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RESEARCH ARTICLE

Hygienic status of meat selling at road side shops in various areas of Karachi, Pakistan

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Abstract

The aim of the present study was to determine the hygienic status of red meat supplied to Karachi, and to observe the antibiotic resistance pattern of bacteria isolated from these samples. The meat samples were collected from open air local retail shops, situated in different areas of Karachi. A total of 50 isolates were selected as representatives of various samples. All the isolates were Gram-negative bacteria belonging to group of enteric bacteria, including Escherichia coli, Salmonella spp. and Klebsiella spp. The antibiotic resistance among these isolates were determined, these includes Amoxil, Cefizox, Gentamicin, Septran (sulphurmethaxole-trimethoprim) and Streptomycin. The mean resistance against these antibiotics was 5, 8.3, 0, 25 and 8.3%, respectively. The prevalence of resistance was the highest for Amoxil followed by Septran, Cefizox and Streptomycin, while all the isolates were sensitive to Gentamicin.

INTRODUCTION

Food borne illnesses are caused by ingestion of toxic or infectious agents (Brusa et al., 2013). Variety of food including meat, are identified as vehicles of illness. In developing countries prevalence of food borne illness is significantly high due to poor hygienic and sanitary conditions. According to world health organization (WHO), about 2.2 million people die annually due to food borne infections together with water born diarrheal diseases (Ahmed and Shimamoto 2013). Microbial food borne diseases affect approximately one third of the world population each year in developed countries (Andargie, et al., 2008). Meat is a perishable food containing wide nutritional composition, a suitable pH and sufficient amount of water that favours the growth of most microorganisms (Oliveira et al., 2013). Consumption of contaminated meat is one of the main sources of food borne illness (Ali et al., 2010). Safety hazards and challenges of meat hygiene have also been increased with increased consumption of meat worldwide (Iyer et al., 2013). Therefore, ensuring the practice of WHO basic hygiene principles, covering the food safety procedures from the farm of origin to ante-mortem and post-mortem inspection, to handling, is necessary until the consumption of food (Ali et al., 2010).

Fresh meat is contaminated with microorganisms by improper processing practices, livestock rearing environments, ill workers and usage of unclean processing tools. The process of culling and transport to the slaughter house, causes shocks in animals, which is responsible for the spreading of microbial species from the gastrointestinal system to muscles of the animals, allowing the survival or proliferation of these microbial species on meat after sectioning and slaughtering (Serrone and Nicoletti 2013).

Both pathogenic and commensal bacteria learned to develop or acquire appropriate weapons in the constantly changing battle against antimicrobial agents, as a result multi drug resistance (MDR) proves to be a perfect tool in the fight for survival (Szmolka and Nagay 2013). Emergence of antibiotic resistance in Enterobacteriaceae has been documented in several studies (Fuentes et al., 2013). Antibiotics are not only used in veterinary for therapy and

prevention of bacterial infection but also used in animal feeds as growth promoter, to increase feed efficacy and decrease waste production (Bogaard & Stobberingh, 1999; Bogaard & Stobberingh 2000).

Bacteria can evolve antibiotic resistance by a number of mechanisms, like changes in cell permeability, horizontal transfer of resistant genes and alterations by mutations of the antibiotic target(s). Antimicrobial resistance helps bacteria to adapt itself in a hostile environment (Beceiro and Alejandro, 2013). Modification of existing genes by accumulation of point mutation or transfer of resistance genes by horizontal gene transfer results in antibiotic resistance (Carattoli b 2003). Plasmids are self-replicating, double stranded, circular or linear extra chromosomal DNA molecules that can confer resistance to the important classes of antibiotics such as aminoglycosides, beta-lactams, chloramphenicol, macrolides, quinolones, sulfonamides, trimethoprim and tetracyclines. Mobile genetic elements including plasmids, transposons and gene cassettes in integrons play an important role in transferring resistance and increasing multi-drug resistance in bacteria (Saenz et al., 2004; Carattoli a 2013).In this study microbial/hygienic status of meat at retail shops located in different areas of Karachi was determined by isolating various gram negative bacteria from those samples and antibiotic resistance patterns were also determined.

Material and Methods:

Media and chemicals

Sample processing and bacterial isolation

Isolation of gram negative bacteria from red meat was performed according to protocol of Andritsos 2013 with slight modification. For isolation, 250 mili grams of meat purchased from various shops of Karachi city. Meat pieces were suspended in 250 mL of sterile saline (pH 7.0) for 30 minutes. 100 µL of saline suspension was spread onto MacConkey's agar plates. Fifty grams negative isolates were selected as representative of overall random sampling for antibiotic resistance determination (Ali et al., 2010).

