*Original Article*

# Foodborne bacterial pathogens recovered from contaminated shawarma meat in northern Jordan

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## Abstract

Introduction: The purpose of the study was to isolate, identify, and determine the antimicrobial resistance of the bacterial pathogens recovered from shawarma (donair) sandwiches served to the public in Jordan.

Methodology: Bacterial contamination of 100 shawarma sandwiches with pathogenic bacteria was studied by culture on selective media, serology, PCR assay, and antimicrobial susceptibility testing.

Results: One hundred and forty-five bacterial isolates were identified. The predominant species was *Escherichia coli* (28.3%), with six isolates of serotype O157:H7, followed by *Salmonella* spp. (25.5%). Higher contamination rates were found in chicken sandwiches. The majority of these bacteria expressed high resistance to several antimicrobials, especially tetracycline and streptomycin. *Citrobacter freundii* was isolated from 15.9% and *Staphylococcus aureus* was isolated from 8.3% of the sandwiches. The presence of these pathogens is of primary concern because some strains are capable of producing a heat-stable enterotoxin that causes food poisoning in humans, and should therefore be taken into account in risk assessment.

Conclusions: Results signify the importance of sustained surveillance of foodborne pathogens in shawarma sandwiches to minimize the risk of contamination. Availability of data on the isolated pathogens and modes of transmission in food from different countries would provide a common ground for reaching international agreement on food safety regulations.

**Key words:** Foodborne Bacterial pathogens; *Salmonella paratyphi*; *E. coli* O157:H7; Serotyping; PCR.

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## Introduction

The importance of food as a vehicle for the transmission of several diseases has been documented, especially in developing countries where hygienic standards are not strictly followed or enforced [1]. The fact that very few illnesses can be linked to food with certainty makes it difficult to estimate the burden of foodborne diseases, and these links are often made only during outbreak situations [2]. Although foodborne diseases do not always result in acute gastroenteritis, food represents an important vehicle for pathogens causing acute gastroenteritis.

More than 250 different foodborne diseases have been described; most of these diseases are caused by a variety of pathogenic bacteria, parasites, and viruses that can be foodborne and can cause food poisoning [3]. Many bacterial species are involved, especially *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Clostridium* spp., and *Listeria* spp., which have been identified worldwide [4]. In addition to traditional foodborne diseases, foodborne bacteria, such as *Escherichia coli* O157:H7, are being increasingly reported. The presence of these microorganisms can lead to many foodborne outbreaks. Most foods become contaminated due to poor sanitation during food preparation, packaging, storage, and serving [5,6].

Shawarma can be originally traced back to Turkey, where it was called "çevirme", which means "turning", but the dish itself is usually called döner kebab, meaning "turning kebab". In Greek, it is called gyros, meaning "turned". It is becoming more popular among consumers of fast foods in Jordan, the Middle East, Europe, Canada, and other countries. Basically, it is a wrap of shredded meat (beef, lamb, or marinated chicken) prepared by alternately stacking strips of fat and pieces of seasoned meat on a rotating vertical skewer. The meat is roasted from the outside, while most of the inside remains rare. Shavings are cut off the block of meat for serving, and the remaining block of meat is kept heated on the rotating skewer. Shawarma sandwiches contain sliced chicken, beef, or

lamb meat with fat, are seasoned with peppers and with tahini (sesame seeds paste and oil) and served in a pita bread wrap. Some restaurants use local mayonnaise to season the sandwiches. Shawarma sandwiches are similar to donairs, and such types of foods are subjected to contamination with pathogens during preparation, processing, and serving [7]. These foods are manipulated extensively during processing and therefore have a potential for high bacterial contamination levels on the surface of the meat, as well as the inside. As a result, there is an increased risk of pathogens surviving and transferring not only by cross-contamination, but also by undercooking as observed in this kind of fast-food industry [8]. Several bacterial indicators are used to evaluate hygiene during the meat slaughtering process, including monitoring the counts of *Escherichia coli* and other Enterobacteriaceae, aerobic bacteria, and *Pseudomonas* [9].

The purpose of this study was to determine the contaminating bacterial pathogens in shawarma sandwiches served to the public, and to evaluate their resistance to commonly used antimicrobials.

