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Isolation, identification and antimicrobial susceptibility profile of enteric bacteria from the hands of food handlers working at different restaurants in Peshawar, Pakistan

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Abstract

Being a source of major contamination, dirty hands can contaminate food, resulting in various food-borne illnesses caused by enteric bacteria**.** This study was designed to isolate enteric bacteria associated with the hands of food handlers working at various restaurants at Ring road, Peshawar, Pakistan. A structured questionnaire was used to collect data on the socio-demographic characteristics and personal hygiene status of the participants. A total of 50 samples were collected using sterile swabs, from the palm of food handlers working at 15 different restaurants. McConkey's agar media was inoculated using these swabs to allow only gram-negative bacteria to grow. The isolated bacteria were then identified and characterized according to Burgy's manual. Based on the results of gram staining and different biochemical tests, only two types of enteric bacteria were identified such as *Escherichia coli* (n=24, 48%) and *Klebsiella pneumonia* (n=20, 40%), while no growth was observed in 6 samples. Antibiotic susceptibility profile revealed that the highest number isolates were resistant to Ceftriaxone (30µg) (43% resistant), and Amoxicillin (30µg)(43% resistant), followed by Ciprofloxacin (5µg)(13% resistant) and Imepenem (10µg)(9% resistant) respectively, while all the isolate were found susceptible to Amikacin (30µg).It was concluded from the current study that worker hygiene status was lacking hygiene standards, as bacterial contamination was observed on the hands of most of the subjects. The poor hygiene status of food handlers could result in food-borne diseases in food consumers and therefore, it is important to adapt standard hygiene practices while handling food.

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Introduction

Enteric bacteria are the gut flora of humans and animals (Worku *et al.*, 2015). Different types of bacteria residing in the humans and animals guts are involved in various metabolic activities that protect the host from foreign pathogenic bacteria, save nutrients and energy and also help in the development of the immune system (Tortora and Funke, 2009). However, food-borne illness-causing enteric bacteria are a major threat worldwide especially in developing countries, resulting in high morbidity and mortality rates and also have a profound impact on socioeconomic conditions (Barrabeig *et al.*, 2010).

Hands are one of the most common sources of contamination since there are always some bacteria present on hands and food can become contaminated by dirty hands if food handlers do not adapt the basic hygienic processes during food handling (Anuradha and Dandekar, 2014). During food-borne diseases, the transmission of pathogenic bacteria to patients does not occur directly from contaminated surfaces, rather, it happens via dirty hands, which come in contact with contaminated surfaces and contaminate food (Assefa *et al.*, 2015).

Due to the frequent exposure of human hands to the environment, various pathogenic bacteria can therefore make their way to the mucous membranes in the eyes, nose and mouth via dirty hands, resulting in food-born infection outbreaks (Tan *et al.*, 2013).

Individuals who ensure food quality and safety throughout the production, processing, storage, preparation, and service processes are known as food handlers and foodborne outbreaks caused by food handlers are a prevalent and ongoing problem around the world (Thidarat *et al.*, 2011). Lack of standard personal hygiene is known to be the 3rd most widely recognized factor involved in causing food-borne infections, according to the centers for disease control and prevention (CDC) (Lillquist *et al.,* 2005). Between 1975 -1998, 42% of food-borne outbreaks were caused by food handlers (Sadiq and Abdullahi, 2008). According to a report published by foodborne disease burden epidemiology reference group, World health organization, 600 million infections and 420,000 deaths occurred worldwide in 2010, due to 31 different foodborne diseases Getie *et al.,* 2019).

In addition, more than 200 diseases have been identified that occur in humans due to the consumption of contaminated food (Getie *et al.,* 2019). There are different stages of food products such as food preparation, processing, and storage, and contamination can occur at any of these stages (Kirk *et al.,* 2015). Being a developing country, most of the people including food handlers in Pakistan live under the line of poverty and a lot of people do not get the opportunity to get an education. Therefore due to the lack of food safety knowledge, food handlers may contaminate the food which consequently results in various food-borne illnesses in people. Study regarding the of detection of *Enteric* bacteria associated with food handlers and surfaces of food manufacturing industry located in Hub city, Pakistan has been carried out before (Ali *et al.,* 2016), however, studies are limited in Peshawar, the capital of Pakistan province of Khyber Pakhtunkhwa (KPK).

