Original Article

Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan

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Abstract

Background: This study was conducted to examine the frequency of contamination in retail meat available in Karachi, Pakistan.
Methodology: Raw meat samples (250) and surface swabs (90) from meat processing equipment and the surrounding environment were analyzed for microbiological contamination.
Results: Out of 340 samples, 84% were found to be contaminated with bacterial species, including *Klebsiella, Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis.* A total of 550 (66%) of the bacterial isolates were potential pathogens. Of these, 342 and 208 isolates were from meat and environmental samples respectively. Food-borne pathogens isolated from meat samples included *Escherichia coli* O157:H7, *Listeria*, *Salmonella* Enteritidis and *Shigella* species whereas environmental samples yielded *Staphylococcus aureus* and *Shigella* species. Four strains of *Brucella* species were also isolated from meat samples**.** Total aerobic counts ranged between 10^8 –10^{10} CFU/g or cm^2. Resistance to a wide range of antibiotics was observed. Resistance rates to ampicillin, amoxicillin, novobiocin and cefaclor were from 62 to 75% in general. Thirty-three percent of *Salmonella* isolates were resistant to ampicillin. No quinolone resistance was observed. Biofilm formation was observed among 88 (16%) pathogenic bacteria including *E. coli, Klebsiella, Enterobacter* species and *Staphylococcus aureus*.
Conclusions: Food-borne pathogens found in retail shops could be sources for horizontal contamination of meat. Our data confirm the circulation of antibiotic resistant and biofilm forming pathogens in raw meat and its environment in retail shops in Pakistan, which could play a role in the spread of antimicrobial resistance amongst food-borne bacteria.

Key words: meat contamination, biofilm, antibiotic resistance

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Introduction

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs [1]. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors [2]. Contaminated raw meat is one of the main sources of food-borne illnesses [3,4]. The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs).

Karachi is a large city with a population of more than 15 million. Due to overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene, food-borne infections are on rise in the city [5]. Raw meat available in open-air local retail shops without appropriate temperature control is purchased by approximately 23% households [6]. Despite insufficient public health laboratories and inferior clinical diagnostic settings, a number of studies reporting outbreaks of infections somehow related with poor hygiene and consumption of contaminated food have been performed. In most cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections; however, a few community-based reports provide evidence of several outbreaks caused by *Salmonella*,

Shigella, *E. coli* and *Listeria* in Pakistan [7-9]. Moreover, antibiotic resistance levels are also elevated among food-borne pathogens such as *Salmonella* and *Shigella* [10,11]. It is not inevitable to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, but the presence of such bacteria in food items and their related environment could play a role in the spread of antimicrobial resistance amongst food-borne pathogens [12]. Therefore, to develop an effective strategy for reducing resistance burden in the community, such studies could provide useful information.

This study was conducted to investigate the microbial quality of raw meat available in common retail shops of Karachi and to determine the hygiene status of various environmental factors associated with meat shops and slaughter houses.

Material and methods

Samples

Thirty individual retail outlets located in Main Saddar Market, a district south of Karachi, Pakistan, were randomly selected for the study. A total of 340 meat and surface samples were collected. Out of the 340 samples, 250 were retail meat samples including meat (n = 145), lungs (n = 30), spleen (n = 30), and mucosal surfaces of intestinal tissue free from fecal material (n = 45). Samples were collected within 12 hours post-slaughter and during early afternoons, in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings. Approximately eight samples were collected from each outlet. Ninety environmental samples were collected comprising surface swabs taken from 15-20cm^2 of the surface of meat-cutting equipment such as knives, wooden boards, weigh scales and meat mincers and from the surrounding environment with a surface area of 30cm^2 as shown in Table 1. From two to five surface swabs were collected from each outlet. Collection was dependent on the size of the premises as well as on the cooperation of the shop owners**.** Butchers working in these outlets lack knowledge regarding the importance of disinfecting and sanitizing; consequently, they clean their shops once in 24 hours with detergent and water. No sanitizer medium was used before sampling. Environmental samples were taken using sterile swabs in 3 ml of peptone water and transported to the laboratory within one hour, of collection, and processed within two hours.

Twenty-five grams of collected meat and organ samples were weighed and transferred to sterile flasks containing 100 ml of phosphate buffer saline (PBS). Samples were homogenized using a meat grinder under aseptic conditions. Environmental swabs, kept in sterile glass tubes containing peptone water, were inoculated on blood agar plates by direct swabbing. To get a total viable count, samples were further diluted serially in PBS and appropriate dilutions were used to inoculate nutrient agar plates. Except where indicated all culture media and antibiotic disks were obtained from Oxoid (Hamshire, UK).

