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Monitoring of *Escherichia coli*, *Salmonella* spp. and Staphylococci in Poultry Meat-Based Fast Food in Saudi Arabia

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Abstract

An investigation was made to survey the possible presence of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. from fast-food shops in Al-Ahsa Province, Kingdom of Saudi Arabia (KSA), as potential reservoir of human infection and antimicrobial resistance. A total of 100 samples of shawarma poultry meat were collected from different localities of the province. Conventional, commercial VITEK 2 and molecular techniques were used for isolates' identification and antibiogram detection. *Staph aureus* was isolated at a rate of 14% and CNS as *Staph. sciuri* and *Staph. xylosus* at 2%. *E. coli* was identified at a rate of 12% and antibiogram analysis showed 41.67% of isolates to be extended-spectrum β-lactamases (ESBL) with evidence of multi-drug resistance (MDR). Molecular analysis of *E. coli* revealed presence of sero-groups O1 and O2, entero-toxigenic (ETEC), shiga-toxigenic, ST540 and the prototypical ETEC strain H10407 which are potential public health hazard. *Salmonella enterica* serovar Enteritidis showed 19% prevalence while S. Typhimurium with 8% prevalence. Anti-microbial sensitivity of 15 strains of S. Enteritidis and 5 strains of S. Typhimurium showed multi-drug resistance (MDR).

Keywords

Fast Food, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., Saudi Arabia

1. Introduction

Food-borne diseases caused by pathogenic bacteria are prevalent and still occur at unacceptably high rates in developed and developing countries. The common pathogens that cause most of foodborne diseases are *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus ce-*

reus, *Salmonella enterica*, *Vibrio* spp., Shiga toxin-producing *E. coli* (STEC) and *Clostridium perfringens* [1] [2]. The large number of fast ready-to-eat food shops represent a problem to Public Health authorities responsible for the safety of food [3]. Consumption of poultry meat is increasing world-wide due probably to nutritious value and personal health issues. Under-cooked poultry meat in fast food shops is a dangerous vehicle for pathogens transmission.

Staph. aureus is a common bacterial species found as a commensal in the nose and on the skin of healthy humans and animals. Staphylococcal food poisoning is a common problem that alarm health-care providers worldwide. Entero-toxigenic strains of *Staph. aureus* secretes enterotoxins in food that consequently may cause poisoning when eaten.

Pathogenicity of Coagulase-Negative Staphylococci (CNS) to man and animals in recent years, prompted extensive studies to determine their role in these diseases [4]. *Staph. sciuri* group is generally considered of doubtful pathogenicity in human infection. However, *Staph. sciuri* has been associated with serious human diseases, such as endocarditis, peritonitis, septic shock, and wound infections [5] [6].

Salmonella enterica

Bacteria of the genus *Salmonella* are Gram-negative, facultatively anaerobic, non-spore forming, usually motile rods belonging to the Family Enterobacteriaceae and primarily associated with animals. The genus currently contains just two species, *Salmonella enterica* (including six subspecies) and *Salmonella bongori*. Most of the *Salmonella* isolates from cases of human infection belong to *Salmonella enterica* subspecies *enterica*. The genus is also further subdivided into approximately 2500 serovars (or serotypes), characterised on the basis of their somatic (O) and flagellar (H) antigens [7].

Until recently, individual serovars were referred to as if they were species, for example *Salmonella typhimurium*. However the current convention is to refer to this serovar as *Salmonella enterica* subsp. *enterica* serovar Typhimurium. But it is customary to shorten this to *Salmonella* Typhimurium. The commonest serovars associated with human disease are S. Typhimurium and S. Enteritidis, but many others have been shown to cause disease, notably S. Infantis, S. Virchow and S. Newport [8].

Given the long history of foodborne salmonellosis, it is not surprising that the need for microbiological testing of food ingredients and food products is very significant. A substantial number of methods, both traditional and rapid, have been developed over the years for the detection and identification of *Salmonella*.

Salmonella isolates are accepted by many researchers as the most circulating and frequent bacterial agents causing disease in avian species. It is associated with high economic losses because of high mortality, morbidity and lowered production in poultry. As well, it is considered as a major food-borne pathogen in most countries of the world especially in developing countries [9] [10].

Contamination by *Salmonella* spp. in poultry products occurs frequently and can be transmitted to humans through contact and consumption of undercooked poultry meat [11] [12].

Escherichia coli is a member of Family Enterobacteriaceae that live as a commensal in the intestinal tract of humans and animals but occasionally may cause infection in the intestinal tract and other body systems. Biochemical reactions are used, traditionally, for identification and confirmation of bacteria to species level. Extra-intestinal pathogenic *Escherichia coli* (ExPEC) is a diverse *E. coli* pathogenic group with genetic diversity which reflect its colonization of widespread ecological niches [13]. Entero-haemorrhagic strains of *E. coli* produce a toxin almost identical to that of *Shigella dysenteriae*, which is responsible for gastroenteritis in man. Some farm animals are sub-clinically infected with *E. coli* O157, related to ExPEC, where their faeces may contain *E. coli* O157 in varying numbers.

