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ANTIBIOGRAM FOR COMMON PATHOGENS RECOVERED FROM DIFFERENT FOODSTUFFS

By

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ABSTRACT

In the present study a total of 250 samples were collected from milk products, meat products, fish,shrimp and liver for detection of most commonfood-bornePathogens.The microbiological and biochemical results revealed that there were 60 *E. coli* and 25 *Salmonella* isolates, these results were confirmed serologically by using specific antisera. Antibiogram for the recovered *E. coli* isolates reveated high resistance to amoxicillin (100%), lincomycin (92%), rifampicin (88%) and sulphamethaxzole trimethoprim (72%), on the contrary, *E. coli* strains were highly sensitive to colistin sulphate (72%),Amoxicillin +clavulinic acid (60%). while *Salmonella* isolates were highly resistant to lincomycin, rifampicin, amoxicillin+clavulinic acid and spectinomycin with an incidence of (100%),(100%), (58.33%) and (50%), respectively. On the other hand, salmonella isolates were highly sensitive to colistin sulphate (100%), enrofloxacin (91.67%) and sulphamethaxzole+ trimethoprim (66%).

Keywords:

Foodstuffs, *Salmonezlla, E.coli*, Antibiogram.

INTRODUCTION

Foodborne diseases have become a major public health problem worldwide due to the significantly increased incidence of foodborne diseases over the last 20 years **(Oliver *et al.*, 2005).** Although it is difficult to estimate the global incidence of foodborne diseases as some of the cases are under-reported especially in developing countries, but the increased

incidence of foodborne diseases were reported in many parts of the world **(Van de Venter, 2000)**. For instance, the outbreak of foodborne disease in Taiwan increased rapidly from 121 in 1995 to 177 in 1996 and since then the incidence keep rising **(Chiou *et al.,* 2000)**. According to report from Centers for Diseases Control and Prevention (CDC), approximately 48 million people in the United States get ill, 128000 people are hospitalized and 3000 people die annually due to foodborne diseases despite United States has the safest food supplies in the world **(Oliver *et al.,* 2005; Centers for Disease Control and Prevention, 2011)**. In addition, about a quarter of the population is at a higher risk for foodborne diseases nowadays **(Oliver *et al.,* 2005).** Generally, foodborne diseases are caused by the consumption of food or water contaminated with pathogens or their toxins. Pathogens that caused foodborne diseases are often referred as foodborne pathogens and they include bacteria, viruses, fungi and parasites **(Zhao *et al.,* 2014)**. There are 31 identified foodborne pathogens in the United State and it is estimated that viruses are the primary causes of illnesses whereas bacteria are the primary causes of hospitalizations and deaths **(Scallan *et al.,* 2011)**. The common foodborne pathogens which are responsible for most of the food borne disease out breaks are Listeria monocytogenes, Escherichia coli O157:H7, Staphylococcus aureus, *Salmonella* enterica, Bacillus cereus, and Shigatoxin-producing Escherichia coli (STEC) **(Oliver *et al.,* 2005; Scallan *et al.,* 2011; Zhao *et al.,* 2014).** The increasing amounts of street foods and the increasing demand for minimally processed ready-to-eat products have begun to concern public health agencies on food safety assurance **(Lee *et al.,* 2014)**. Foodborne pathogens are present in various foods such as fruits, vegetables and ready-to-eat products which are consumed without any further treatment **(Chung *et al.,* 2010; Lee *et al*., 2014).** This may lead to foodborne diseases if food safety issues are not taken into consideration. Also, foodborne diseases are often associated with the consumption of raw or undercooked foods such as seafood, meat and poultry **(Wingstr *et al.,* 2006)**. It is essential to analyze the food for the presence of foodborne pathogens in order to ensure a safe food supply and to minimize the occurrence of foodborne diseases. The conventional methods for detecting the foodborne bacterial pathogens present in food are based on culturing the microorganisms on agar plates followed by standard biochemical identifications **(Mandal *et al.*,2011)**.

Conventional methods are usually inexpensive and simple but these methods can be time consuming as they depend on the ability of the microorganisms to grow in different culture

pre-enrichment media, selective enrichment media and selective plating media. Usually conventional methods require 2 to 3 days for preliminary identification and more than a week for confirmation of the species of the pathogens **(Zhao *et al.*, 2014)**. Conventional methods are laborious as they require the preparation of culture media, inoculation of plates and colony counting **(Mandal *et al*., 2011)**. Furthermore, conventional methods may be limited by their low sensitivity **(Lee *et al.*, 2014)**. False negative results may occur due to viable but non-culturable (VBNC) pathogens. The failure to detect foodborne pathogens would increase the transmission risk of pathogens.

