Colour	Blood	Mucus
FH1	F	В	-	-
FH2	F	В	-	-
FH3	F	В	-	-
FH4	F	В	-	-
FH5	F	В	-	-
FH6	F	В	-	-
`H7	S	В	+	-
H8	F	В	-	-
Ή9	F	В	-	-
FH10	U	В	-	-
FH11	S	В	-	-
FH12	S	В	+	+
TH13	S	В	-	+
TH14	U	В	-	-
H15	S	В	-	-
H16	U	В	+	-
H17	F	b	-	-
H18	S	В	-	-
Н19	F	В	+	+
H20	S	В	-	-
H21	F	b	-	-
H22	S	В	-	-
H23	F	В	_	_
H24	F	b	-	+
H25	U	В	+	-
126	F	В	+	-
H27	F	В	-	+
H28	U	В	+	-
H29	U	В	_	_
H30	S	В	_	+
H31	S	В	_	+
H32	F	b	_	_
Н33	F	b	+	_
H34	F	В	-	+
H35	F	b		+

KEY: F- Formed; S- Semi formed; U- Unformed; B- Brown; b- Black; + - Presence - -absence.

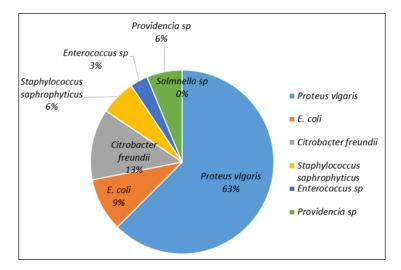


Fig. 1: Distribution of bacterial isolates grown on Salmonella-Shigella Agar

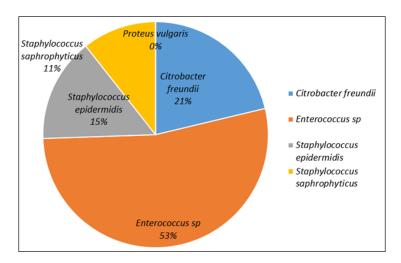


Fig. 2: Distribution of bacterial isolates grown on MacConkey Agar

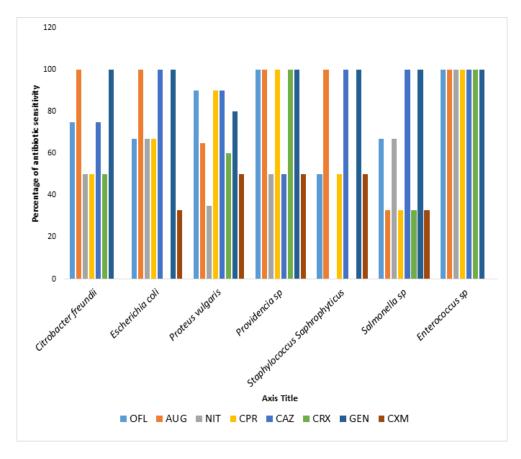


Fig. 3: Antibiotic sensitivity pattern of the enteric organisms

Table 2: Biochemical characterisation of the bacterial isolates on SSA

FHI	S/N	GR	SHAPE	COA	CAT	K	A	H ₂ S	G	CIT	H_2S	IND	МОТ	Identification	
FH2															
FH3	1111	_	Rod	_			'	_		'	_	_	'		
FH4	FH2	-	Rod	+	+	-	+	-	+	-	-	+	+	Escherichia coli	
FH5		-	Rod	-	+	-	+	-	+	-	-	+	+	Escherichia coli	
FH6		-		-	+	-	+	-	+	-	-	+	+	Escherichia coli	
FH7	FH5	-	Rod	-	+	+	+	+	+	+	+	+	+	Proteus vulgaris	
FH8	FH6	-	Rod	-	+	-	+	+	+	+	+	+	+	Proteus vulgaris	
FH10	FH7	-	Rod	-	+	-	+	+	+	+	+	+	+	Proteus vulgaris	
FH10	FH8	-	Rod	-	+	+	+	+	-	+	+	+	+	Proteus vulgaris	
FH11	FH9	-	Rod	-	+	+	+	+	+	+	-	+	+	Providencia sp.	
FH12	FH10	-	Rod	-	+	+	+	+	-	+	+	+	+	Proteus vulgaris	
FH12 + Cocci - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +<	FH11	+	Cocci	-	+	+	+	-	+	+	-	+	-		
FH13 - Rod - + + + + + + + Proteus vulgaris FH14 - Rod - + + + + + + + + + + + + + + + + + + + + + + Proteus vulgaris FH15 - Rod - - + + + + + + Proteus vulgaris FH16 - Rod - - + + + + + Proteus vulgaris FH17 - Rod - - + + + + + + Proteus vulgaris FH18 + Cocci - - + + + + + + Proteus vulgaris FH20 - Rod - - + + +	FH12	+	Cocci	-	+	+	+	+	-	+	+	+	+	Staphylococcus	
FH14 - Rod - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td>FH13</td> <td>_</td> <td>Rod</td> <td>_</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>_</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td></td>	FH13	_	Rod	_	+	+	+	+	_	+	+	+	+		
FH15 - Rod - + + + + + + + + Proteus vulgaris FH16 - Rod - - + + + + + + + Proteus vulgaris FH17 - Rod - + + + + + + + Proteus vulgaris FH17 - Rod - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +		_		_					+					_	
FH16 - Rod - - + + + + + + + + + + - + - + - + - + + - + + - + + - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td></td> <td>_</td> <td>Rod</td> <td>_</td> <td>+</td> <td></td> <td>+</td> <td>+</td> <td></td> <td>+</td> <td></td> <td>+</td> <td></td> <td>_</td>		_	Rod	_	+		+	+		+		+		_	