Anti-microbial susceptibility testing is vital for control of food-borne infectious bacteria. The emergence of isolates presenting resistance to several antibiotics *i.e.* multi-drug resistance (MDR) is of concern because these drugs are crucial to the successful treatment. A bacterial species exhibits MDR when showing resistance to more than three unrelated antibiotics.

Antimicrobial resistance (AMR) has emerged as a global health problem. Hence, contamination of poultry feeds with bacteria may lead to increase in AMR strains. From 48 studies, only one of the studies did not report multidrug resistance. At least 18 bacterial spp. were reported to be resistant to many locally available antimicrobial agents. A total 16 residue studies reported high levels of drug residues in the form of prevalence or concentration above the recommended international limit [14]. A trial in Canada to change from conventional use of antimicrobials in broiler production to reduction practices indicated that restriction of antibiotic use should start immediately [15]. AMR specific interventions are in early stages of development in EU as there are many data gaps. Epidemiological studies at food production environments linked to One Health at EU are needed [16]. Poultry reared at backyard in California showed AMR of *Salmonella* similar to commercial poultry imposing zoonotic risks and food contamination; flock surveillance programme is needed [17]. Most common serotypes in poultry products were *Salmonella* Enteritidis and *Salmonella* Typhimurium. Highest AMR was recorded for nalidixic acid and ampicillin which highlight the need to control misuse of antibiotics in poultry [18]. Pharmacokinetics/Pharmacodynamics (PK/PD) models, establish rational dosage regimens, are suggested for veterinary antimicrobials to face misuse and abuse of antibiotics. They would achieve efficacy in prevention AMR, prevention and treatment of bacterial infection [19]. Environment link to AMR was studied by trapping 493 flies to isolate *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus* spp. Target bacteria were isolated and 35.3% of flies carried AMR species and 9.0% harboured multidrug resistant isolates. Therefore flies can indicate environmental

contamination of AMR [20]. In the EU, increased cases of human salmonellosis after 2014 led to investigate conditions in poultry production. Although evidence from the study is inconclusive, there is conclusive evidence that increased stocking density, stress and larger farms result in spread of *Salmonella* in laying hens [21].

Multilocus sequence typing (MLST) provides an efficient genotyping tool for molecular epidemiology analysis. *E. coli* strains with identical MLST profiles (known as sequence types or STs) may possess distinct genotypes. This enable different ecological- or pathological-typical lifestyles. However, STs are not uniform with regard to genetic properties or ecological/pathological-typical behaviors [14].

In Al-Ahsa Province, KSA, *Salmonella enteritidis* was reported as the cause of an outbreak of food poising by eating chicken shawarma [15].

Conventional methods are used routinely for identification of microorganisms with increased number of misidentification making it continuously unreliable. The VITEK 2 Automated System (bioMérieux) is a widely used instruments in clinical microbiology laboratories for identification of bacteria and evaluation of anti-microbial susceptibility profile [16]. Further, molecular bacteriological techniques are receiving attention from clinical microbiology laboratories. Partial 16S rRNA gene sequencing has been used for accurate bacterial identification [17] [18].

In Al-Ahsa Province, KSA, data about food-borne pathogenic bacteria is meagre, hence the present study aimed for identification and characterization of Staphylococci, *E. coli* and *Salmonella* spp. from fast-food shops.

2. Materials and Methods

2.1. Collection of Samples

The survey was carried out in Al-Ahsa Province, Eastern Region, KSA. A total of 100 samples were collected from fast-food shops in different localities of the Province. Sandwiches of shawarma poultry are prepared by making slices of poultry meat, layer on vertical spit, slow-roasted, mix with vegetables and delivered fresh for breakfast to consumers. Samples were put into sterile plastic bags and sent to the laboratory in an ice box within maximum two hours from the time of purchase. Processing in the laboratory by aseptically weighing 25 gm of sample in sterile stomacher bags (Seward Medical StomacherR Bags), diluted with 225 ml of sterilized 0.1% w/v peptone water (Oxoid) and macerated in a stomacher for 3 min.

2.2. Staphylococci

A selective medium for Staphylococci Baird Parker agar (BD Diagnostics, Franklin Lakes, NJ, USA), supplemented with egg yolk tellurite emulsion, is used for primary isolation and incubated at 37˚C overnight. Recovered single colonies were streaked onto 5% citrated sheep blood agar plates and incubated at 37˚C

overnight. Cultural characteristics, morphology and biochemical tests were done to identify Staphylococci. Confirmation of the identification of isolates and antimicrobial susceptibility profile to a wide range of antimicrobial drugs were done by VITEK 2 technique (bioMerieux, France).