Microbiological analyses

Diluted meat samples were inoculated on nutrient agar by pour plate method for total viable count. Plates were incubated at 37^{o}C. For the isolation of Gram-negative bacteria, samples were cultured on Eosine Methylene Blue agar, MacConkey`s agar, and Sorbitol MacConkey's agar and incubated at 37^{o}C aerobically. For the detection of *Salmonella*, one gram of each sample was also inoculated in Selenite F broth (Difco, Michigan, USA) and incubated for 18 hours at 37^{o}C. Tubes were further sub-cultured on Xylose lysine deoxycholate medium and incubated for 18 hours at 37^{o}C. Sorbitol MacConkey's agar was especially used for initial screening of *E. coli* O157:H7. Colourless, non-sorbitol fermenting colonies were tested by serotyping. Sheep blood agar (5%), Mannitol salt agar (Merck, Darmstadt, Germany) and 6.5% NaCl Mueller Hinton agar were inoculated and incubated at 37^{o}C in a CO_2 enriched environment for the isolation and identification of Gram-positive organisms.

For isolation of *Brucella* species, approximately one gram of grounded meat samples were also inoculated in 50 ml of brain heart infusion (Merck, Darmstadt, Germany) and tripticase soy broth supplemented with 5 µg/ml of nalidixic acid, 25 IU/ml bacitracin, 100 µg/ml cyclohexamide and 5I U/ml of polymyxin B (Sigma, Hampshire, UK) and incubated at 37^{o}C under 5% CO_2 enriched atmosphere for one month. Broth cultures were sub-cultured on a weekly basis on 5% sheep blood agar [13].

To test for *Listeria*, nutrient agar plates were incubated for additional two weeks at 37^{o}C and inspected for characteristic colonies. Additionally, 10 ml of trypticase soy broth supplemented with 25 µg/ml of nalidixic acid and 105 µg/ml of polymyxin B was inoculated with one gram of meat and

Table 1. Aerobic mesophils counts on meat and environmental samples of retail shops in Karachi, Pakistan

Samples	Sample type	No. of samples (n)	Total viable count (log of CFU/g or cm^2)	Range of viable count (log of CFU/g or cm^2)	
				Maximum	Minimum
Retail Meat	Meat	145	10.2	10.5	5.2
	Lungs	30	6.66	6.8	3.5
	Spleen	30	6.2	6.9	2.2
	Intestinal tissue	45	8.7	9.2	8.1
Surface swabs from meat cutting equipments	Knives	25	10.2	10.7	7
	Weighing scales	7	9.2	9.6	8.9
	Wooden boards	20	8.5	10.1	5.2
	Meat mincer	4	7.5	7.2	7.6
Surface swabs from environment	Customer platforms	22	5.0	3.4	6
	Floors	4	8.6	7.2	9.8
	Walls	4	7.0	7	7.2
	12 inch long Steel meat anchors	4	8.2	8.5	5

g – gram, CFU - Colony

incubated at 37°C for two weeks. Sub-culturing was performed every three days on 5% sheep blood agar. Any Beta hemolytic colonies were subjected to serotyping using Listeria O poly antisera (Becton Dickinson, Oxford, UK).

Bacterial identification was conducted by standard biochemical methods [13]. For Gram-negative organisms, the identification battery included oxidase, citrate, urea hydrolysis, sulphide indole motility (SIM), and triple sugar iron (TSI). In some cases, API 20E strips (bioMerieux, Inc. Polska, Poland) were used for confirmation, whereas identification of Gram-positive organisms was based on Gram staining, catalase, tube coagulase, DNase and characteristic pigment production. Serotyping was performed for the identification of *Salmonella*, *Shigella* and *E. coli* O157:H7 using specific antisera (Becton Dickinson, Lahore, Pakistan. Antibiotic susceptibility pattern was determined by Kirby Bauer disc diffusion method [14] using a wide range of commonly used antibiotics including ampicillin (10µg), ofloxacin (5µg), cefaclor (30µg), amoxicillin (25µg), trimethoprim (5µg), doxycycline (30µg), cephalaxin (30µg), roxithromycin (30µg), novobiocin (5µg), streptomycin (10µg), tetracycline (30µg), lincomycin (10µg) and cefazolin (30µg). No control strains were used.