FH17 - Rod - + - + + + + - + Citrobacter freundii FH18 + Cocci - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <	FH16	_	Rod	_	_	+	+	+		+	+	+			
FH18		_	Rod	_	+		+	+		+		+			
FH19 - Rod - + + + + + + + - + - H + + + + + - - + - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td></td>															
FH20 - Rod - - + + + + + + - + Salmonella sp FH21 - Rod - - + + + + + + + + - + Proteus vulgaris FH22 - Rod - - + + + + + + + Proteus vulgaris FH23 - Rod - - + + + + + + Proteus vulgaris FH24 - Rod - - + + + + + + Proteus vulgaris FH25 - Rod - - + + + + + + Proteus vulgaris FH25 - Rod - - + + + + + + + Proteus vulgaris FH27<	FH18	+	Cocci	-	-	+	+	+	-	+	+	+	+	Enterococcus sp	
FH20 - Rod - - + + + + + + - + Salmonella sp FH21 - Rod - - + + + + + + + + - + Proteus vulgaris FH22 - Rod - - + + + + + + Proteus vulgaris FH23 - Rod - - + + + + + + Proteus vulgaris FH24 - Rod - - + + + + + Proteus vulgaris FH25 - Rod - - + + + + + Proteus vulgaris FH26 - Rod - - + + + + + + + Proteus vulgaris FH27 - Rod	FH19	-	Rod	-	+	+	+	+	-	+	+	+	+	Proteus vulgaris	
FH22 - Rod - + + + + + + + Proteus vulgaris FH23 - Rod - - + + + + + + Proteus vulgaris FH24 - Rod - - + + + + + + Proteus vulgaris FH25 - Rod - + + + + + + Proteus vulgaris FH25 - Rod - - + + + + + + Proteus vulgaris FH26 - Rod - + + + + + + Proteus vulgaris FH27 - Rod - + + + + + + + Proteus vulgaris FH28 - Rod - + + + + + + <	FH20	-	Rod	-	-	+	+	+	+	+	+	-	+	_	
FH23 - Rod - - + + + + + + + Proteus vulgaris FH24 - Rod - - + + + + + + + Proteus vulgaris FH25 - Rod - + + + + + + + Citrobacter freundii FH26 - Rod - - + + + + + Providencia sp FH27 - Rod - + + + + + + Proteus vulgaris FH28 - Rod - + + + + + + + Proteus vulgaris FH29 - Rod - + + + + + + + + Proteus vulgaris FH30 - Rod - + + + + + + <	FH21	-	Rod	-	-	+	+	+	+	+	+	-	+	Salmonella sp	
FH24 - Rod - - + + + + + + + Proteus vulgaris FH25 - Rod - + - + + + + + + Proteus vulgaris FH26 - Rod - - + + + + + + Proteus vulgaris FH27 - Rod - + + + + + + Proteus vulgaris FH28 - Rod - + + + + + + Proteus vulgaris FH29 - Rod - + + + + + + + Proteus vulgaris FH30 - Rod - + + + + + + + + + Proteus vulgaris FH31 - Rod - - + <	FH22	_	Rod	-	+	+	+	+	+	+	+	+	+	Proteus vulgaris	
FH24 - Rod - - + + + + + + + Proteus vulgaris FH25 - Rod - + - + + + + + + Proteus vulgaris FH26 - Rod - - + + + + + + Providencia sp FH27 - Rod - + + + + + + Proteus vulgaris FH28 - Rod - + + + + + + Proteus vulgaris FH29 - Rod - + + + + + + + Proteus vulgaris FH30 - Rod - + + + + + + + + Proteus vulgaris FH31 - Rod - - + + <td< td=""><td>FH23</td><td>-</td><td>Rod</td><td>-</td><td>-</td><td>-</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>Proteus vulgaris</td></td<>	FH23	-	Rod	-	-	-	+	+	+	+	+	+	+	Proteus vulgaris	