## Methodology

*Sample collection and microbiological analysis*

A total of 100 shawarma sandwiches were collected as served to the consumer; 80 chicken (more popular) and 20 beef sandwiches were randomly obtained from 80 local fast-food restaurants. They were collected from five different cities in northern Jordan: Irbid (47 samples); Jarash (10 samples); Al-Ramtha (5 samples), located on the Syrian border north of Irbid; Al-huson (3 samples) located seven kilometers south of Irbid; and Amman (35 samples), the capital of Jordan located in the north-western region of the country. Each sample was transferred to the research laboratory in an icebox, and was kept in the refrigerator until processed within four hours of collection. Twenty-five grams of meat were weighed aseptically from each sandwich, cut into small pieces by a sterile blade, cultured in 225 mL of sterile nutrient broth (Oxoid, Basingstoke, UK) and incubated aerobically at 37°C for 24 hours. One broth culture from each sample was incubated in anaerobic jar with a CampyGen pack at 37°C for 24 hours for the enrichment and isolation of *Campylobacter* spp.

A loopful from the aerobic nutrient broth was then subcultured on several selective media to isolate different pathogens. These media included *Campylobacter* selective agar; mannitol salt agar for *Staphylococcus aureus*; Colombia blood agar for *Listeria* spp. and other pathogens; sorbitol MacConkey agar for the Shiga toxin-producing *Escherichia coli* (STEC), also commonly referred to as verocytotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC) serotype O157:H7; and enrichment in selenite broth for *Salmonella* spp. All plates were incubated at 37°C for 24 hours, followed by subculture on salmonella-shigella (SS) agar, xylose lysine dextrose (XLD) agar, and brilliant green agar for *Salmonella* spp. All subculture plates were incubated aerobically at 37°C for 24–48 hours except for *Campylobacter* selective agar, which was incubated at 42°C for 72 hours in anaerobic jar with an Oxoid CampyGen pack (Thermo Fisher Scientific Inc., Waltham, MA, USA), which generates a microaerobic atmosphere (5% O2, 10% CO2, 85% N2) required for the growth of *Campylobacter* spp.

*Identification of pathogens*

All isolates picked from each selective media were biochemically identified by the Remel RapID system (Thermo Fisher Scientific Inc., USA) as described by the manufacturer.

*Salmonella* spp. was isolated from each sample. Colorless colonies with black center from SS agar were biochemically identified and confirmed serologically using antisera specific to somatic and flagellar antigen [10].

Several non-sorbitol fermenting colonies were picked from sorbitol MacConkey agar and were identified biochemically by Remel RapID systems. Confirmation of *E. coli* O157:H7 requires identification of the H7 flagellar antigen, and these isolates were confirmed serologically for the presence of O157 and H7 antigens using *E. coli* antisera for O157 and H7 (BD, Sparks, USA) [10].

Colonies grown on *Campylobacter* selective agar were identified using a catalase test, oxidase test, hydrolyses of hippurate, and growth on sheep blood agar at 42°C. *Campylobacter* speciation was based on hippurate hydrolysis, indoxyacetate hydrolysis, $H_2S$ production, and susceptibility to 30 µg nalidixic acid [11].

Colonies grown on Colombia blood agar plates were identified by beta-hemolysis in CAMP test that was performed following standard procedures by streaking bacteria along with *S. aureus* on blood agar plates containing 5% blood and observing zones of hemolysis. Fermentation of mannitol, rhamnose, and xylose in addition to biochemical testing using the

commercial API *Listeria*, API Rapid ID 32 Strep was done.

Mannitol-fermenting colonies were identified as *S. aureus* based on catalase production, coagulase test, and salt tolerance [10].

*Antimicrobial susceptibility testing*

The susceptibility of the isolates were tested against a panel of antimicrobial agents by a standard disk diffusion method using Mueller-Hinton agar (Oxoid, UK) according to Clinical and Laboratory Standards Institute guidelines 2002 [12]. The plates were incubated for 24 hours at 37°C, and the diameter of the inhibition zone around each of the eight antimicrobial disks was measured. Multidrug resistance was reported as a single isolate resistant (intermediate or complete) to three or more antimicrobials.

*Molecular characterization of the isolates*

DNA extraction was performed using Wizard Genomic DNA purification kit (Promega, Madison, USA), following the manufacturer's instructions. The extracted DNA was stored in 100 µL of rehydration solution at -20°C until it was tested.