Biofilm formation assay

Isolated bacteria were processed to determine biofilm formation by crystal violet assay according to Shanks *et al.* [15] with modifications. Twenty-four-hour-old bacterial cultures with a final inocula of 1 x 10^6 in trypticase soya broth were dispensed into 96-well polystyrene plates and incubated at 37°C for 24 and 72 hours. Plates were washed with PBS three times, dried at 60°C and stained with crystal violet for one minute. Later the wells were filled with 10% glacial acetic acid and subjected to absorbance measurement at 450nm. Absorbance (A_{450}) more than 1 was considered as positive. Reference strain of *Pseudomonas aeruginosa* (ATCC 27853) was used as positive control.

Results

Meat and surface samples included in this study showed high viable bacterial counts as shown in table 1. Gram-negative bacteria such as *E. coli*, *Enterobacter* and *Klebsiella* predominantly constituted the total viable count, whereas frequently observed Gram-positive bacteria included *Bacillus subtilis*, *Micrococcus* species, and *Staphylococcus* species. In general, a total of 550 potential pathogenic bacterial isolates were obtained from 340 samples collected, out of which 342 were

isolated from meat samples and 208 from surface swabs. As shown in table 2, out of 342 bacterial pathogens isolated from meat samples, 120 (35%) were identified as *Escherichia coli* and 51 (15%) of these *E coli* isolates were characterized as serotype 0157;H7, which is known to cause hemorrhagic colitis. Other potentially pathogenic isolates were *Listeria* species 14 (4%), *Klebsiella* 27 (8%), *Enterobacter* species 51 (15%), and *Staphylococcus aureus* 24 (7%). Table 2 shows the detailed distribution of potential pathogens in meat samples and environmental surface swabs.

Table 2. Frequency of potential bacterial pathogens in samples

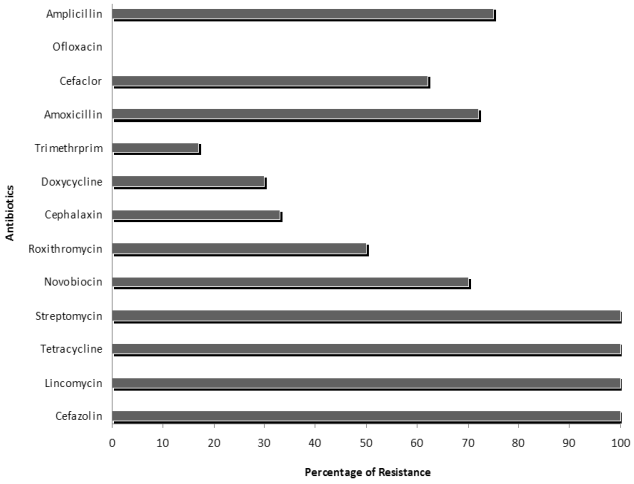
Microorganisms	Number of pathogenic isolates (n) from	
	Meat samples	Surface swabs
Escherichia coli	120(35)	50 (24)
Escherichia coli O157:H7	51 (15)	ND
Listeria	14 (4)	ND
Klebsiella	27 (8)	33 (16)
Enterobacter	51 (15)	50 (24)
Staphylococcus aureus	24 (7)	31 (15)
Salmonella Enteritidis	24 (7)	ND
Shigella	27 (8)	4 (2)
Brucella	4 (1)	ND
Citrobacter freundii	ND	17 (8)
Kurthia	ND	11 (5)
Sporosarcina	ND	12 (6)
Total	342	208

Numbers in parentheses represent percentages; ND – not detected

Antibiotics susceptibility profile showed the prevalence of cefazolin, lincomycin, streptomycin and tetracycline resistance against all potentially bacterial pathogens (Figure 1). Resistance against ampicillin, amoxicillin, novobiocin and cefaclor was observed 72%, 75%, 70% and 62% respectively. Fifty percent of the isolates were resistant against roxithromycin whereas 33% were resistant against cephalxin. No quinolone resistant pathogen was isolated. Methicillin and vancomycin resistance was not observed. Table 3 illustrates the rate of antibiotic resistance among commonly isolated bacteria.

The presence of antibiotic resistant pathogens in retail meat and its associated environment further stimulated interest to determine their biofilm formation ability. A total of 88 (16%) isolates were able to form biofilm. Biofilm formation was predominantly observed in enteric bacteria including

Figure 1. Antibiotic resistance profile of bacterial pathogens isolated from meat and environmental samples



E. coli (n = 35), *Klebsiella* species (n = 38), and *Enterobacter* species (n = 25). A few *Staphylococcus aureus* (n = 16) isolates were also able to form biofilm.