FH26 - Rod - - + + - + + + + + Providencia sp FH27 - Rod - + + + + + + + + Proteus vulgaris FH28 - Rod - + + + + + + + Proteus vulgaris FH29 - Rod - + + + + + + + Proteus vulgaris FH30 - Rod - + + + + + + + Proteus vulgaris FH31 - Rod - + + + + + + + + Proteus vulgaris FH31 - Rod - - + + + + + + + Proteus vulgaris FH32 - Rod <td< td=""><td>FH24</td><td>-</td><td>Rod</td><td>-</td><td>-</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td></td></td<>	FH24	-	Rod	-	-	+	+	+	+	+	+	+	+		
FH27 - Rod - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td>FH25</td> <td>-</td> <td>Rod</td> <td>-</td> <td>+</td> <td>-</td> <td>+</td> <td>-</td> <td>+</td> <td>+</td> <td>+</td> <td>-</td> <td>+</td> <td></td>	FH25	-	Rod	-	+	-	+	-	+	+	+	-	+		
FH28 - Rod - + + + + + + + + + + + + + + + + Proteus vulgaris FH30 - Rod - + + + + + + + Proteus vulgaris + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +	FH26	-	Rod	-	-	+	+	-	+	+	+	+	+	Providencia sp	
FH28 - Rod - + + + + + + + + + + + + + + + + - - + Citrobacter freundii FH30 - Rod - + + + + + + + + + + Proteus vulgaris FH31 - Rod - - + + + + + + + - + + Proteus vulgaris FH31 - Rod - - + + + + + + + + - + + Proteus vulgaris FH32 - Rod - - + + + + + + + + + + + + + + + + + + + + + + + +	FH27	-	Rod	-	+	+	+	+	-	+	+	+	+	Proteus vulgaris	
FH29 - Rod - + + + + + + - - + Citrobacter freundii FH30 - Rod - + + + + + + + + Proteus vulgaris FH31 - Rod - - + + + + + - + + Proteus vulgaris FH32 - Rod - - + + + + + + + Proteus vulgaris FH33 - Rod - + + + + + + + + Proteus vulgaris FH34 - Rod - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +	FH28	-	Rod	-	+	+	+	+	+	+	+	+	+		
FH30 - Rod - + + + + + + + + + + + + + + + + + + + - + + - + + + + + + + - + + + - - + + + + + + + + + - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td>FH29</td> <td>-</td> <td>Rod</td> <td>-</td> <td>+</td> <td>+</td> <td>+</td> <td>-</td> <td>+</td> <td>+</td> <td>-</td> <td>-</td> <td>+</td> <td>Citrobacter</td>	FH29	-	Rod	-	+	+	+	-	+	+	-	-	+	Citrobacter	
FH31 - Rod - + + + + + - + + - + + - + + - + + - + + - - + + + + + + + + - + + + - - + + + + + + + + + - - + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + <td>FH30</td> <td>_</td> <td>Rod</td> <td>_</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>_</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>•</td>	FH30	_	Rod	_	+	+	+	+	_	+	+	+	+	•	
FH31 FH32 - Rod - - + + - + + Proteus vulgaris FH33 - Rod - + + + + + + + Proteus vulgaris FH34 - Rod - + + + + + + Proteus vulgaris	FH31	-	Rod	_					_						
FH33 - Rod - + + + + + + + + + + + Proteus vulgaris FH34 - Rod - + + + + + + + + + + Proteus vulgaris	FH31													I	
FH33 - Rod - + + + + + + + + + + + Proteus vulgaris FH34 - Rod - + + + + + + + + + + Proteus vulgaris	FH32	-	Rod	-	-	+	+	-	+	+	-	+	+	Proteus vulgaris	
FH34 - Rod - + + + + + + + + + Proteus vulgaris	FH33	-	Rod	-	+	+	+	+	+	+	+	+			
	FH34	-		-	+	+	+	+	+	+	+	+	+		
	FH35	-	Rod	-	+	+	+	+	-	+	+	+	+	Proteus vulgaris	