*Salmonella* spp. confirmation of biochemically identified isolates was done by PCR using ST11 and ST15 primers (F: 5'-AGCCAACCATTGCTA AATTGCCGCA-3'; and R: 5'-GGTAGA AATTCCCAGCGG GTACTGG-3'), which amplify a fragment of 429 bp that is specific for *Salmonella* spp. [13].

*E. coli* O157:H7 identification was confirmed using the primers F: 5'-ACGTTACGACGTGTT GCTGGG ATC-3', and R: 5'-TTGCCACAG ACTGCGTCAGTTAGG-3', which amplify a 123 bp fragment of the *stx1* gene [14].

For *Campylobacter* spp., the genes coding for 16S rRNA were amplified by PCR using the primers in the conserved regions within the 16S rRNA gene. Primers sequences were as follows: forward primer, PLO6 (5'-GGTTAAGTCCCGCAACGAGCGC-3') and reverse primer CAMPC5 (5'-GGCTGATCTACGATT ACTAGCGAT-3'), which amplify a 283 bp fragment. PCR amplification conditions were carried out as previously described [15].

All PCR amplifications were carried out in a Gene AMP 9700 thermal cycler (Perkin Elmer, Akron, USA).

Well-characterized *Campylobacter*, *E.coli* O157:H7, and *Salmonella* strains were used as the PCR-positive controls and a PCR mix with no DNA template was included as a negative control in every PCR run. Ten microliters of the PCR products as well as 100 bp molecular marker (Promega, USA) were loaded into 1% agarose gel containing 1 µg of ethidium bromide per mL. DNA bands were visualized by UV transillumination.

## Results

*Bacterial isolates*

Cultures of shawarma samples following enrichment and subculture on selective media as explained in the methods above resulted in the growth of several bacterial species (Table 1). A total of 37 *Salmonella* isolates were recovered from both meat samples; 30 (81%) were recovered from chicken meat samples, while only 7 (19%) were recovered from beef meat samples. *Salmonella paratyphi A* was the most predominant *Salmonella* species (19/37, 51.4%) in both chicken and beef samples (Table 2); *S. choleraesuis* was isolated more frequently from chicken samples (11/30, 36.7%) than from beef samples (1/7, 14.3%), whereas *S. pullorum* (6/30, 20.0%) was detected in chicken samples only.

Serotyping of the 37 *Salmonella* isolates showed that 30 (81%) were positive with the O antisera, whereas 31 (84%) were positive with the H antisera, provided that all *S. pullorum* were nonmotile.

The total number of *E. coli* isolates that were non-sorbitol fermenting, colorless colonies growing on sorbitol MacConkey agar was 41. Of these, only six isolates were positive with both O157 and H7 antisera and hence were considered to be the O157:H7 serotype.

Twenty-three *Citrobacter freundii* (15.8%), 14 *Morganella morganii* (9.6%), and 12 *Staphylococcus aureus* (8.3%) isolates were detected in both chicken and beef sandwiches. However, all species were isolated more frequently from chicken sandwiches than from beef. Other bacterial species were found at low frequencies in the samples (Table 1).

No *Campylobacter* or *Listeria* species were recovered from either type of sandwich.

*Antimicrobial susceptibility testing*

A high level of antibiotic resistance among the *Salmonella* isolates was observed against streptomycin and tetracycline (87% and 89%, respectively) (Table 3).

**Table 1.** Bacterial species isolated from shawarma sandwiches (n = 100)

| Bacteria | Meat type | | Total no. of isolates (%) |
|---|---|---|---|
| | Chicken *(%) | Beef *(%) | |
| *Salmonella* spp. | 30 (26.3) | 7 (22.6) | 37 (25.5) |
| *Escherichia coli* | 33 (29.0) | 8 (25.8) | 41 (28.3) |
| *Citrobacter freundii* | 17 (14.9) | 6 (19.4) | 23 (15.9) |
| *Morganella morganii* | 11 (9.6) | 3 (9.7) | 14 (9.6) |
| *Staphylococcus aureus* | 10 (8.8) | 2 (6.5) | 12 (8.3) |
| *Serratia marcescens* | 7 (6.1) | 1 (3.2) | 8 (5.5) |
| *Proteus mirabilis* | 4 (3.5) | 0 (0.0) | 4 (2.8) |
| *Proteus vulgaris* | 1 (0.9) | 2 (6.4) | 3 (2.1) |
| *Hafnia alvei* | 1 (0.9) | 2 (6.4) | 3 (2.1) |
| **Total** | **114** | **31** | **145** |

*Percentages were calculated out of the total number of isolates in the meat type.