Discussion

Observations showed heavy bacteriological load carried by meat carcasses with total viable counts ranging from 10^6 –10^{10} CFU/g. The presence of a high number of viable bacteria, an indicator of he expected shelf life of meat, increases the chances of meat spoilage in a short time as described by the Agriculture and Consumer Protection Department; FAO (http://www.fao.org/DOCREP/004/T0279E/T0279E03.htm, last accessed April 01, 2010). Similar observations are also recorded from neighboring countries, namely India and Bangladesh [3,16].

The presence of bacteria in meat has been widely reported from different parts of the world [17,18]. Some groups recognized the presence of viable bacteria, especially Gram-negative organisms from 10^6 to 10^9, as an indication of open-air meat spoilage [19], while others argued this assertion and considered the presence of a high number of background organisms as a pathogen-reduction strategy due to the organisms' antagonistic effect against pathogenic bacteria and thus safer for meat quality. Therefore, it is considered that fresh meat that contains 10^5–10^6 of background organisms are inherently safer than those that contain less bioload; however, this hypothesis applies only to harmless bacteria [20]. In order to address the issue in the view of our local scenario, the organisms were identified. Results indicated the predominance of Gram-

Table 3. Distribution of antibiotic resistance among commonly isolated Gram-negative bacteria from meat and environmental samples

	***E. coli* (n = 170)**		***E. coli* O157:H7 (n = 51)**		***Klebsiella* (n = 60)**		***Enterobacter* (n = 101)**		***Salmonella* (n = 24)**	
Antibiotics	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Amplicillin	122	71	35	69	60	100	76	75	8	33
Amoxicillin	124	73	32	62	58	97	76	75	8	33
Cefaclor	102	60	26	51	50	83	55	55	5	21
Ofloxacin	0	0	0	0	0	0	0	0	0	0
Trimethoprim	25	15	10	20	10	17	20	20	4	17
Doxycycline	51	30	15	29	15	25	36	36	6	25
Cephalaxin	60	35	17	33	18	30	35	35	5	21
Roxithromycin	55	32	26	51	28	47	39	39	6	25

negative organisms such as *Salmonella*, *Shigella*, and *Escherichia coli* as reported by other groups [21]. These organisms are already involved in various infectious disease outbreaks in Karachi [22-24]. The presence of zoonotic bacteria such as *Brucella* and *Listeria* indicates poor ante-mortem inspection of the animals as well as unhygienic meat processing [25,26].

The frequency of potential pathogens in the surrounding environment and surfaces of retail shops was also examined. High viable counts and the presence of potential pathogens on meat-processing equipment, as well as on the walls and floors of retail shops, represent their environmental hygiene status. However, it is interesting to note that consumer platforms or counters of the shops were cleaner than the floors and walls with an average of 10^5 CFU per cm^2, which might be due to mopping of this area several times in a day. The presence of bacterial pathogens in meat-processing equipment and associated surfaces may contribute to the contamination of meat. Previously, it has been demonstrated that mincing meat with dirty equipment significantly increases the level of contamination in minced meats as compared to that in whole carcasses; furthermore, the process of mincing has the potential to introduce pathogens such as *Listeria monocytogenes* [27]. On the other hand, food-borne pathogens which are able to disseminate from contaminated meat to such surfaces [28] can spread infections in the community.

It is already known that bacteria form biofilm on hydrated surfaces [29]. Biofilm forming bacteria are usually resistant to a wide range of antibiotics [30,31]. To find the prevalence of drug resistance bacteria, assays for susceptibility profiles and biofilm formation were performed. Resistance of bacterial isolates to a battery of available antibiotics and the biofilm formation ability of these isolates was commonly observed. The problem may be attributed to a number of possible sources, including the natural resistance of species to certain antibiotics [31], possible transfer of antibiotic resistance among species, and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, which could also select for resistant strains [11]. However, no control strains were used for antibiotic susceptibility profiles, which can be considered as limitation of the study to reach valid conclusion.

This study presents the contamination status of retail meat and its surrounding environment as well as demonstrates the role of raw food as a reservoir of antibiotic resistance bacteria that can be transferred to humans, thereby constituting a health problem. The application of hygiene practices along the food chain and prudent use of antibiotics in animal husbandry are therefore essential to control further emergence of antibiotic resistance.

According to an FAO survey conducted in 1996, meat output in Pakistan is increasing day by day, in response to growing domestic demand (Meat and meat products, FAO, http://www.fao.org/docrep/004/w1690e/w1690e11.htm Last accessed April 02, 2010). Therefore it is important to ensure the practice of WHO basic hygiene principles, which cover food safety procedures from the farm of origin, to ante-mortem and post-mortem inspection, to handling until the food is consumed. The scientific community should join regulatory authorities to spread awareness about basic hygiene principles. It is especially important to

provide training to meat handlers regarding food safety.

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