During food production and preparation, since both healthy and infected workers stay at work for several days, the possibility therefore exists for healthy individuals to be continually exposed to these infected workers. ¹⁶

These infected workers could be in the asymptomatic stage and still harbour millions of infectious organisms in their stools without any symptom of infection. To prevent pathogen transmission therefore one needs to adhere strictly to effective hand washing barrier creation to prevent such pathogens from being transmitted to foods. ¹⁶ In a study from Japan on *Salmonella* carriage rate amongst food workers, 331,644 faecal specimens were collected

from workers in hotels, supermarket, food factories, and restaurants; only 0.032% of the faecal samples harboured *Salmonella*, and the most common serovars were Agona, Corvallis, Infantis and Enteritidis. ¹⁷

The majority of the food worker-associated outbreaks reviewed by Todd in 2008 involved transmission of the pathogen to food by food handlers' hands. In fact hand contact was described as a factor in 40% of the 816 outbreaks, and the investigators specifically mentioned that the food handlers were not wearing gloves in 1.3% of the outbreaks. A study conducted by Senthilkumar, 2005 showed that the food handlers played prominent role

Table 3: Biochemical characterization of bacterial isolates

S/N	GR	SHAPE	COA	CAT	K	A	H_2S	G	CIT	H_2S	IND	MOT	Identification
FH1	-	Rod	-	-	-	+	-	+	+	-	-	+	Citrobacter freundii
FH2	+	Cocci	-	-	-	+	-	+	+	-	-	+	Enterococcus sp
FH3	+	Cocci	-	+	-	+	-	+	-	-	+	+	Staphylococcus epidermidis
FH4	+	Cocci	-	+	-	+	-	+	+	-	+	+	Staphylococcus epidermidis
FH5	-	Rod	-	+	+	+	+	+	+	+	+	+	Proteus vulgaris
FH6	+	Cocci	-	+	-	+	+	+	+	+	+	+	Staphylococcus epidermidis
FH7	-	Rod	-	-	-	+	+	-	+	+	+	+	Proteus vulgaris
FH8	+	Cocci	-	+	-	+	+	-	+	+	+	+	Staphylococcus epidermidis
FH9	-	Rod	-	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH10	+	Cocci	-	+	+	+	+	-	+	+	+	+	Staphylococcus epidermidis
FH11	+	Cocci	-	+	-	+	-	+	+	+	+	+	Staphylococcus epidermidis
FH12	+	Cocci	-	+	-	+	+	+	+	+	+	+	Staphylococcus epidermidis
FH13	+	Cocci	-	+	-	+	+	-	+	+	+	+	Staphylococcus epidermidis
FH14	-	Rod	-	+	-	+	-	+	+	-	+	+	Citrobacter freundii
FH15	+	Cocci	-	+	-	+	+	-	+	+	+	+	Staphylococcus epidermidis
FH16	+	Cocci	-	-	+	+	-	-	+	+	+	+	Staphylococcus epidermidis
FH17	_	Rod	_	+	_	+	+	_	+	+	_	+	Proteus vulgaris
FH18	+	Cocci	-	+	-	+	+	-	+	+	-	+	Staphylococcus epidermidis
FH19	_	Rod	_	+	+	+	+	_	+	+	+	+	Proteus vulgaris
FH20	-	Rod	-	+	-	+	+	+	+	+	+	+	Proteus vulgaris
FH21	-	Rod	-	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH22	-	Rod	-	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH23	-	Rod	-	-	-	+	+	-	+	+	+	+	Proteus vulgaris
FH24	+	Cocci	-	-	-	+	+	+	+	-	+	+	Enterococcus sp
FH25	-	Rod	-	-	-	+	+	-	+	+	-	+	Proteus vulgaris
FH26	-	Rod	-	-	-	+	+	-	+	+	+	+	Proteus vulgaris
FH27	+	Cocci	-	-	+	+	+	-	+	+	+	+	Enterococcus sp
FH28	+	Cocci	-	-	-	+	+	-	+	+	+	+	Enterococcus sp
FH29	+	Cocci	-	-	+	+	+	-	+	+	-	+	Enterococcus sp
FH30	-	Rod	-	-	-	+	+	+	+	+	+	+	Proteus vulgaris
FH31	-	Rod	-	+	-	+	+	-	+	+	-	+	Proteus vulgaris
FH32	-	Rod	-	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH33	-	Rod	-	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH34	_	Rod	_	+	-	+	+	-	+	+	+	+	Proteus vulgaris
FH35	+	Cocci	-	+	-	+	+	-	+	+	+	+	Staphylococcus epidermidis

