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Prevalance And Antibiotic Resistance of Pathogens in Ready to Eat Street Veneded Meat Products in Lahore City

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ABSTRACT

In this modern era, majority of the people are busy in their work and do not have enough time to prepare their own food, so they turn towards Ready to Eat food. Meat products are amongst the most commonly consumed RTE foodstuffs. The lack of food handling and hygiene practices among food handlers can lead to contamination or improper cooking of food resulting in foodborne illnesses. Lahore is the second largest city of Pakistan with numerous street vendors of RTE meat products including barbecued items. These barbecued items are often undercooked which can result in incomplete elimination of pathogens. *E. coli* and *Salmonella* spp. are one of the most common bacteria which are present in uncooked or undercooked meat and if consumed can lead to various health hazards. Additionally, frequent exposure of livestock to antibiotics for either therapeutic or non-therapeutic reasons has led to resistance in the bacteria which can be a major problem for humans. In this study food handlers were surveyed regarding their knowledge of food safety and hygiene practices. Afterwards, samples of RTE Seekh kebabs were collected from street vendors in different areas of Lahore and were assessed for prevalence of bacterial pathogens using various media and presumptive identification through biochemical tests. After assessment, the detected pathogens were tested for resistance against different antibiotics on the MH Agar through Kirby-Bauer method. The response of the food handlers to survey showed a positive response of less than 50% of individuals in majority of the questions. Further, the results revealed that out of thirty samples, three showed the presence of *E. coli* and no sample showed the presence of *Salmonella* spp. Furthermore, biochemical testing also revealed the presence of *Shigella dysentriae* and *Citrobacter freundii* in some samples. All the *E. coli* strains showed resistance towards vancomycin and amikacin. Whereas, *S. dysentriae* showed resistance to vancomycin and intermediate resistance to ampicillin. *C. freundii* was found to be susceptible towards majority of the antibiotics and intermediately resistant against chloramphenicol. Overall, the contamination of samples through improper cooking or handling can result in health hazards which calls for proper trainings of food handlers and also of farmers regarding prudent use of antibiotics to cope with the problem of antibiotic resistance.

1. INTRODUCTION

Animal food derivatives such as eggs, milk and meat are one of the best sources of proteins as they help in the development of the body by repairing, maintaining and growing the muscles (Abass *et al*., 2020). Although meat consists of more vitamins, minerals and proteins than any other source but they are high in water activity which is approx. 0.99 so they offer an optimum ground for bacteria to grow. One of the most active food contamination points in Pakistan are bus stations and railway stations where many local vendors are available and due to improper handling there is a lot of contamination of food which is being sold there (Alimi, 2016). Lahore is the second most populous city of Pakistan with the population of 11.13 million which only next to Karachi's (Shirazi & Kazmi, 2014). With this much population and increasing trend towards ready to eat products, majority of the people prefer to eat from the street vendors as it is low in cost and people like to eat on the go to save their time. Barbeque is a slow cooking process in which meat is mixed with various herbs and spices and then put on a skew followed by cooking them on charcoal, this slow cooking for a longer duration produces smoke which acts as a dehydration agent for the surface while keeping the inside moist and juicy. Due to large number of consumers, majority of the people cooking the food work haphazardly which can lead to the food being prepared remain undercooked at various places, such cases are relatively high if we talk about barbequed items like seekh kebabs etc. which when consumed can lead to foodborne infections (Sohaib & Jamil, 2017). Keeping in view the extensive consumption of ready to eat meat products and the imprudent use of antibiotics in Pakistan, the objectives of this study were to assess the prevalence of various food borne pathogens in ready to eat meat products being sold by the street vendors in the city of Lahore. Additionally, the resistance of the prevalent pathogens towards various antibiotics was also assessed during the study.

3. MATERIALS & METHODS

3.1 Assessment of Food Safety Awareness of Food Handlers:

The awareness of food handlers regarding food safety was assessed through a questionnaire based survey filled by each vendor. The questionnaire was designed to check the knowledge of hygiene and handling practices of food handlers. The survey, in addition to responses from food handlers, also recorded the observations made by the surveyor (Ma *et al*., 2019)

3.2 Sample Collection:

For assessment of pathogen prevalence, 30 different samples of seekh kabab were taken from the local vendors in Lahore which were selling RTE meat products. The samples were collected in a zip lock bag which had previously been sterilized using UV chamber (UVITEC Cambridge, UK) for 15 minutes at 312 nm. The collected samples kept in a refrigerator overnight at 4°C until further processing at the subsequent day.

3.3 Preparation of Sample:

The work was done aseptically in a biosafety cabinet to prevent any contamination and all the materials including reagent bottles, petri plates and test tubes were sterilized through autoclave prior to use. The sample was prepared according to the method described by (Adzitey, Assoah-peprah, et al., 2020) with few modifications. The seekh kebab sample weighing 25 grams in the zip lock bag was homogenized manually and then transferred in 225 ml of sterilized buffered peptone water (BPW) followed by incubation in shaker for 2 hours at 37°C.