For Enterobacteriacaeae, the following media MacConkey Agar (MCA) for *E. coli* detection, Sorbitol MacCankoky Agar (SMAC) for STX-EC. Purple colonies on MCA were identified as *E. coli*, whereas white colonies on SMAC were presumptive STX-EC and were therefore stereotyped by PCR for confirmation. On the other hand, Serial dilutions (10-fold) of the samples were made in 1% sterile peptone water (Difco, UK). Then 0.1 mL of each dilution was inoculated into *Salmonella* enrichment medium, Selenite F broth (Oxoid, UK), and incubated at 37˚C for 24 - 48 h. Thereafter, the growth was transferred to the media recommended for *Salmonella* spp. including: MCA, Xylose lysine deoxycholate agar (XLD) (Oxoid, UK) and Deoxycholate citrate agar (DCA) (Oxoid, UK), and incubated at 37˚C for 24 h. *Salmonella* colonies, characterized by producing non-lactose fermenting pale colored colonies on MCA, and with black centres on DCA medium and pink-red colonies with black centres on XLD medium, were picked up and sub-cultured. The colonies were confirmed as Gram negative bacteria using Gram staining procedures, and glycerol cultures of all of the isolates were prepared and stored at −80˚C for further analysis. The isolated bacterial strains were subjected to identification using biochemical tests and VITEK 2 technique (bioMerieux, France). Antimicrobial sensitivity test was done by VITEK 2 technique. The tested anti-microbial agents are: Ampicillin, Amoxicillin, Piperacillin, Cefotaxime, Ceftazidime, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Norofloxacine, Fosfomycin, Nitrofurantoin, Trimethoprim/sulfamethoxazole.

2.3. Molecular Analysis

1) *Staph. aureus*:

DNA was extracted by sub-culturing *Staph. aureus* isolates into Luria Bertani broth and incubated at 37˚C for 18 hours. A volume of 1 ml was centrifuged at 3500g/3min in micro-centrifuge tubes and the supernatant (S/N) was discarded. The precipitate was suspended in 200 µl of extraction buffer (0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 8.0, 1.25% SDS) and mixed well. The tube was incubated at 63˚C for 3 min, brought to room temperature, washed at 3500g/3min and S/N was removed gently to a new tube. An equal volume of absolute ethanol was added to precipitate DNA, washed for 3 min at 3500x g and dried by air flow.

PCR on 16S rRNA using 27F and 1492R primers was performed by Macrogen Inc. (Seoul, South Korea). Sequences of the primers were:

27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'

1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

2) Enterobacteriacaeae:

DNA was extracted by sub-culturing *E. coli* and *Salmonella* spp. isolates into Luria Bertani broth and incubated at 37˚C for 18 hours. A volume of 1 ml was centrifuged at 3500 g/3 min in micro-centrifuge tubes and the supernatant (S/N) was discarded. The precipitate was suspended in 200 µl of extraction buffer (0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 8.0, 1.25% SDS) and mixed well. The tube was incubated at 63˚C for 3 min, brought to room temperature, washed at 3500 g/3min and S/N was removed gently to a new tube. An equal volume of absolute ethanol was added to precipitate DNA, washed for 3 min at 3500x g and dried by air flow.

PCR on 16S rRNA using 27F and 1492R primers was performed by Macrogen Inc. (Seoul, South Korea). Sequences of the primers were:

Primer Type Type 2 Sequence (5 to 3) Reference;

27F Universal AgAgTTTgATCMTGGCTCAg;

1492R Universal TACggYTACCTTgTTACgACTT [19].

3) 16S ribosomal RNA gene sequence analysis:

Determined sequences were compared with sequences available in GeneBank, EMBL, and DDBJ databases using the BLAST algorithm,15 through the National centre for biotechnology information server (http://www.ncbi.nlm.nih.gov) and with sequences available in Ribosomal database project (RDP-II), release 9.59, by use of Sequence match algorithm 9. In order to assign isolate to a species, each derived sequence aligned by the BLAST algorithm, yielded at least 99% similarity score with identified species in the BLAST search, and the highest S-ab value with identified species in the Sequence match search.

3. Results

Staphylococci

Staph aureus was isolated from 14 samples at a rate of 14% by conventional methods and confirmed by VITEK 2 technique. CNS identified by VITEK 2 technique as *Staph. sciuri* and *Staph. xylosus* from two samples (2%).