KEY: S/N- Sample number, GR- Gram reaction, COA- coagulase, CAT- catalase, K- alkaline, A- acid, H_2S - hydrogen sulphide, CTI- citrate, IND- indole, MOT- motility, FH- Food handlers.

Table 4: Antibiotic sensitivity and resistance patterns of bacterial isolates

S/N	Organisms	OFL	AUG	NIT	CPR	CAZ	CRX	GEN	CXM
FH1	Citrobacter freundii	S	S	S	R	S	R	S	R
FH2	Escherichia coli	S	S	S	S	S	R	S	R
FH3	Escherichia coli	I	S	I	I	S	R	S	R
FH4	Escherichia coli	S	S	S	S	S	R	S	S
FH5	Proteus vulgaris	S	R	S	S	S	R	S	S
FH6	Proteus vulgaris	R	S	S	S	S	S	S	S
FH7	Proteus vulgaris	S	R	R	S	S	R	S	R
FH8	Proteus vulgaris	S	S	R	S	S	R	S	S
FH9	Providencia sp.	S	S	R	S	S	S	S	S
FH10	Proteus vulgaris	I	S	R	S	S	S	S	R
FH11	Staphylococcus saprophyticus	I	S	R	I	S	I	S	R
FH12	Staphylococcus saprophyticus	S	S	R	S	S	R	S	S
FH13	Proteus vulgaris	S	S	R	S	S	S	S	S
FH14	Proteus vulgaris	S	S	I	S	S	S	S	R
FH15	Proteus vulgaris	S	S	S	S	S	S	S	R
FH16	Proteus vulgaris	S	S	S	S	S	S	S	R
FH17	Citrobacter freundii	I	S	R	R	S	R	S	R
FH18	Enterococcus sp	S	S	S	S	S	S	S	R
FH19	Proteus vulgaris	S	R	I	S	S	R	S	R
FH20	Salmonella sp	S	S	S	I	S	R	S	R
FH21	Salmonella sp	S	R	S	S	S	S	S	S
FH22	Proteus vulgaris	S	S	I	S	S	S	S	R
FH23	Proteus vulgaris	S	Ι	S	S	R	R	S	R

in transmission of typhoid bacilli through different food products and water and the carrier state of typhoid was observed in the age group 15-45 years.

5. Conclusions

The prevalence of enteric bacteria in this finding emphasizes that food handler harboring enteric bacterial pathogens asymptomatically are the potential sources of food borne infections. Most of the isolates showed sensitivity to ofloxacin, augmentin, ciprofloxacin, ceftazidime and

gentamicin. Moreover, 33.3% of *Salmonella* species showed multidrug resistance. The findings point out that the rise in antimicrobial resistance is still an ongoing public problem in treating enteric bacteria associated infections. Thus, health education is essential to create awareness about food borne infection linked with unhygienic food handling and preparation, Moreover, continuous supervision and follow up should be undertaken.

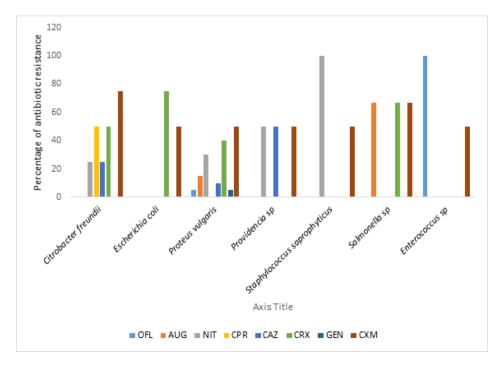


Fig. 4: Antibiotic resistance pattern of the enteric bacteria

6. Recommendation

Antibiotics that were shown to inhibit the growth of the organisms should be used in the treatment of various infections in humans. Indiscriminate prescription and use of antibiotics should be discouraged in both community and hospital setting by continuous public enlightenment on rational antibiotic use as well as adoption of strict national antibiotic policy to regulate the prescription, sale and use of antibiotics.