Understating the hygienic practices amongst food handlers could be linked to the evaluation of the bacteria residing in the hands of food handlers since the information obtained could be used to quantitatively analyze the behavior of food handlers regarding food-related and personal hygiene (Assefa*et al.*, 2015) The current study was, therefore, an attempt to understand the bacterial hand contamination of food handling individuals working in various restaurants at Ring road, Peshawar, Pakistan. We also characterized the isolates and determined their antibiotic susceptibility profile.

Material and methods

Study area and population

The current study was conducted to evaluate the current personal hygienic status of food handlers working at various restaurants located on Ring road, Peshawar, KPK Pakistan.

Inclusion and exclusion criteria

Food handlers who were engaged in food preparation, serving, and Cleaning were included in this study. The front desk manager, clerical staff and food handlers who had any disease currently or in the previous two weeks were excluded.

Experimental place

The practical work was performed in the Microbiology laboratory, Institute of Biological Sciences, Sarhad University of Information Technology Peshawar.

Samples and data collection

A total of 50 samples was collected from the hands of food handlers working at 15 different restaurants, located on Ring road district Peshawar, Khyber Pakhtunkhwa, Pakistan, using sterile swabs. A questionnaire was used to obtain information related to gender, age, level of education, employment status, marital status and personal hygiene practices such as smoking, use of sanitizer and antibiotics, of the study subjects. Using sterile gloves, Restaurants chefs, dishwashers and waiters were sampled from each restaurant.

The samples were collected in duplicate or triplicate from each individual. Three to four samples were collected from different parts of the hands of each food handler for bacteriological analysis from each restaurant. Cotton swabs, moistened in normal saline, were used to collect samples from different parts of the palm. Cautions were taken during sample collection to prevent contamination of the swab, and the swabs were placed back into their container and sealed with adhesive tape and labeled. The samples were immediately transported to Microbiology Lab, Sarhad University Peshawar for further processing and culturing.

Sample processing

During culturing, swabs were inoculated on McConkey's agar medium and incubated at 37°C for 24 hrs. Growth of selected bacterial species was examined as lactose fermenter and lactose non-fermenter after 24 hrs. To isolate selected pure bacterial cultures, sub-culturing was done for all the isolates using Nutrient agar media. The purified cultures were preserved in 75%glycerol.

Identification of the Isolates

Identification of bacteria was made on the basis of morphology, colony characteristics, gram staining techniques and various biochemical tests such as motility, spore, catalase, indole, oxidase and triple sugar iron test according to Burgy's manual of determinative bacteriology (Holt *et al.*, 2013).

Antibiotic susceptibility profile of isolated strains

The antibiotic susceptibility profile of all the isolates was determined following – Bauer disc diffusion method (Bauer *et al.,* 1996). Prior to inoculation of the bacteria, all the plates were incubated for about 20 minutes to dry off the excessive moisture. Subsequently, each isolate was picked using a loop and was inoculated into normal saline. The mixture was compared to 0.5 McFarland standard and then swabbed on the surface of Mueller – Hinton agar. Each disc was aseptically placed on the surface of all the media plates. Antibiotic discs used were Ceftriaxone (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Amikacin (30µg) and Augmentin (30µg). All the plates were kept in an incubator at 35°C for 24 hours. The next day, the zone of inhibition formed around each disc was measured. The isolates were classified as resistant, intermediate, or susceptible in accordance with the interpretive standard of the clinical and laboratory standards institute (CLSI).

Results and discussion

Characterization of the Isolates

The results of the current study showed that 44(88%) samples were positive for bacterial growth, while 6(12%) samples were negative, as no growth was observed on nutrient media, which shows that 88% of the food handlers were contaminated. Such a high level of contamination could bedue to a lack of knowledge and poor personal hygiene of food handlers. The current day results agree with the findings of (Dahiru*et al.,* 2016).

Table 1. Results of the microscopic and biochemical analysis carried out for the identification of the *enteric* bacteria isolated from the hands of food handlers.