Antibiogram analysis of all isolates showed 5 strains (35.7%) to be MRSA with the following profile: Benzylpenicillin MIC ≥ 0.5 R; Ampicillin MIC ≥ 0.4 R; Oxacillin MIC ≥ 4 R; Gentamicin MIC ≤ 0.5 S; Ciprofloxacin MIC ≤ 0.5 S; levofloxacin MIC ≤ 0.12 S; Moxifloxacin MIC ≤ 0.25 S; Erythromycin MIC 2 IR; Clindamycin MIC 0.5 S; Quinupristin/Dalfopristin MIC 1 S; Linezolid MIC 2 S; Vancomycin MIC 2 S; Tetracycline MIC 2 S; Tigecyclin MIC ≤ 0.12 S; Nitrofurantoin MIC 64 IR; Rifampicin MIC 1 S; Trimethoprim/Sulfa MIC ≥ 320 R (**Table 1**).

Non-MRSA isolates were R to Ciprofloxacin.

All isolates were confirmed by molecular analysis. A dendrogram showing relatedness of CNS isolates as *Staph. sciuri* was identified and confirmed from two samples (**Figure 1**).

1) Enterobacteriacaeae:

Table 1. Antimicrobial susceptibility testing of MRSA isolates from fast food shops in Al-Ahsa province, KSA.

No.	Antimicrobial Agent	MIC	Reaction
1	Benzyl penicillin	≥0.5	R
2	Ampicillin	≥0.4	R
3	Oxacillin	≥4	R
4	Gentamicin	≤0.5	S
5	Ciprofloxacin	≤0.5	S
6	levofloxacin	≤0.12	S
7	Moxifloxacin	≤0.25	S
8	Erythromycin	2	IR
9	Clindamycin	≤0.5	S
10	Quinupristin/Dalfopristin	1	S
11	Linezolid	2	S
12	Vancomycin	2	S
13	Tetracycline	2	S
14	Tigecyclin	≤0.12	S
15	Nitrofurantoin	64	IR
16	Rifampicin	1	S
17	Trimethoprim/Sulfa	≥320	R

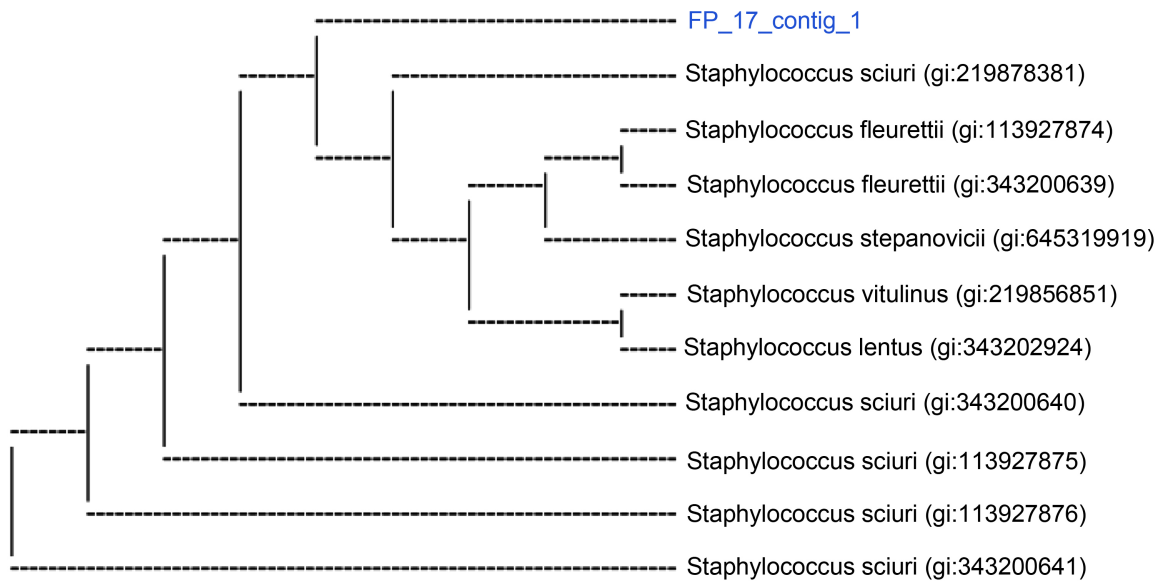

Figure 1. Dendrogram showing genetic relatedness among the isolated CNS strains based on 16Sr DNA sequences analysis.

E. coli was isolated and identified from 12 specimens at a rate of 12% by conventional methods and VITEK 2 technique biochemical confirmation.

Antibiogram analysis, by VITEK 2 technique, showed 5 strains (41.67%) of all isolates to be extended-spectrum β-lactamases (ESBL) positive with the follow-

ing profile:

ESBL MIC: Pos; Ampicillin: MIC ≥ 32 R; Amoxicillin MIC ≤ 2 S; Piperacillin MIC ≥ 128 R; Cefotaxime MIC ≥ 64 R; Ceftazidime MIC ≥ 1 IR; Cefepime MIC ≥ 64 R; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0.25 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≥ 64 R; Gentamicin MIC ≥ 16 R; Ciprofloxacin MIC ≥ 4 R; Norofloxacine MIC ≥ 16 R; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC 64 IR; Trimethoprim/Sulfa MIC ≥ 320 R (Table 2).