Recently, different rapid methods with high sensitivity and specificity have been developed to overcome the limitations of conventional methods for the detection and identification of foodborne pathogens. Furthermore, researchers are still developing novel methods with improvements in terms of rapidity, sensitivity, specificity and suitability for in situ analysis and distinction of the viable cell **(Zhao *et al.*, 2014).** Rapid detection methods are important, particularly in food industry, as they are able to detect the presence of pathogens in raw and processed foods immediately. Rapid methods are also sensitive enough to detect pathogens that present in low numbers in the food. Sensitivity is important because a single pathogen present in food has the risk to cause infection. Rapid methods are more time-efficient, labor-saving and able to reduce human errors **(Mandal *et al.*, 2011).** Nevertheless, each of the rapid method has its own advantages and limitations. Generally, rapid detection methods are categorized into nucleic acid-based, biosensor-based and immunological-based methods **(Zhao *et al.*, 2014).** This review examines these recent rapid detection methods and their applications in foodborne bacterial pathogens detection and along with their advantages and limitations.

MATERIAL AND METHODS

Samples:

A total of 250 samples were collected from different food samples from different region in Giza, Cairo, Fayoum and Beni-Suef Governorates. The samples include raw milk, milk products (cheese, yoghurt) meat, minced meat ,meat products (luncheon and burger) liver, fish, chichen meat and shrimps. These samples were collected from Jan to august during 2016.

Table (1): Collective food Samples.

Source of samples	Number of samples	Source of samples	Number of samples
Milk	**25**	**Burger**	**10**
Yogurt	**15**	**Luncheon**	**15**
cheese	**10**	**Fish**	**15**
Meat	**25**	**Shrimps**	**20**
Minced Meat	**35**	**Liver**	**25**
Poultry Meat	**55**		
Total	**250**		

Isolation and identification of *E. coli and Salmonella* (Collee *et al.,* 1996).

The collected samples were cultivated under aseptic condition into Rappaport Vassiliadis and MacConkey's broth. All inoculated media were incubated aerobically at 37°C for 24 hrs. in *E.coli* and 42°C for 24 hrs in incase of *Salmonella* .Then loop full from the inoculated broth were streaked onto Tryptone Soya agar (TSA) and MacConkey's agar then, incubated aerobically at 37°C for 24-72 hrs. The colonies were examined for their cultural characters and morphological appearance as well as lactose or non-lactose fermenting. The separate colonies were picked up for purification on TSA and incubated aerobically at 37°C for 24 -72 hrs. The lactose fermenting colonies were inoculated onto eosin methylene blue agar medium. Colonies showed characteristic green metallic sheen on EMB agar were picked up and identified. On the other hand, the non-lactose fermenting colonies were inoculated onto Xylose lysine deoxycholate (X.L.D.) and *Salmonella*-Shigella (S.S.) agar media. The inoculated plates were incubated at 42°C for 24 - 48 hrs. Red colonies with black center and pale colonies with black center respectively were picked up and identified.Foreach plate, one single colony representing typical colonial appearance and morphological character was picked up and inoculated into 0.5% semi-solid agar by stabbing. Motility of the isolated bacteria was recorded after incubation at 37°C for 24 hrs.The cultivated semisolid was kept at 4°C for further investigations (biochemical serological and sensitivity). Smears from pure colonies were stained with Gram's staining technique and examined microscopically identifying their morphology according to the staining reaction, shape, size, sporulation, and arrangement. These colonies that revealed to be Gram negative medium size, non-capsulated

and non-sporulated were further examined biochemically, serology and sensitivity to 25 *E. coli* isolates, 12 *Salmonella* isolates from food samples against 11 chemotherapeutic agents.