The BPW used during analysis was prepared and dissolved in 1 liter of distilled water with the final pH of 7.0±0.2. The BPW was then autoclaved at 121°C for 15 minutes at 1 atm.

3.4 Total Plate Count:

The total plate count of samples of evaluated on nutrient agar (CM003 Oxoid, England). The nutrient agar was prepared by dissolving 28g of media in one liter of distilled water followed by autoclaving at 121°C for 15 minutes at 1 atm. The sample (100µl) from each tube was spread on nutrient agar plates using glass spreader. The plates were covered, wrapped with parafilm and incubated at 37°C for 24 hours in an inverted position. Then, bacterial colonies were counted and the plate showing colonies between 30-300 was selected for final results (Zainuri *et al*., 2020).

3.5 Detection of E. coli:

The detection of *E. coli* was carried out using MacConkey agar (100205 Merck, Germany) and EMB agar (CM0069 Oxoid, England). 1ml of the sample was taken and transferred to 10 ml of EC Broth (*E. col*i Coliform broth) for enrichment purpose and incubated further for 24 hours at 37°C. After incubation, using a sterilize wire loop, two loops full of EC broth were taken and streaked onto the MacConkey Agar. The plates were observed for *E. coli* which gives pink colonies on MacConkey (Ema, 2022). A single pink colony was subcultured on to Levine's Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours for the confirmation of *E. coli* by observing colonies with Green metallic sheen (Qamar *et al*., 2018)

3.5.1 Identification of E. coli:

After 24 hours the colonies which gave green metallic sheen were subjected to biochemical tests which included gram staining, KOH test, VP test and Indole test using QTS (24) strips (DESTO, Pakistan).

Figure 3.1 growth of *E. coli* on MacConkey Agar.

3.6 Detection of Salmonella spp:

The detection of *Salmonella* spp. was done according to standard method described by the Chinese National Standard Method (GB 6789.4-2010) with some modifications. The prepared sample was taken after 24 hours and 1ml of the sample was transferred to 10 ml of Selenite Cystine (SC) Broth (Oxoid, England) (Wu & Haubert, 2021). Afterwards, two full wire loops of SC Broth were streaked on Xylose Lysine Desoxycholate (XLD) Agar and Bismuth Sulphite (BS) Agar. After 24 hours black colonies on BSA and black colonies with red surroundings on XLD were assumed to be *Salmonella* spp (Ni *et al*., 2017). For further verification, these colonies were picked and streaked on a *Salmonella-Shigella* (SS) Agar (CM0099 Oxoid,England) (Gunasegaran *et al*., 2011).

3.6.1 Identification of Salmonella spp:

The identification of *Salmonella* spp was done using various biochemical tests including urease test, Triple Sugar Iron Test, Indole Test, VP Test and Hydrogen Sulfide Test through QTS 24 Strips (DESTO, Pakistan). The confirmed *Salmonella* were then used in the antimicrobial susceptibility test (Cai *et al*., 2016).

3.7 Other Pathogens

Some bacterial pathogens were not found to fall in the category of *E. coli* or *Salmonella* as they were found to differ morphologically from said organisms. These organisms were further tested biochemically using QTS strip test for presumptive identification as per instructions from the manufacturer.

3.8 Antimicrobial Susceptibility Test of E. coli:

For antimicrobial Susceptibility test, Kirby-Bauer method was used on the Mueller Hinton (MH) Agar (103872, Merck, Germany). The method was carried out according to Committee for Clinical Laboratory Standard (CLSI, 2015). The bacterial strains identified through biochemical testing were transferred to test tube containing 5mls of nutrient broth (Biosciences, Singapore) and incubated at 37°C for 18 hours. Afterwards, the bacterial suspension was adjusted to 0.5 Mcfarland turbidity standards. Antimicrobial discs with known concentrations (chloramphenicol 30µg, streptomycin 10µg, ampicillin 10µg, vancomycin 30µg, ceftriaxone 30µg, tetracycline 10µg, amikacin 30µg) were placed carefully on the MHA at each quadrant. The plates were then incubated for 18-24 hours at 37°C and zone of inhibition was measured in millimeters using a standard scale. The bacterial strains were classified either, resistant, intermediate or susceptible by comparing them to the published interpretive chart by National Committee for Laboratory Standards (Kapena *et al*., 2020).

4. RESULTS

4.1 Food Safety Awareness Status of Street Vendors:

During the survey, majority of the food handlers responded positively towards there adherence to hygienic practices, however the data observed by the surveyor was in contrast. During the survey, 100 % of the food handlers responded positively towards the question about hand washing. However, the surveyor observed that only 27% of the food handlers were washing hands. Majority of the food handlers were not able to respond positively regarding their knowledge about procurement, storage and disposal of food.

