Basrah Journal of Veterinary Research,Vol.15, No.3,2016
Proceeding of 5th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

ISOLATION OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI;* THE IMPORTANT FOOD BORNE PATHOGENS FROM SEVERAL RESTAURANTS IN BASRAH CITY, IRAQ.

Nidham M. Jamalludeen*

* Department of Microbiology,College of Medicine ,University of Basrah
Basrah, Iraq

Key words: Restaurant, *Staphylococci*, *E. coli*, Basrah

ABSTRACT

The goal of this research was to isolate, identify and characterize *Staphylococcus aureus*, *Escherichia coli* and some other foodborne pathogens from randomly public distributed restaurants in Basrah city, Iraq. Of the 134 bacterial isolates from restaurants samples, 36 were confirmed as *S. aureus* and out of 141 isolates, 72 were confirmed as *E. coli* using different selective and enrichment bacteriological media. Data results of other microorganisms have been excluded from this study. However, result from this work indicates that preventive and disinfectant plans should be considered to ensure contamination free restaurants for the better health of all consumers.

INTRODUCTION

Bacterial food borne infections occur when food that is contaminated with bacteria is eaten and the bacteria continues to grow in the intestines, setting up an infection which causes illness. *Salmonella, Campylobacter,* hemorrhagic *E. coli, Staphylococci* and *Listeria* all cause infections (1). Moreover, Contamination of food at restaurants, with pathogenic bacteria is mostly due to processing, handling, and unhygienic conditions. For example, microorganisms may spread to food by hands that are not washed after using the toilet. They also may spread to

raw meat during processing so that it is contaminated when brought into the kitchen. Because of this, it is important to make sure hands and working surfaces are thoroughly washed after contact with raw meat, fish and poultry and before working with foods that require no further cooking (1, 2).

The bacterium of *Staphylococci*, *Escherichia*, *Candida* and other food borne pathogens are wide spread in nature and have been isolated separately from an enormous range of environmental sources such as plant surfaces, meat, poultry, air, water, soil, and dairy products (3). These bacteria are able to cause mild to life threatening diseases, which also includes food borne illnesses. Multiple species in these microorganisms are having capability to produce diseases with contaminated food. Of these, *Staphylococcus aureus* is reported to be a third most important cause of foodborne diseases in the world among the foodborne pathogens (4, 5). *E. coli* frequently contaminates food organism and it is a good indicator of fecal pollution (6, 7, 8). Existence of *E. coli* in food indicates the presence of enteric pathogens, which constitute a public health hazard. Enteropathogenic *E. coli* can cause severe diarrhoea and vomiting (9). There is huge evidence that food handlers whose work involves touching unwrapped foods to be consumed raw or without further cooking or other forms of treatment are those most commonly implicated in foodborne outbreaks. The unhygienic handling of such foods constitutes a particularly grave risk (1, 10). The risk of foodborne illness has clearly noticeable over the last 20 years, with nearly a quarter of the population at higher risk for illness today. Thus, preventing illness and death associated with foodborne pathogens remains a major public health challenge. Therefore, this study was aimed to isolate and identify some pathogenic microorganism that capable to contaminate and transfer through food processing chain from several restaurants in Basrah city, Iraq.

MATERIALS AND METHODS

Collection of Samples:

Swab samples from food processing surfaces, food handlers, and portion of salads were collected during the period of two months from different restaurants at the Basrah region, Iraq. The collected samples were examined for the existing of several pathogenic microorganisms that can be transmitted through food consumption. Samples were collected aseptically, transferred to sterile plastic bags and then were directly transported to the laboratory under cold conditions. Samples were processed within 1 hour at the microbiology laboratory of Basrah Medical College.

Microbiological analysis:

The swabs and a portion of salad sample after (homogenized with 100 ml sterile normal saline) were cultured into Blood Agar (BA), Mannitol Salt Agar (MSA), MacConkey Agar (MA), and Eosin Methylen Blue agar (EMB) (Oxoid, UK). Cultured media were incubated at 37 ^{O}C for 24 hours, further investigation were done using biochemical tests. Bacteria from suspected colonies were also stained with Gram stain for the primary identification; and a technique mentioned by (11) was used for isolation and identification of *Staphylococcus aureus*. Sample was streaked on MSA and BA and the plates were incubated at 37 ^{O}C for 24 hours. Appearance of golden yellow colonies on MSA and hemolytic colonies on BA were considered to be presumed *S. aureus*. *E. coli* was isolated by culturing samples into selective medium Eosin Methylene Blue (EMB) agar and MacConkey Agar and incubated at 37 ^{O}C for 24 hours. Morphologically typical colonies producing metallic sheen and lactose fermentor had been selected for further identification (11).