Materials used for real- time PCR:

DNeasy Blood & Tissue Kit (Qiagen cat # 69504)

Brilliant II QPCR Master Mix (Agilent Catalog #600804)

Primers and probes:

(**Forward primer**) *5′ -CGTTCTGAACCTTTGGTAATAA- 3′* Sal-F

Sal-R (**Reverse primer**) *5′ -CGTTCGGGCAATTCGTTA- 3′*

Probe) *FAM - GGCGGTGGGTTTTGTTGTCTTCT-TAMRA*(Sal-TM

Primers and probe in case of *E.coli*

(Forward primer) *5′ -CAATGGTGATGTCAGCGTT- 3′*

(Reverse primer) *5′ -ACACTCTGTCCGGCTTTTG- 3′*

(Probe) *FAM –TTGCAACTGGACAAGGCACCA-BBQ*

The PCR mix was performed as follow:

Brilliant II QPCR Master Mix……..…	12.5 µl
Sense primer……	0.1 µl
Antisense primer…….……	0.1 µl
Fame labeled probe…….……	0.2 µl
Diluted reference Rox dye……..……	0.185 µl
Template DNA……	5.00 µl

RESULTS

Occurrence of *E. coli* and *Salmonella* recovered from food samples:

Microbiological, biochemical, and serological characterization of 60 *E.coli* and 25 *Salmonella* isolates from food samples as showed in (Table 2, 3and 4) and Fig. (1).

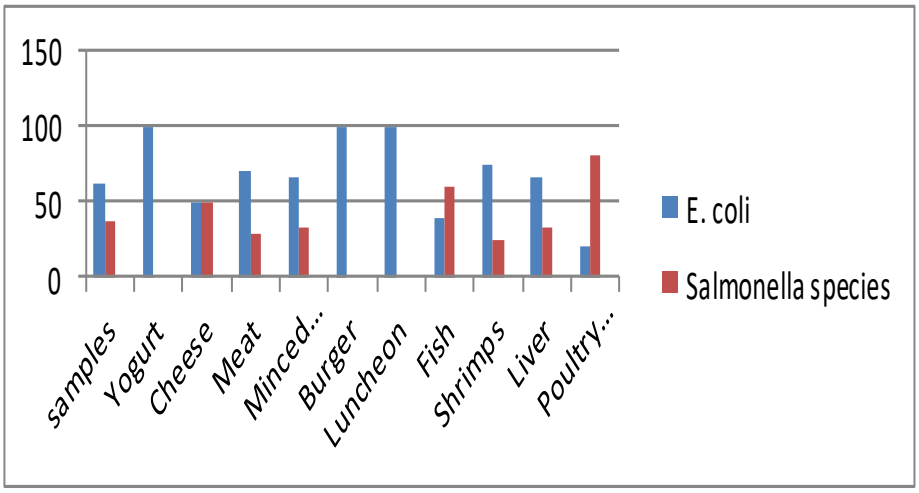


Fig. (1): Incidence of bacterial isolates in relation to the type of collected food samples.

Table (2): Prevalence rate of single bacterial isolates obtained from food samples.

Bacterial isolates	n=isolates	%
E. coli	60	24
Salmonella	25	10
Total	85	34

% was collected in relation to the total number of the examined samples

Table (3): Results of serogrouping of *E. coli* isolates recovered from food samples.

Serogroups	n= isolates	%
E. coli O_{157}	12	34.3
E. coli O_{126}	7	20
E. coli O_{44}	4	11.4
E. coli O_{125}	5	14.3
E. coli O_{164}	3	8.6
Untyped	4	11.4
Total	35	100

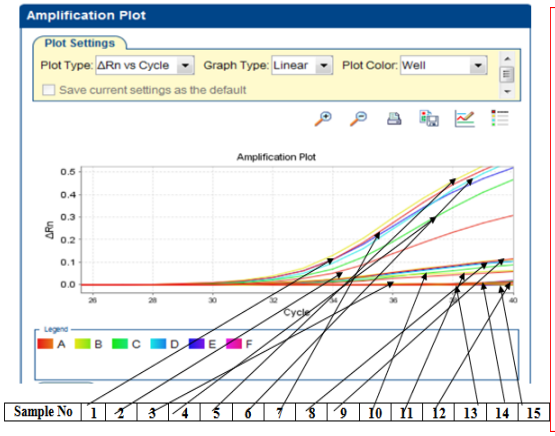
Table (4): Results of serological identification of *Salmonella* isolates from food samples.