4.2 Total Plate Count:

It was observed that 40% of the samples had TPC in range of 10^6 cfu/g and 20% of the samples had TPC in range of 10^7 cfu/g. These amounts of bacterial concentration in ready to eat meat products are considered unsatisfactory for consumption. Majority of the samples exceeded the satisfactory level recommend for total plate count by Standards, thus making the food unsafe for the consumer to some extent.

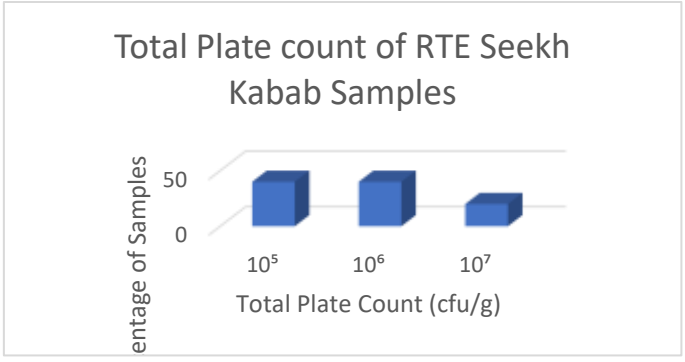

Figure 4.2 Total Plate Count of RTE Seekh Kebab Samples.

4.3 Prevalence of various Foodborne pathogens in RTE Meat product:

Out of the 30 samples only 3 showed the presence of *E. coli* (10%) and no sample showed the presence of *Salmonella* spp (0%). Although 2 samples showed the presence of *Citrobacter fruendii* (6.66%) and 1 sample showed the presence of *Shigella* (3.33%) as shown in figure 4.3.

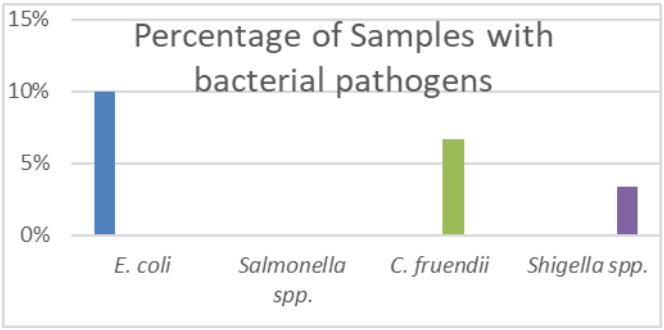

Figure 4.3 Percentage of samples detected with various food borne pathogens.

The prevalence of antibiotic resistance was found to be the highest for *E. coli* which was observed to be resistant against against Vancomycin (VA 30) and Amikacin (AK 30). Furthermore, *S. dysentriae* was found to be resistant against Vancomycin (VA30). Whereas, *C. freundii* was susceptible towards majority of the antibiotic discs used. The table 4.2 shows the Antibiotic resistance pattern found in the analyzed samples of RTE meat product (seekh kabab).

Table 4.2 Antibiotic resistance pattern found in RTE meat products.

Sample	S 10	C 30	T 10	VA 30	AM 10	CRO 30	AK 30	CIP 5
5	S	S	I	**R**	I	S	**R**	S
7	S	S	I	**R**	I	S	**R**	S
10	S	I	S	S	S	S	S	S
16	I	S	S	**R**	I	S	S	S
17	S	S	I	**R**	I	S	I	S
27	S	I	S	S	S	S	S	S
AK 30= Amikacin 30µg, C 30= Chloramphenicol 30µg, T10= Tetracycline 30µg, AM 10= Ampicillin 10µg, S 10= Streptomycin 10µg, VA 30= Vancomycin 30µg, CIP 5= Ceprofloxacin 5µg, CRO 30= Ceftriaxone 30µg.								
R= Resistance, S= Susceptible, I= Intermediate.								