2) Antibiogram profile of 7 strains (58.33%) identified as ESBL negative:

ESBL MIC: Neg; Ampicillin: MIC ≤ 2 S; Amoxicillin MIC ≤ 2 S Piperacillin MIC ≤ 4 S; Cefotaxime MIC ≤ 4 S; Ceftazidime MIC ≤ 1 S; Cefepime MIC ≤ 1 S; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0.25 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≤ 2 S; Gentamicin MIC ≤ 1 S; Ciprofloxacin MIC ≥ 4 R; NorofloxacineMIC ≥ 16 R; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC ≤ 16 S.

Trimethoprim/Sulfa MIC ≥ 320 R (Table 3).

Molecular technique analysis of *E. coli* isolates documented the following strains as shown on (Table 4).

Escherichia coli ETEC H10407, complete genome

Escherichia coli ETEC H10407, complete genome

Escherichia coli O25b:H4-ST131 str. EC958 chromo

Escherichia coli strain P33 16S ribosomal RNA gen

Escherichia coli strain LW1655F+ 16S ribosomal RN

Table 2. Antimicrobial susceptibility testing of *E. coli* ESBL positive isolates from fast food shops in Al-Ahsa province, KSA.

No.	Antimicrobial Agent	MIC	Reaction
1	Ampicillin	≥32	R
2	Amoxicillin	≤2	S
3	Piperacillin	≥128	R
4	Cefotaxime	≥64	R
5	Ceftazidime	≥1	IR
6	Cefepime	≥64	R
7	Ertapenem	≤0.5	S
8	Imipenem	≤0.25	S
9	Meropenem	≤0.25	S
10	Amikacin	≥64	R
11	Gentamicin	≥16	R
12	Ciprofloxacin	≥4	R
13	Norofloxacine	≥16	R
14	Fosfomycin	≤16	S
15	Nitrofurantoin	64	IR
16	Trimethoprim/Sulfa	≥320	R

Table 3. Antimicrobial susceptibility testing of *E. coli* ESBL negative isolates from fast food shops in Al-Ahsa province, KSA.

No.	Antimicrobial Agent	MIC	Reaction
1	Ampicillin	≤2	S
2	Amoxicillin	≤2	S
3	Piperacillin	≤4	S
4	Cefotaxime	≤4	S
5	Ceftazidime	≤1	S
6	Cefepime	≤1	S
7	Ertapenem	≤0.5	S
8	Imipenem	≤0.25	S
9	Meropenem	≤0.25	S
10	Amikacin	≤2	S
11	Gentamicin	≤1	S
12	Ciprofloxacin	≥4	R
13	Norofloxacine	≥16	R
14	Fosfomycin	≤16	S
15	Nitrofurantoin	≤16	S
16	Trimethoprim/Sulfa	≥320	R

Escherichia coli UMNK88, complete genome
Escherichia coli JJ1886, complete genome
Escherichia coli O145:H28 str. RM12581, complete
Escherichia coli O25b:H4-ST131 str. EC958 chromo
Escherichia coli strain ST540, complete genome
Escherichia coli O103:H2 str. 12009 DNA, complete genome
Escherichia coli O104:H4 str. 2009EL-2071, complete genome

S. *enterica* serovar Enteritidis showed highest prevalence 19% while S. Typhimurium with 8% prevalence in food samples by VITEK 2 technique biochemical confirmation and molecular method.

Antibiogram of 15 strains of S. Enteritidis showed the following profile:

Ampicillin: MIC ≤ 1 S; Amoxicillin MIC ≤ 1 S; Piperacillin MIC ≤ 1 S; Cefotaxime MIC ≥ 32 R; Ceftazidime MIC ≤ 1 S; Cefepime MIC ≤ 1 S; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0. 5 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≥ 32 R; Gentamicin MIC ≥ 32 R; Ciprofloxacin MIC ≥ 32 R; Norofloxacine MIC ≤ 0.5 S; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC 256 R; Trimethoprim/Sulfa MIC ≥ 320 R (Table 5).

Antibiogram of 5 strains of S.Typhimurium showed the following profile:

Ampicillin: MIC ≤ 1 S; Amoxicillin MIC ≤ 1 S; Piperacillin MIC ≤ 4 S; Cefotaxime MIC ≥ 32 R; Ceftazidime MIC ≥ 32 R; Cefepime MIC ≥ 32 R; Ertapenem MIC ≤ 0.5 S; Imipenem MIC ≤ 0. 5 S; Meropenem MIC ≤ 0.25 S; Amikacin MIC ≥

Table 4. Molecular technique analysis of *E. coli* isolates from fast food shops in Al-Ahsa province, KSA.