Serotype	n=isolates	%
S. Kentucky	**9**	**29**
S. Enteritidis	**7**	**27**
S. typhimurium	**9**	**44**
Total	**25**	**100**

***In vitro* antibiotic resistance pattern:**

Results of *in-vitro* sensitivity testing of 25 *E. coli* isolates, 12 *Salmonella* isolates from food samples against 11 chemotherapeutic agents. The majority of *E. coli* isolates were highly resistant to amoxicillin (100%), lincomycin (92%), rifampicin (88%) and sulpha methaxzole trimethoprim (72%). on contrary, *E. coli* strains were highly sensitive to colistin-sulphate (72%), amoxicillin+clavulinic acid (60%), and (56%) for each of spectinomycin. *Salmonella* species were highly resistant to lincomycin, rifampicin, amoxicillin+clavulinic acid and spectinomycin with an incidence of (100%), (100%), (58.33%) and 50%, respectively. On the other hand, *salmonella* isolates were highly sensitive to colistinsulphate (100%), enrofloxacin (91.67%), and sulphamethaxzole+ trimethoprim (66%).

Result of Real-TimePCR:samples from (1-22) indicated positive to *E.coli* and *Salmonella*



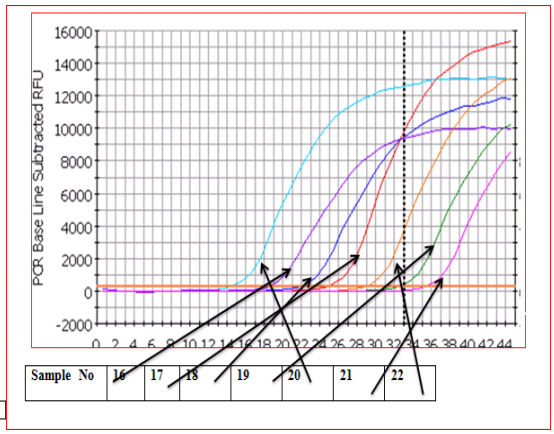


Photo (1) **photo (2)**

Real-Time PCR. All samples indicated positive to *E.coli* and *Salmonella.*

DISCUSSION

Public health agencies are concerned with food safety due to spreading of food markets and increasing numbers of fast food outside home all over the world **(Kennedy and wall, 2007**). The methods of handling, manufacturing of food products and sale are entirely depending on the traditional systems. Such systems could supply a favorable environment for bacterial contamination. **El-Mahmood *et al,(* 2007)**. Large numbers of microbes can get access to food of animal origin as *S. aureus, E. coli, Salmonella spp, Shigella spp, Y. enterocolitica, B. abortus, C. jejuni, B. cereus,* and *L. monocytogenes* **(Garbutt *et al*, 1997)**. *E. coli* and *Salmonella* were most common contaminates and they consider an important factor of gastrointestinal infection including food borne illness and food poisoning **(Somooro, 2003)**. In the present study results revealed that *E. coli* was isolated from all samples raw milk and milk products (cheese and yoghurt), minced meat and meat products (burger and luncheon,), fish and shrimps. The result showed in (Table 1), (2) revealed *E. coli* with a percentage of (32%), (20) and (33.3%) from (raw milk, milk products) and (meat, meat products). **Fatin (2004)** isolated *E. coli* from beef burger (50%), but low in luncheon (13%) than our results. **Hassanien (2007)** in case of *E. coli* isolated from beef burger (15%) but low incidence in case of luncheon (2.5%). **Ali *et al,* (2010)** isolated (35%) *E.coli* from meat sample. *E. coli* was founded from meat with percentage of (20%) by **Petternel *et al,* (2014). *Gousia et al.,* (2011)** isolated 157 *E. coli* from 428 meat products (36.6%). **Abdalslam *et al.,* (2014)** identified *E.coli* from meat products with percentage of (50%). Our results are similar to **Al-Zogibi *et al.,* (2015)** results who recovered (40%) of *E.coli* from raw meat samples. In our work the incidence of *E. coli* isolated from milk and milk products (yoghurt and cheese) with a percentage of (32%) (Raw milk, yoghurt and chess with a percentage of 33.3 %, 20 % respectively agreement with **Gardew *et al.,* (2012)** who isolated *E. coli* (29.6%) from raw milk. **Al-Zogibi *et al.,* (2015)** founded *E. coli* in raw milk (15.93%) and from market milk with a percentage of (36.66%). As in **Mohammed E.E.S.A. (2014)** *Salmonella* strains was isolated from examined samples from processed meat products (Kabab and Kofta), operating surfaces, workers hands and tools all the confirmed *Salmonella* results of serogrouping of 35 *E.coli* isolates were illustrated in (Table 4) revealed that 6 O-serogroups were obtained. The serovar O_{157} was the most prevalent with a rate of 34.3% followed by serogroups O_{126} (20%) and O_{125} (14.3%). Also, the serogroups O_{44}, and O_{164} were identified at rates of 11.4%, and 8.6%, respectively, while 11.4% of *E. coli* isolates were untyped with the available