**Table 2.** Distribution of *Salmonella* spp. isolated from shawarma sandwiches (n = 100)

| ***Salmonella* spp.** | **Total** | | **Shawarma meat** | | | |
|---|---|---|---|---|---|---|
| | | | **Chicken** | | **Beef** | |
| | **No. of isolates** | **%** | **No. of isolates** | **%*** | **No. of isolates** | **%*** |
| *S. paratyphi A* | 19 | 51.4 | 13 | 43 | 6 | 86 |
| *S. cholerasuis* | 12 | 32.4 | 11 | 37 | 1 | 14 |
| *S. pullorum* | 6 | 16.2 | 6 | 20 | 0 | 0 |
| **Total** | **37** | **-** | **30** | **-** | **7** | **-** |

*Percentages were calculated out of the total number of isolates in the meat type.

**Table 3.** Susceptibility test of *Salmonella* isolates (n = 37)

| **Antimicrobial agents (concentration in µg)** | **Susceptible** | | **Intermediate** | | **Resistant** | |
|---|---|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| Gentamicin (12) | 36 | 97.3 | 0 | 0.0 | 1 | 2.7 |
| Ampicilin (10) | 28 | 75.7 | 2 | 5.4 | 7 | 18.9 |
| Amoxicillin (25) | 27 | 73.0 | 4 | 10.8 | 6 | 16.2 |
| Nalidixic acid (30) | 23 | 62.2 | 4 | 10.8 | 10 | 27.0 |
| Chloramphinicol (30) | 22 | 59.5 | 3 | 8.1 | 12 | 32.4 |
| Trimethoprime/sulfamethozole (25) | 21 | 56.8 | 2 | 5.4 | 14 | 37.8 |
| Streptomycin (10) | 5 | 13.5 | 0 | 0.0 | 32 | 86.5 |
| Tetracycline (30) | 4 | 10.8 | 0 | 0.0 | 33 | 89.2 |

However, the majority of the isolates were moderately susceptible to ampicillin, amoxicillin, nalidixic acid, chloramphenicol, and cotrimoxazole. On the other hand, resistance to tetracycline was absolute among all the 41 *E. coli* isolates, whereas resistance to the rest of antimicrobials tested was low.

*PCR results*

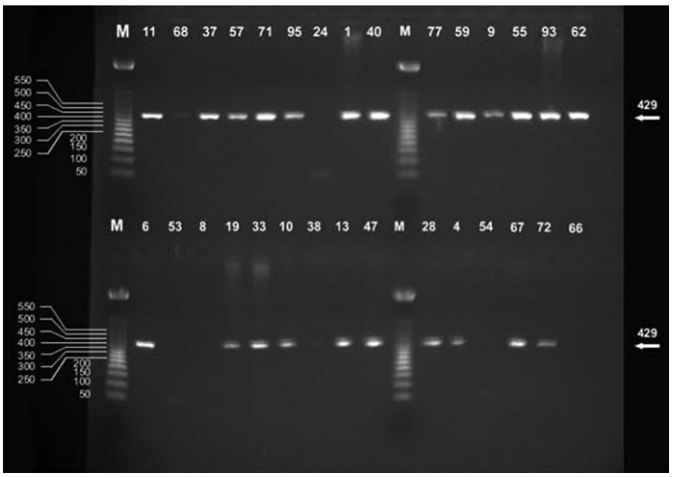
Among the 30 randomly selected *Salmonella* isolates that were tested by PCR assay, 24 (80%) isolates showed the fragment of 429 bp specific for *Salmonella* spp. (Figure 1). Most of the *Salmonella* isolates were from the chicken sandwiches, and *Salmonella paratyphi A* was predominant (Table 2).

Twenty out of the 41 *E. coli* isolates were randomly selected and tested by PCR; six (30%) isolates were positive for the *stx1* gene, and these same isolates were positive with O157:H7 antisera. These isolates were recovered from the chicken sandwiches.