Biochemical Identification of *Enteric* Isolates		
Characteristics	*Escherichia coli*	*Klebsiellapneumonia*
Gram Staining	-ve	-ve
Shape	Rod	Rod
Motility	+ve	-ve
Spore	-ve	-ve
Catalase	+ve	+ve
Indole	+ve	+ve
Oxidase	-ve	-ve
Triple sugar iron	+ve	+ve

They also showed about 89% of food handlers were contaminated. Andargie*et al.,* 2008 reported that 100% of the study participant were contaminated. Our results however are not in line with the study of Assefa *et al.*, 2015, in which only 49.6% of study subjects were contaminated.

Table 2. Socio-demographic characteristics of the food handlers.

Included Variables			
Variables categories		Frequency	Percent (%)
Age	16-20 years	9	18
	21-25 years	15	30
	30-35 years	7	14
	36-40 year	4	8
	41-45 years	3	6
	45-50 years	1	2
	≥50 years	1	2
Education	Primary	6	12
	Middle	9	18
	High	11	22
	Higher Secondary	6	12
	Illiterate	18	36
Intoxicants	Smokers	12	24
	Snuff	28	56
	Other Intoxicants	4	8
Residence	Home	24	48
	Hostel	26	52
Marital Status	Married	15	30
	Unmarried	35	70
Use of Sanitizer	Used	15	30
	Not Used	35	70
Use of Antibiotics	Used	7	14
	Not Used	43	86
Duties of personnel's	Cook	21	42
	Waiters	17	34
	Dish Washers	7	14
	Food Cutting	1	2
	Helpers	2	4
	Cleaners	2	4
Salary	10000-15000	36	72
	16000-20000	10	20
	21000-25000	2	4
	26000-30000	1	2
	31000-35000	1	2

This difference might be due to socioeconomic condition, low level of education, lack of personal hygiene and low standard of food practices. Based on the results of biochemical tests, 24 (48%) isolates were identified as *E. coli* while 20 (40%) isolates were identified as *K. pneumonia*. The results of the gram staining and biochemical tests are given in Table 1. The development of various food-borne diseases can be attributed to ingestions of pathogenic microbes or biotoxins produced by various pathogens (Ali *et al.,* 2016). It is an established fact that most of the *E. coli strains* do not cause any diseases but some strains are well-known causative agents of different diseases like diarrhea, fever, vomiting and hemolytic uremic syndrome (Ma *et al.,* 2019). *K. pneumonaie* is not only a major hospital-acquired pathogen but an important food pathogen and has been frequently found in foods including raw vegetables, powdered infant formula, meat, fish, and street food and the and the number of foodborne illness outbreaks caused by *K. pneumoniae* has increased in recent years (Zhang *et al.,* 2018). *E. coli* and *K. pneumonia* has been isolated from the hands of food handlers before. Mouna *et al*., 2015 carried out a study to evaluate the hygiene status and etiology of food handlers.

Table 3. Antimicrobial Susceptibility Profile of *Enteric* Isolates.

Antimicrobial Susceptibility Profile of Enteric Isolates			
Antibiotics	Resistant Isolates	Intermediate Isolates	Susceptible Isolates
Amoxicillin	19 (43%)	7 (15%)	18 (40%)
Ceftriaxone	19 (43%)	8 (18%)	17 (38%)
Imipenem	4 (9%)	0 (0%)	40 (90%)
Ciprofloxacin	6 (13%)	0 (0%)	38 (86%)
Amikacin	0 (0%)	0 (0%)	44 (100%)

They identified 12 different genera of bacteria including *K. pneumoniae* and *E. coli*. Another study reported the isolation of *E. coli* and *Salmonella typhi* from the hands of food handlers in Africa (Lambrechts *et al.,* 2014*)*.

Socio-demographic characteristics of the subjects

A total of 50 subjects were involved in this study and the socio-demographic characteristics of all participants have been shown in Table 2. All the subjects in this study were male with minimum and maximum ages of 16 and 56 respectively. Most of the participant were illiterate (36%), who have never attended school, while the education background of other participants was found as primay (12%), middle (18%), high (22%), higher secondary (12%).

The marital status was observed as follow; 70% were unmarried and 30% were married individuals. In addition, the highest number of participants were cook (42%), followed by waiters (34%), dish washers (14%), food cutters (2%), Helpers (4%) and cleaners (4%). Regarding their income (salary), most of the participants (72%) were earning between 10000-15000 Pakistani rupees/month (PKR/Mo), 20% were earning between 16000-20000 PKR/Mo, 4% were earning, 21000-25000 PKR/Mo, 2% were working on the salary between 26000-30000 PKR/Mo while the remaining 2% were making the highest salary i.e, between 31000-35000 PKR/Mo.