No	Description	Gene	Length	Score (bits)	E value	Identities	Percentage	Strand	AC
1	*Escherichia coli* O25b:H4-ST131 str. EC958 chromosome	complete genome	5,109,767	2721 (1473)	0.0	1475/1476	99%	Plus/Plus	emb|HG941718.1|
2	*Escherichia coli* O145:H28 str. RM12581	complete genome	5,585,611	2721 (1473)	0.0	1475/1476	99%	Plus/Plus	gb|CP007136.1|
3	*Escherichia* coli NA114	complete genome	4,971,461	2712 (1468)	0.0	1468/1468	100%	Plus/Minus	gb|CP002797.2|
4	*Escherichia* coli JJ1886	complete genome	5,129,938	2706 (1465)	0.0	1467/1468	99%	Plus/Plus	gb|CP006784.1|
5	*Escherichia coli* PMV-1 main chromosome	complete genome	4,984,940	2706 (1465)	0.0	1467/1468	99%	Plus/Minus	emb|HG428755.1|
6	*Escherichia coli* 0127:H6 E2348/69	complete genome	4,965,553	2700 (1462)	0.0	1462/1462	100%	Plus/Plus	emb|FM180568.1|
7	*Escherichia coli* 536	complete genome	4,938,920 (1465)	2706	0.0	1468/1469	99%	Plus/Plus	gb|CP000247.1|
8	*Escherichia coli* strain chicken11	16S ribosomal RNA partial sequence	1478	2695 (1459)	0.0	1461/1462	99%	Plus/Plus	gb|JX041515.1|
9	*Escherichia coli* strain LW1655F+	16S ribosomal RNA partial sequence	1517	2726 (1476)	0.0	1476/1476	100%	Plus/Plus	gb|AY616658.1|
10	*Escherichia coli* strain ST540	complete genome	4,875,682	2721 (1473)	0.0	1475/1476	99%	Plus/Minus	gb|CP007391.1|
11	*Escherichia coli* SE15 DNA	complete genome	4,717,338	2712 (1468)	0.0	1468/1468	100%	Plus/Minus	dbj|AP009378.1|
12	*Escherichia coli* strain KVP110	16S ribosomal RNA partial sequence	1479	2700 (1462)	0.0	1467/1469	99%	Plus/Plus	gb|JX290090.1|
13	*Escherichia coli* ABU 83972	complete genome	5,131,397	2700 (1462)	0.0	1467/1469	99%	Plus/Plus	gb|CP001671.1|
14	*Escherichia coli* strain KVP107	16S ribosomal RNA partial sequence	1482	2700 (1462)	0.0	1467/1469	99%	Plus/Plus	gb|JX290087.1|
15	*Escherichia coli* 042	complete genome	5,241,977	2695 (1459)	0.0	1466/1469	99%	Plus/Minus	emb|FN554766.1|
16	*Acinetobacter baumannii* strain AC29	complete genome	3,935,134	2717 (1471)	0.0	1471/1471	100%	Plus/Minus	gb|CP007535.1|
17	*Shigella flexneri* strain E58	16S ribosomal RNA partial sequence	1523	2706 (1465)	0.0	1467/1468	99%	Plus/Plus	gb|HQ407235.1|
18	*Shigella dysenteriae* strain FBD012	16S ribosomal RNA partial sequence	1542	2700 (1462)	0.0	1462/1462	100%	Plus/Plus	gb|EU009183.1|

Table 5. Antimicrobial susceptibility testing of S. Enteritidis isolates from fast food shops in Al-Ahsa province, KSA.

No.	Antimicrobial Agent	MIC	Reaction
1	Ampicillin	≤1	S
2	Amoxicillin	≤1	S
3	Piperacillin	≤1	S
4	Cefotaxime	≥32	R
5	Ceftazidime	≤1	S
6	Cefepime	≤1	S
7	Ertapenem	≤0.5	S
8	Imipenem	≤0.5	S
9	Meropenem	≤0.25	S
10	Amikacin	≥32	R
11	Gentamicin	≥32	R
12	Ciprofloxacin	≥32	R
13	Norofloxacine	≤0.5	S
14	Fosfomycin	≤16	S
15	Nitrofurantoin	256	R
16	Trimethoprim/Sulfa	≥320	R

Table 6. Antimicrobial susceptibility testing of S. Typhimurium isolates from fast food shops in Al-Ahsa province, KSA.