5. DISCUSSION

Meat is one the best sources of protein known to human (Idowu *et al*., 2021). Many societies around the globe use it in various cuisines and dishes because of its high nutritional content, it is cooked by various methods before being served like, barbecuing, roasting, frying, steaming (Cömert & Gökmen, 2020). Out of all of these barbecuing is mostly preferred because it is slow cooking and is done over the flame directly or indirectly for a longer period of time giving a characteristic aroma and flavor to the product as compared to other techniques (Sobral *et al.*, 2018). Street vended barbecued meat has a risk to human health because it contains a very large number of micro-organisms including *Staphylococcus aureus, E. coli* and coliform *(*Dagalea *et al.*, 2021). In countries like Pakistan which are developing, barbecue is mostly sold by the street vendors which are lacking proper handling techniques which results in poor quality of food and safety of the food (Savvaidis *et al.*, 2022). Moreover, due to high demand, insufficient heating of the meat and uneven heat distribution are some of the factors which can cause food infections and contaminations. The reduction in the cooking time can lead to the improper cooking which can lead to microbial hazards and chemical hazards (Ologhobo *et al*., 2010).Safety of the RTE meat products highly depends on how the food is being prepared, cooked and its post processing hygiene practices. Majority of the meat is re-contaminated, especially at the post processing stage and can lead to outbreaks of various foodborne diseases (Ansari, 2015). In the post cooking areas the presence of pathogens serves as one of the important point for contamination of food along with the environment and equipment surfaces in the process of RTE meat (Syne *et al.*, 2013). Therefore, it is important to investigate such meat products which are which are being sold by street vendors without apparent adherence to food safety practices. This study was thus designed to find the prevalence of various bacterial pathogens in Ready to Eat (RTE) Street Vended Meat Product which were being sold by the Local Vendors of Lahore. The meat product selected for this study was seekh kabab.

The absence of *Salmonella* spp. in this research was found similar to that observed by Tavakoli & Riazipour (2008) who stated the absence of *Salmonella* spp. Contamination in grilled chicken. Similarly, another study by Fang *et al*. (2003), showed that they did not find any contamination with *Salomella*

spp. In ready to eat food products. According to (Murphy *et al*, 2004) the absence of *Salmonellae* in heat treated products can be due to thermal treatment of the meat during processing which can be effective for killing heat sensitive bacteria. In addition to heat treatment (Sha & Xiong, 2020) suggests that using spices, herbs like black pepper, garlic, mustard during the marination not only enhances the flavor, aroma and palatability but also plays a vital role in the killing bacteria due to their antimicrobial activity.

In case of *E. coli*, our study revealed the prevalence in 3 samples out of 30 samples collected which makes up 10% of the total sample collected. The results of this study are similar to the study done by Manguiat & Fang (2013), who found that 27 (12.5%) out of 216 samples of street vended meat based foods were contaminated by *E. coli*. This can be due to the surface contamination, inadequate cooking, post- cooking, cross contamination through utensils, spices and raw material introduced during processing (Sharaf & Sabra, 2012b). Fang *et al*. (2003) stated that the lengthy gaps between the preparation and food consumption and neglecting to cook at the essential temperature which is required to kill the microorganisms is one of the most important reasons for food contamination.

In a study done by Ahmed *et al*. (2023) out of 150 samples of duck meat, 25 (16.67%) samples were contaminated with *C. freundii*, which is higher than the number of samples contaminated with *C. freundii* in this study. After *Enterbactero cloacae*, *C. freundii* is the second most common pathogen which was isolated from food items which is transferred via fecal oral transportation as stated by Liu *et al*. (2017).

Additionally, our study reported one sample to have been contaminated with *Shigella dysentriae* out of 30 samples. A study conducted by Cetinkaya *et al*. (2008), stated that a total number of 416 samples were collected in order to detect the presence of *Shigella* and *Salmonella* which included RTE meat, salads, minced meat and raw milk but there was no *Shigella* detected in any of the samples. The reason for contamination of street vended RTE meat product with *Shigella*, can be the lack of running water at vending sites, unsanitary handling practices and dishwashing which is usually done in multiple dishes and buckets. Some food handlers even wash their hands after returning from the toilet with using any soap with same water (Ologhobo *et al*., 2010).

The antimicrobial resistance of food pathogens in this study showed that *E. coli* has a very high resistance to Vancomycin because all of the three isolated strains from the samples showed no zone of inhibition towards Vancomycin and Amikacin. A study done by Manguiat & Fang (2013) showed that *E. coli* isolated from chicken samples (5) showed resistance to tetracycline, chloramphenicol, ampicillin and sulphamethoxazole/ trimethroprim.

On the basis of the results of the current work, it is concluded that some of the street vended RTE meat product (seekh kabab) being sold by the local vendors in Lahore showed the prevalence of food borne pathogens which included a *E. coli* along with some cases of *C. freundii* and *Shigella dysentriae*. *E. coli* showed resistance to two different antibiotics Vancomycin and Amikacin while intermediate resistance towards Tetracycline. In order to control the spread of foodborne pathogens, it is very important for the street vendors to have knowledge about the food handling practices as many of the pathogenic bacteria are transferred to the cooked RTE meat products due to unsanitary hands which are used to put raw minced meat on the skewers and use the same hand to remove the cooked meat from the skewers. This study shows that the microbiological profile of some samples from street vendors in Lahore is questionable and requires strict action by the authorities and there is a strong need to educate the vendors which are dealing with the RTE meat products to maintain good hygiene practices, good handling practices and training of personnel in order to keep their food free from various food borne pathogens which can cause illness in consumers.

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