## Discussion

While numerous potential vehicles of transmission exist, commercial chicken meat has been identified as one of the most important food vehicles for *Salmonella* [16]. Meat could be contaminated from cutting boards [17]; other ingredients could be contaminated from the hands of food handlers [18]. Furthermore, seasoning sauces and mayonnaise used in sandwich dressing could also be contaminated. This may explain the relatively higher prevalence of *Salmonella* spp. in chicken shawarma (30/37, 81%) than beef shawarma (7/37, 19%), and may explain the *Salmonella* outbreak in Jordan in 2005 that was attributed to contaminated mayonnaise [19]. These results are consistent with the Centers for Disease Control and Prevention (CDC) report in which nontyphoidal *Salmonella* spp. were listed as being among the top five pathogens contributing to domestically acquired foodborne illnesses in United States (11%) [20]. *Salmonella paratyphi A* was the predominant species and its presence was most likely due to the poor hygiene of workers involved in food preparation, which indicates a need for more hand-washing or use of gloves in food preparation. Higher contamination levels with *Salmonella paratyphi* A (50%) and *E. coli* O157:H7 (75%) resistant to many antimicrobials were also reported in Lebanon [1]. Another *Salmonella* spp., *Salmonella* Thompson, was reported in 18 out of 46 probable cases who consumed chicken shawarma prepared at a local restaurant in Ontario, Canada [21].

**Figure 1.** Agarose gel of PCR product of *Salmonella* using genus-specific primers that amplify a 429 bp.




*Salmonella* spp. is widespread in poultry production in Europe; however, its prevalence varies considerably depending on country and type of production. Poultry meat and eggs represent an

important source of human infection with *Salmonella* spp., and serovars *S. Typhimurium* and *S. enteritidis* are the most commonly reported [22].

The poultry industry and exports of baby chickens to Saudi Arabia have expanded in Jordan in recent years. However, Jordan is still importing poultry from a few countries (*e.g.*, Brazil) and signed a trade agreement in 2012 to import more poultry and poultry by-products from Ukraine. Jordan imports beef from Brazil, Australia, Dubai, and Sudan.

The Ministry of Health in Jordan is responsible for the surveillance of foodborne diseases, notification of individual cases, and the outbreak reporting system. However, limited data are available to assess the burden of illness resulting from foodborne pathogens in Jordan. In a 2003–2004 study by the World Health Organization in collaboration with the Ministry of Health, a large burden of foodborne pathogens was discovered and the magnitude of under-reporting and under-diagnosis of foodborne illness at all stages of the surveillance system was reported [23].

Food safety activities in Jordan have proven to be inadequate, as evidenced by the recurrent outbreaks of food poisoning from mayonnaise and shawarma [23]. Constraints to food safety include limited human resources, inadequate consumer awareness, the overlap of responsibilities across the food chain, the lack of regulation of street foods and food handlers, the lack of multidisciplinary inspection teams, and limited laboratory services.

*E. coli* (28.3%) was the most frequently isolated pathogen in our study; however, it was the second most frequently isolated pathogen from 13.6% of the shawarma purchased from local restaurants in Nigeria [24]. Moreover, *E. coli* (16%), *Salmonella* spp. (8%) and *Citrobacter* spp. (4%) were isolated from shawarma samples collected from different supermarkets and shops in Giza governorate in Egypt [25]. In a study of retail raw meat samples (chicken, turkey, pork, and beef) from the greater Washington, D.C. area in the US [26], *E. coli* was isolated from 38.7% and 19.0% of chicken and beef samples, respectively, which is close to the 29% and 25.8% rates of isolation from chicken and beef samples, respectively, in our study.

Six out of the 41 (14.6%) *Escherichia coli* O157:H7 that were recovered from chicken meat were positive for the verotoxin gene. This serotype was first identified as a pathogen in 1982 and is an important food safety concern worldwide [27]. It is rapidly increasing as a foodborne pathogen primarily associated with the consumption of contaminated ground beef, lamb, pork, or poultry [28]. It is the predominant EHEC serotype both in the US and the United Kingdom [29,30]. Since this serotype is heat sensitive, its isolation indicates uneven cooking and consumption of undercooked meat. The non-O157 Shiga toxin-producing *E. coli* (STEC) serogroup is not nearly as well understood, partly because food outbreaks due to this group are rarely identified, and because it is less likely than *E. coli* O157 to cause severe illness in general [20].