No.	Antimicrobial Agent	MIC	Reaction
1	Ampicillin	≤1	S
2	Amoxicillin	≤1	S
3	Piperacillin	≤4	S
4	Cefotaxime	≥32	R
5	Ceftazidime	≥32	R
6	Cefepime	≥32	R
7	Ertapenem	≤0.5	S
8	Imipenem	≤0.5	S
9	Meropenem	≤0.25	S
10	Amikacin	≥32	R
11	Gentamicin	≥32	R
12	Ciprofloxacin	≥32	R
13	Norofloxacine	≥16	R
14	Fosfomycin	≤16	S
15	Nitrofurantoin	256	R
16	Trimethoprim/Sulfa	≥320	R

32 R; Gentamicin MIC ≥ 32 R; Ciprofloxacin MIC ≥ 32 R; Norofloxacine MIC ≥ 32 R; Fosfomycin MIC ≤ 16 S; Nitrofurantoin MIC 256 R; Trimethoprim/Sulfa MIC ≥ 320 R (Table 6).

4. Discussion

The present study aimed to explore prevalence of pathogenic bacteria in poultry-based fast-food shops and their anti-microbial susceptibility pattern. In Staphylococci, *Staph aureus* was isolated at rate of 14% and *Staph. sciuri* at 2%. Poultry meat may be contaminated with *Staph aureus* at preparation stage or from food-handlers. *Staph aureus* is non-spore-former but can cause contamination of food products during food processing. It can grow in a wide range of temperatures (7˚C to 48.5˚C; optimum 30˚C to 37˚C), pH (4.2 to 9.3; optimum 7 to 7.5), and concentration of sodium chloride up to 15% NaCl. *Staph aureus* can tolerate desiccation to survive in almost dry and stressful environments such as the human nose and skin [20]. Staphylococcal food poisoning has been reported and investigation in one outbreak pointed to the food-handler who transmitted the pathogen to the food [21]. Another study documented that highest occurrence of *Staph aureus* was on hands of abattoir workers (79.6%) and beef carcasses (59.9%) [22].

This is the first report of isolation of *Staph. sciuri* which belongs to CNS from food samples in Saudi Arabia. Food poisoning was reported to be caused occasionally by other Staphylococcus species such as *Staph. intermedius* [23]. An epidemiological survey among CNS in ready-to-eat meats was done. PCR has been established as a reliable tool for identification of various microorganisms such as bacteria, fungi and yeasts at the genus and species level. PCR identification of foodborne pathogens has many advantages such as accuracy, simplicity, sensitivity and reproducibility. It has been established that by PCR methods, isolated CNS contained toxigenic and antimicrobial-resistance genes [24]. Other workers reported presence of classical enterotoxin and antibiotics resistance genes in CNS from poultry [25]. Recently *Staph. sciuri* is considered as emerging human pathogen most frequently causing wound infections and carrying antibiotic resistance genes [26].

Globally, the status of antimicrobial resistant of microbes in the food chain and their capacity to be dispersed through the international food trade, is increasing.

MRSA isolates in the present study, showed resistance to Benzylpenicillin, Ampicillin, Oxacillin, Erythromycin, Nitrofurantoin and Trimethoprim. On the other hand, non-MRSA showed similar resistance pattern plus being resistant to ciprofloxacin. This indicates multi-drug resistance among the isolates with potential to transmit resistance through the food chain. It has been reported that MRSA isolates from retail meat, a mastitic cow and CA-MRSA isolates were closely related according to *spa* type. They were identical according to PFGE pattern, ST, and SCC*mec* and had the characteristics of ST8/SCC*mec* Ivl [27].

E. coli was isolated and identified from 12 specimens at a rate of 12%. Anti-microbial analysis of all isolates showed 41.67% of strains to be ESBL positive. ESBLs are a large group of plasmid-mediated enzymes which induce resistance to most beta-lactam antibiotics [28]. Antibiogram analysis of this group, as shown in the results, indicates multi-drug resistance among isolates. Resistance was determined for Ampicillin, Piperacillin, Cefotaxime, Ceftazidime, Cefepime, Amikacin, Gentamicin, Ciprofloxacin, Norofloxacine, Nitrofurantoin and Trimethoprim. While ESBL negative strains were resistant to Ciprofloxacin, Norofloxacine and Trimethoprim. This is interesting for the study area as it highlights the prevalence of multi-drug resistance among *E. coli* isolates from poultry fast-food with potential of transmission to humans. Multi-drug resistance was reported for 64.91% of *E. coli* isolates from poultry meat [29]. Another study indicated that ESBL/plasmid mediated AmpC-type cephalosporinase (pAmpC) *E.coli* in the broiler production pyramid is prevalent, with measureable transfer between subsequent production levels [30]. The problem of transfer of antimicrobial resistance from animals to man is stressed with the emergence of *E. coli* strains harbouring transferable-plasmids carrying multiple antimicrobial resistance genes [31]. Another study documented that ESBL-*E. coli* in retail chicken meats could be a potential reservoir for multidrug resistance determinants and that some are potentially harmful to humans [32].