Food handlers should have a good personal hygiene which means that they should always clean their hands before and after touching food so as not to transfer the microorganisms in their hands to food in order to reduce the rate of transmission to other susceptible human.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

- Barrabeig RA, Buesa J, Bartolomé R, Pintó R, Prellezo H, Demissie T, et al. Prevalence and antimicrobial susceptibility patterns of Shigella and Salmonella Species among patients with diarrhoea attending Gondar town health institutions. Sci J Public Health. 2010;2(5):469– 75
- Batt RM, Rutgers HC, Sancak AA. Enteric bacteria: Friend or foe? J Small Animal Pract. 1996;37(6):261–7.

- Aklilu A, Kahase D, Dessalegn M, Tarekegn N, Gebremichael S, Zenebe S. Prevalence of intestinal parasites, salmonella and shigella among apparently health food handlers of Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia. BMC Res Notes. 2015;8(1):17.
- Khurana S, Thapar R, Sharma M, Malla N. Intestinal bacterial and parasitic infections among food handlers in a tertiary care hospital of North India. *Trop Gastroenterol*. 2008;29(4):207–9.
- Tiruneh MDM. Bacterial Profile and Antimicrobial Susceptibility Pattern among Food Handlers at Gondar University Cafeteria, Northwest Ethiopia. J Infect Dis Ther. 2013;01(02):1–6.
- Abera B, Biadegelgen F, Bezabih B. Prevalence of Salmonella typhi and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia. *Ethiop J Health Dev.* 2010;24(1):46–50.
- Smith SI, Agomo CO, Bamidele M, Opere BO, Aboaba OO. Survey of food handlers in bukas (a type of local restaurant) in Lagos, Nigeria about typhoid fever. *Health*. 2010;02(08):951–6.
- Oladipo I. Incidence of antibiotic resistance in some bacterial pathogens from Street Vended food in Ogbomoso, Nigeria. *Pak J Nutr.* 2010;9(11):1061–8.
- Ahmed S. Bacteriological and Parasitological Assessment of Food Handlers in the Omdurman Area of Sudan. *J Microbiol Immunol Infect*. 2010;43(1):70–3.
- Andargie G, Kassu A, Moges F, Tiruneh M, Huruy K. Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town, Northwest Ethiopia. *J Health, Popul Nutr.* 2009;26(4):451–5.
- Bassyouni H, Hefzy H, Wegdan A. Perception of food safety and prevalence of Staphylococcus aureus and Salmonella species Carriers among Fayoum University Food handlers. *Life Sci J.* 2012;9(4):1–7.
- Oundo JO, Kariuki SM, Boga HI, Muli FW, Iijima Y. High Incidence of EnteroaggregativeEscherichia coliAmong Food Handlers in Three Areas of Kenya: A Possible Transmission Route of Travelers' Diarrhea. J Travel Med. 2008;15(1):31–8.
- Smith SI, Agomo CO, Bamidele M, Opere BO, Aboaba OO. Survey of food handlers in bukas (a type of local restaurant) in Lagos, Nigeria about typhoid fever. *Health*. 2010;02(08):951–6.
- Fadlalla I, Abdel A, Ibrahim M. Antimicrobial susceptibility of Salmonella serotypes isolated from human and animals in Sudan. J

- $Public\ Health\ Epidemiol.\ 2012; 4(1): 19-23.$
- Okojie OH, Wagbatsoma VA, Ighoroge AD. An assessment of food hygiene among food handlers in a Nigerian University campus. Nigerian Post- graduate. *Med J.* 2005;12(2):93–6.
- 16. Todd ECD, Greig JD, Bartleson CA, Michaels BS. Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 6. Transmission and Survival of Pathogens in the Food Processing and Preparation Environment. J Food Prot.
- 2009;72(1):202-19.
- Murakami K, Ishihara T, Horikawa K, Oda T. Features of Salmonella serovars among food handlers in Kyushu, Japan. N Microbiol. 2007;30(2):155–9.

Cite this article: Abubakar MU, Alao FO, Abubakar SH, Garba MA, Yakub AU. Isolation of enteric bacteria from asymptomatic food handlers. *Indian J Microbiol Res* 2020;7(3):247-257.