The *E. coli* O157:H7 serotype has been reported in at least four documented outbreaks associated with the consumption of chicken and beef shawarma served at local restaurants in Canada [21], as well as in outbreaks linked to chicken dishes in the UK [31]. It has been suggested that these organisms may have been introduced into the donairs by meat that originated from infected animals or from cross-contamination during preparation. Inadequate or uneven cooking time or slicing too deeply into the meat stack led to the survival of pathogens in the sliced meat, and resulted in the serving of undercooked meat slices.

Antimicrobial susceptibility tests of *Salmonella* spp. and *E. coli* isolates showed that more than half of the isolates were resistant to three antimicrobials or more, especially to tetracycline and streptomycin. All the 41 *E. coli* isolates, including the serotype O157:H7, were resistant to tetracycline. These antibiotics are commonly added to animal feed in relatively low concentrations for therapeutic and prophylactic purposes and as growth promoters, which may select resistant bacterial strains [32].

*Staphylococcus aureus* was isolated from 12 (8.3%) of the sandwiches, mainly from the chicken sandwiches (10 out of 12). Similar shawarma contamination rates with *S. aureus* were reported in Sudan [33], and in the US in chicken (41%) and beef (37%) samples [34]. It is well known that *S. aureus* is a major foodborne pathogen in many countries and is probably responsible for even more individual and family cases than documented. Its presence in sandwiches is of primary concern because some strains are capable of producing a highly heat-stable enterotoxin that causes food poisoning in humans and should be taken into account in risk assessment. Possible sources of contamination of the sandwiches with this pathogen include the hands of food handlers, who handle the food items bare-handed, which indicates poor handling or sanitation.

The poor *Campylobacter* growth on culture plates was unexpected and could be explained by the reduced growth potential of these microaerophilic bacteria in meat exposed to the air during transportation and meat display. *Campylobacter* is known to be sensitive to acid conditions, and the seasoning used in the sandwiches may have resulted in pH reduction in the pathogen environment. However, this interesting finding needs further investigation.

*Citrobacter freundii* was recovered in the current study from 23 (15.9%) samples. It was also reported in an outbreak of gastroenteritis in the US Air Force Academy associated with consumption of chicken salad [35], and in egg shells in Korea [36]. This bacterium is an infrequent, but is an established cause of diarrhea in humans, and is likely to emerge as an important foodborne pathogen since it has the ability to produce or acquire a number of heat-stable enterotoxins.

As for the other bacterial species that were isolated in the current study, *Serratia* spp. (5.5%) and *Morganella morganii* (9.6%) were mainly recovered from chicken (Table 1). They could emerge as foodborne pathogens as they were previously isolated from outbreaks. An earlier study reported the isolation of multidrug-resistant *Morganella morganii* in US retail poultry and beef [37], and *Serratia* spp. (14.3%) were among the most prevalent contaminants. A study of fresh beef meat samples from local markets in Nigeria [34] reported *Citrobacter freundii* (13.9%), *Serratia marcescens* (11.1%), and *Proteus vulgaris* (2.8%) at rates that are close to our results. The

sources of contamination and their importance in the current study could not be determined.

## Conclusions

Our results indicate that shawarma sandwiches serve as potential vehicles for the transmission of several antimicrobial-resistant pathogenic bacteria, especially *Salmonella* spp. and *E. coli* O157:H7 among other possible emerging foodborne pathogens, and are therefore a potential public health hazard. This supports the importance of sustained surveillance of foodborne pathogens in such sandwiches to minimize the risks of contamination through controls implemented during production, cooking, and serving. Food contamination with pathogens will always be a health risk. However, foodborne illnesses are preventable when the responsibility of food safety is shared between regulatory bodies, academia, the food industry, and the consumer. The globalization of the risks associated with foodborne illness has increased with trade in food and international travel, which resulted in greater interdependence in terms of food safety. Availability of epidemiologic data about the prevalent pathogens and their transmission in food from different countries would provide a common ground for reaching international agreement on food safety regulations.

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