Molecular analysis of *E. coli* strains, in the present study, showed presence of sero-groups O1 and O2, ETEC, shiga-toxigenic *E. coli* and other strains as displayed on the results. Other workers reported that whole genome sequencing on 100 ESBL/pAmpC-EC isolated from broilers, revealed that isolates carried resistance genes to four antimicrobial classes plus cephalosporins. In addition these uncommon ESBL/pAmpC-*E. coli* lineages, previously reported in diverse hosts, including humans, could emerge in poultry [33]. [34] sequenced the O-antigen clusters of various *E. coli* serogroups O1 and O2 strains. It indicated that the O1 and O2 are encoded by different sets of O-antigen encoding genes and identified potential new O-groups. Moreover, *E. coli* strains belonging to serogroups O1 and O2 are frequently associated with human infections, especially extra-intestinal infections. In the present study, two strains were identified as ETEC H10407 which are known to produce both heat-labile (LT) and heat-stable (ST) enterotoxins. Isolation of ETEC from two outbreaks of diaerrhea in broiler chicks potentially may transmit infection to man, has been reported [35]. Other workers indicated that the prototypical ETEC strain H10407 harbours LT and ST enterotoxins along with the colonization factor antigen I adhesin and other classical virulence factors. In humans, enterotoxin is functionally and structurally similar to the diaerrhea-inducing cholera toxin produced by *Vibrio cholerae* [36].

Poultry salmonellosis is a challenge to Veterinary and Human Medicine with substantial increase in its incidence. Prevalence of S. Enteritidis, in the present study, was 19% in food samples. *Salmonella* Enteritidis is commonly associated with poultry products, whereas S. Typhimurium has a wider species range, in-

cluding ruminants, swine and poultry [37]. As well, diverse epidemiological studies indicated importance of poultry foodstuffs in transmission of *Salmonella* to man [38]. Salmonellosis has been the most frequent cause of food-borne outbreaks in Europe [39] while in the USA, CDC estimated more than 1 million annual cases of food-borne salmonellosis [40].

Anti-microbial sensitivity tests, in the present study, indicated high percentage of anti-microbial resistance among S. Enteritidis strains, 15 strains exhibited MDR. Reaction to cephalosporin showed resistance to cefotaxime and sensitivity to the rest, which are not in routine use in the study area. There is resistance to aminoglycosides, ciprofloxacin, nitrofurantoin and trimethoprim/sulfamethoxazole. On the other hand, isolates were sensitive to several β-lactams antibiotics (ampicillin, amoxicillin and piperacillin).

Isolation and identification of frank food poisoning bacterial species, in the present study, from poultry meat shawarma could raise concern about the preparation method of this popular dish. Other workers in Lebanon, reported that meat-based fast foods, especially Shawarma, could be a public health hazard, as they may act as a potential vehicle for many antimicrobial-resistant bacteria [41]. The findings of the present study, indicate that fast-food, in the study area, carried food poisoning pathogenic bacterial species with MDR. Therefore, it is advisable to carry out further surveys, based on the present results, to address the problem extent pinpointing weak points of transmission and suggest preventive measures. Such measures could be to adopt an integrated "One Health" approach to survey antimicrobial resistance in enteric bacteria from humans, retail meat, and food animals. Moreover, surveillance areas should be expanded, adding new bacterial species and antibiotics [42].

5. Conclusions

In the reported study, conventional, commercial VITEK 2 and molecular techniques were used for identification of isolates from poultry meat sandwiches and anti-microbial sensitivity testing. *Staph aureus* was isolated at a rate of 14% and *Staph. sciuri* and *Staph. xylosus* of CNS at 2%. Anti-microbial susceptibility test indicated 35.7% of isolates to be MRSA. *E. coli* was identified at a rate of 12% and antibiogram analysis showed 41.67% of isolates to be ESBL with evidence of multi-drug resistance. *E. coli* revealed presence of sero-groups O1 and O2, entero-toxigenic (ETEC), shiga-toxigenic, ST540 and the prototypical ETEC strain H10407. *Salmonella enterica* serovar Enteritidis showed 19% prevalence while S. Typhimurium with 8% prevalence. Anti-microbial sensitivity of 15 strains of S. Enteritidis and 5 strains of S. Typhimurium showed multi-drug resistance (MDR).

To recapitulate, poultry meat sandwiches in the study area, were contaminated with *Staph aureus* and CNS, *E. coli*, S. Enteritidis and S. Typhimurium. The isolated strains exhibited MDR which indicates that besides potential transmission of food poisoning agents, they may transmit anti-microbial resistance. More investigations to explore the problem and formulate preventive measures,

are unavoidable.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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