Antibiotics susceptibility profile of isolated strains

The antibiotic susceptibility profile of the isolates was determined according to the method of Kirby Bauer disk diffusion method.

The result interpretation was carried out according to standard CLSI and the isolates were divided as resistant, intermediate and susceptible (Table 3). Amikacin was the most effective antibiotic tested, as none of the isolates was resistant to it, followed by Imipenem (9% resistant), Ciprofloxacin (13% resistant), Ceftriaxone (43% resistant) and Amoxicillin (43% resistant) (Fig. 1).

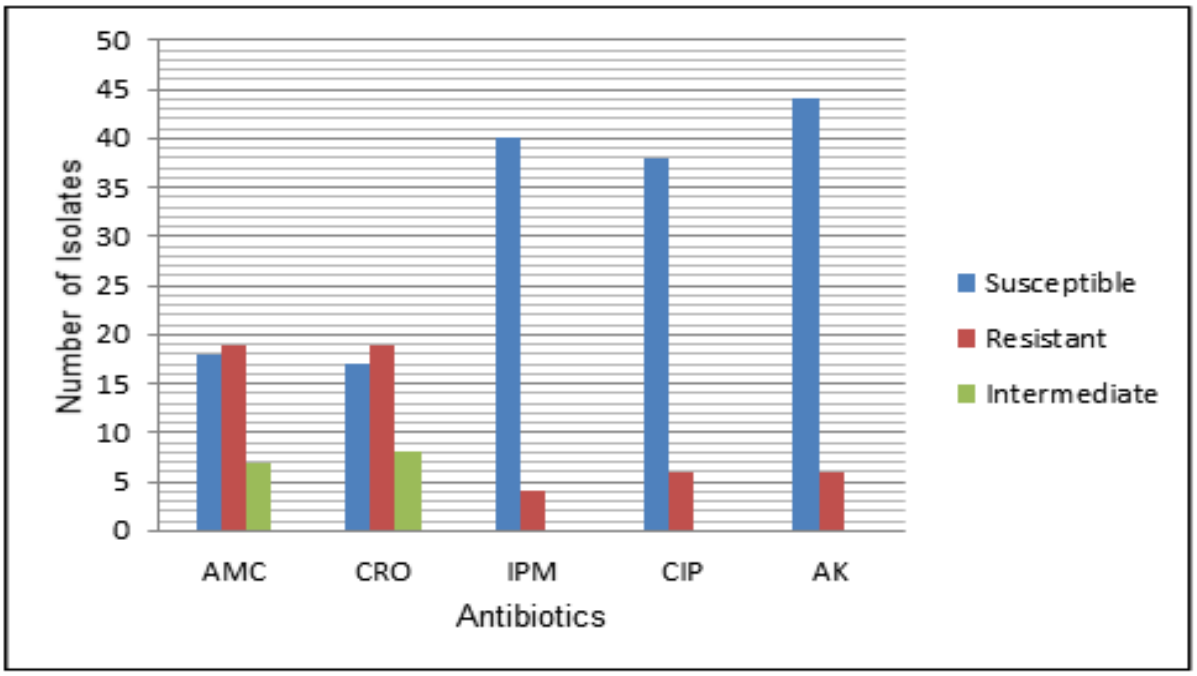

Fig. 1. The antimicrobial microbiology profile of *enteric* isolates.

Conclusion

This study reveals that bacterial contamination among food handlers of these various restaurants was very high 88% of food handlers were found contaminated. Two types of enteric bacteria were identified as *E. coli* with the occurrence of 48% and *K. pneumonia* with the occurrence of 40%. Lack of food safety knowledge, lack of personal hygiene, snuff, and other intoxicants are some of the factors that might contribute food to food contamination. Therefore, preventing the transmission of various pathogenic bacteria from food handlers to food consumers via contaminated food is the need of the hour. The current study, therefore, recommends that, since the contamination level was quite high, standard hygienic practices should be adapted by providing necessary training and education to food handlers.

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