Biochemical examination:

Four colonies from pure culture of suspected bacteria were selected to be cultured and identified by the various biochemical tests. Confirmation of Genus, *Staphylococcus* was done by Gram staining and several biochemical tests including Catalase , Oxidase , Indole, Methyl red, Voges-Proskauer tests. In addition, Nitrate reduction, acid from different sugars, and haemolysis on blood agar were also used for identification following the method described by Forebs *et al*. (12). The suspected species of *S aureus* was also confirmed by Coagulase test as mentioned by Monica (13) (Table 1)

Table 1. Biochemical tests reaction for *S. aureus*

Biochemical test	Reaction
Catalase	+
Oxidase	-
Indole Production	-
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	+
Glucose	+
Mannitol	+
Maltose	+
Lactose	+
Raffinose	-
Sucrose	+
Haemolysis	+
Coagulase	+

On the other hand, Gram staining, Catalase , Indole, Methyl red, Voges- Proskauer tests, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests (Table 2) were used to confirm *E.coli* isolates

Table 2. Biochemical tests reaction for *E. coli*

Biochemical test	Reaction
Lactose fermentation	+
Catalase	+
Simmon's Citrate	-
Indole Production	+
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	-
Urease	-
Glucose	+
Mannitol	+
Lactose	+
Salicin	+
Sucrose	+

RESULTS

The research findings are related to isolate of *S. aureus* and *E. coli* from several samples taken from restaurants around Basrah city. The results were summarized in Table 3 which describes the sampling data that consists of various numbers of samples analyzed and confirmed as *S.aureus* and *E. coli*. Out of 134 isolates, only 36 isolates were confirmed as *S. aureus*; out of 141 isolates 72 were confirmed as *E. coli* on the basis of morphological and biochemical characterization (Table 1, 2). According to these results a high contamination with *E. coli* and *S. aureus* was found in samples collected from different restaurants distributed at the Basrah area.

Table 3. *S. aureus* and *E. coli* detected in examined samples

Organism	Number of sampling			Number of bacterial isolates taken			Number of positive samples		
	Food processing surfaces	Food handlers	Salads	Food processing surfaces	Food handlers	Salads	Food processing surfaces	Food handlers	Salads
S. aureus	33	20	33	61	27	46	18	8	10
E. coli	33	20	33	54	34	53	21	14	37

DISCUSSION

The presence of foodborne pathogens in food processing area or in restaurant equipments as well as food handler's arm, nose or skin are considered a big problem for the public health. Contaminated food plays an overwhelming role in transmission of infection. Results of this study indicate contamination of several restaurants with important pathogenic bacteria together with the normal flora which may lead to outbreak infection. *E. coli* and *S. aureus* were found to be the most prevalent species among the bacterial species identified in the samples. Their accurate identification is important and the pathogenicity of these species needs to be studied, as many species of these bacteria are known for their enterotoxigenic potential (14, 15). Coagulase positive *S. aureus* was previously isolated from nasal swabs of 10 apparently healthy humans (16). *S. aureus* is a among the most important nasocomial pathogens because of both the diversity and the severity of the infections. It causes superficial and deep skin and soft tissue infections, endocarditis and bacteraemia and a variety of toxin mediated diseases such as gastroenteritis, staphylococcus scalded-skin syndrome and toxic shock syndrome (17). On the other hand, *S. aureus* releases a toxin such as enterotoxin. As less than 1.0 μg of this toxin

present in contaminated food produces symptoms of illness. It has been found that this level of toxin can be released at 10^5 cells /g of food (18).The incidence of the species of *E. coli* itself in restaurants, as a possible cause of food borne disease, is not significant if *E. coli* is normally a ubiquitous organism (19), yet the pathogenic strains if present could be harmful to consumers. The results of the existing study indicate that strict preventive and standards disinfectant should be performed to ensure contamination free restaurants for the good health of all consumers. For this care is required from the point of food preparing to the point of consumption.

In conclusion, *S. aureus* and *E. coli* were found to be most prevalent species identified in the samples taken from different restaurants distributed randomly in the Basrah city. These organisms are very important foodborne pathogens and should be eliminated from food chain.

Acknowledgments

The author would like to thank Ms. Mareim Nabeel for her kindly assist in samples processing.

عزل جراثيم المكورات الذهبيه والاشريكيا القولونيه الممرضه التي تنتقل عن طريق الغذاء من عدة مطاعم منتشره في محافظة البصرة-العراق

نظام محمد جمال الدين

قسم الاحياء المجهريه ، كلية الطب،جامعة البصره ،البصره ،العراق

الخلاصه

تهدف هذه الدراسه الى عزل وتشخيص جراثيم المكورات الذهبيه والاشريكيا القولونيه الممرضه والتي عادة ما تنتقل عن طريق تناول الاغذيه الملوثه بها من عدة مطاعم عامه منتشره عشوائيا في مدينة البصره-العراق. فقد تم تاكيد عزل 36 عزله من جراثيم المكورات الذهبيه من مجموع 134 عزله جرثوميه ناميه على اوساط زرعيه غنيه، انتخابيه ومفرقه مستخدمه لعزل

هكذا جراثيم و72 عزله من جراثيم الاشريكيا القولونيه من مجموع 141 عزله ناميه على الاوساط الزرعيه.تم استبعاد نتائج عزلات لمجموعه من الكانات الدقيقه الاخرى من هذه الدراسه. تشير نتائج هذا العمل الى ضرورة اجراء واتباع خطط الوقايه الصحيه اللازمه واستخدام المعقمات الضروريه لاسطح اماكن تحضير وجبات الاغذيه من البدايه وحتى وصولها للمستهلك لغرض الحصول على منتج نهائي خالي من الكانات الممرضه للاستخدام الادمي.

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