antisera. Affections. results were nearly similar to that obtained by **Araujo *et al.* (2002)** who found that 97% of soft cheese samples from Brazil contained *E.coli* of the same sero groups O_{44} $O_{125,}$ while **Murinda.(2002)** showed that 80 % of *E. coli* isolate from meat and meat by product were enteropathogenic (O_{157}, O_{164}, $O_{12,}$ O_{44}). The results of serotyping of *Salmonella* isolates recovered from food samples were shown in (Table 4) which revealed that 37 *Salmonella* isolates were obtained, all the isolates were serotyped as 11 *S.* Kentucky (29%), 10 *S. Enteritidis* (27%) and 16 *S.* Typhimurium (44%). The obtained results run parallel to that obtained by **Abouzeed *et al.* (2000)** who isolated *S.* Typhimurium with an incidence 35.6%. **Hegazi (2002)** detected *S.*Enteritidis and *S.* Kentucky at rates of 62.16%, and 5.41%, respectively. Results of *in-vitro* sensitivity testing of 25 *E. coli* isolates, 12 *Salmonella* isolates from food samples against 11 chemotherapeutic agents were summarized that, the majority of *E. coli* isolates were highly resistant to amoxicillin (100%), lincomycin (92%), rifampicin (88%) and sulphamethaxzoletrimethoprim (72%). on contrary, *E.coli* strains were highly sensitive to colistin-sulphate (72%), amoxicillin+clavulinic acid (60%), and (56%) for each of spectinomycin. The obtained results nearly similar to that obtained by **Ramaswamy *et al.* (1982);** found that all *E. coli* isolates were highly resistant to rifampicin, erythromycin, kanamycin and chloramphenicol. These results disagreed with the findings of **Akond *et al.* (2009)** who reported that none of *E. coli* strains showed resistance to gentamicin and enrofloxacin. These results are similar to **Gurra *et al.* (2000)** who studied the resistance profiles of 333 *Salmonella* strains against 15 antimicrobial agents and reported that,the most frequent degrees of resistance were to sulfadiazine, tetracycline, streptomycin, spectinomycin, ampicillin, and chloramphenicol (ranging from 46 to 22%); 13% were resistant to these six drugs and **Kruy *et al.* (2010)** who found that high multiple antimicrobial resistance profiles were observed for *Salmonella* Corvallis, *Salmonella* Enteritidis, *Salmonella* Anatum, *Salmonella* Typhimurium and other *Salmonella* serovars to amoxicillin (8.3-90%), sulfonamide (8.5-75%) and tetracycline (15.4-90%). Only *Salmonella* Stanley was resistant to tetracycline in a moderate rate (9%). On the other hand, *Salmonella* strains were highly sensitive to colistin sulphate (100%), enrofloxacin (91.67%), 75% for each sulphamethaxzole+ trimethoprim and fosfomycin. These results are partially similar in agreement with that of **Lestari *et al.* (2009)** reported that all *Salmonella* isolates were susceptible to amikacin, ceftriaxone, and ciprofloxacin; however, decreased susceptibility to quinolones (7.1%) or extended-spectrum cephalosporins (45.2%) was observed; and **Kuang**

***et al.* (2015)** investigated the susceptibility of serotypes of *Salmonella* samples isolated from chickens. In antimicrobial susceptibility tests, 41.14% of *Salmonella* spp. was susceptible to all antimicrobial agents, 48.14% were resistant to at least one, and 34.72% were resistant to more than three classes. Strains were highly resistant to sulfamethoxazole-trimethoprim (39.61%), nalidixic acid (39.17%), doxycycline (28.22%), and tetracycline (27.58%). Resistance to cephalosporins and fluoroquinolones ranged from 5.25 to 7.44% and 19.04 to 24.51%, respectively. Among penicillin-resistant and cephalosporin-resistant strains, 25 isolates produced extended-spectrum β-lactamases (ESBLs).

Real-Time PCR was applied on 22 samples of different food stuff for direct detection of food borne pathogens (*E.coli* and *Salmonella*). As illustrated in photo (1&2) positive results were indicated by obtaining the *Salmonella* and *E.coli*-specific PCR product using the specific primers, where DNA was detected in all inoculated samples 12 *Salmonella* and 10 *E.coli* in case of Real-Time PCR**.**

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