Assay for antibiotic resistance profile:

Agar-well diffusion method

Agar-well diffusion method was performed to determine the antibiotic resistance profile. Bacterial cultures were developed in nutrient broth for about 3 – 4 hours. 100 µL of fresh culture was added in 4 mL of soft agar (1% agar), mixed well and then poured on nutrient agar plate. Once solidified, wells were made with the help of sterile borer (10 mm), 100 µL of test compound antibiotics at two concentrations (100µg/mL and 500µg/mL) were added into the wells and plates were incubated overnight at 37 ^{o}C (Osterblada et al., 1999; Alzoubi et al., 2014).

Curing experiment:

To determine the location of the genetic factors (plasmid borne or chromosomal) responsible for antibiotic resistance, the curing experiment was performed using UV light and high temperature. Only those isolates showing resistance to more than one antibiotic were selected for curing. 0.1 mL isolate suspension was inoculated in a flask of 50 mL of broth containing 0.2 mL of antibiotic Amoxil, was incubated at 37^{o}C for overnight. Next day, 1 mL of 1:100 was poured in three Petri plates. Two different time intervals were selected for UV exposure 30 seconds and 60 seconds. Unexposed samples served as control. The exposed samples were also subjected to heat shock for 15-30 minutes at 42 ^{o}C. Spots were inoculated on nutrient agar plated with or without antibiotic. Cured colonies were obtained on plain nutrient agar plates without antibiotics (Bayley et al., 1977; Sasirekha and Shivakumar 2012).

Results

Meat samples used for isolation shows variability in hygienic conditions, as few samples were high in gram negative bacteria compared to other samples. Percentage of isolates at different shops in Karachi is shown in Table 1. These meat samples included 3 genera of Gram-negative organisms such as Escherichia coli (58%), Salmonella spp (22%) and Klebsiella spp (20%).

All 50 isolates were tested against 5 antibiotics. Among these meat isolates, 66.6% strains were not resistant to any antibiotics tested, while 33.4% strains were resistant to at least 1 of the 5 antibiotics tested Figure 1.

Among the antibiotic resistant meat isolates, 25% strains were resistant to single drug while 75% were multi-drug resistant at two concentrations (100 µg/mL and 500 µg/mL). Susceptibility against different antibiotics are shown in Figures 2-5, highest for Amoxil (50%), followed by Septran (25%), Cefizox (8.3%) and Streptomycin (8.3%) and Gentamicin (0%), which showed no resistance at all to any of the tested isolates. Isolates showing resistance to more than one antibiotic is shown in Table 2.

Table 1: Areawise characterization of isolates:

S.No	Location	Isolates collected	Percentage(%)
1	Orangi town	KMS-1 to KMS-6	12
2	North Karachi	KM-7 to KMS-13	14
3	Sujani town	KMS-14 to KMS-19	12
4	Manghopir	KMS-20 to KMS-27	16
5	Gulshan-e-iqbal town	KMS-28 to KMS-34	14
6	New Karachi-I	KMS-35 to KMS-39	10
7	New Karachi-II	KMS-40 to KMS-45	12
8	New Karachi-III	KMS-46 to KMS-50	10

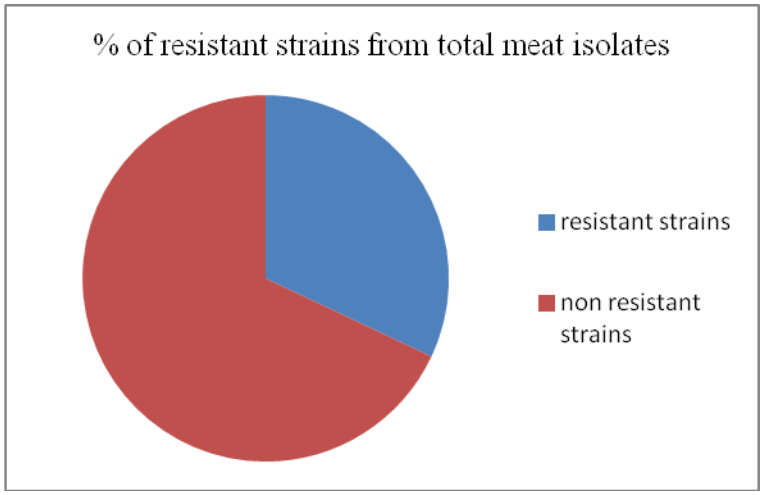


Figure1. Percentage of antibiotic resistant and non resistant strains in meat isolates.

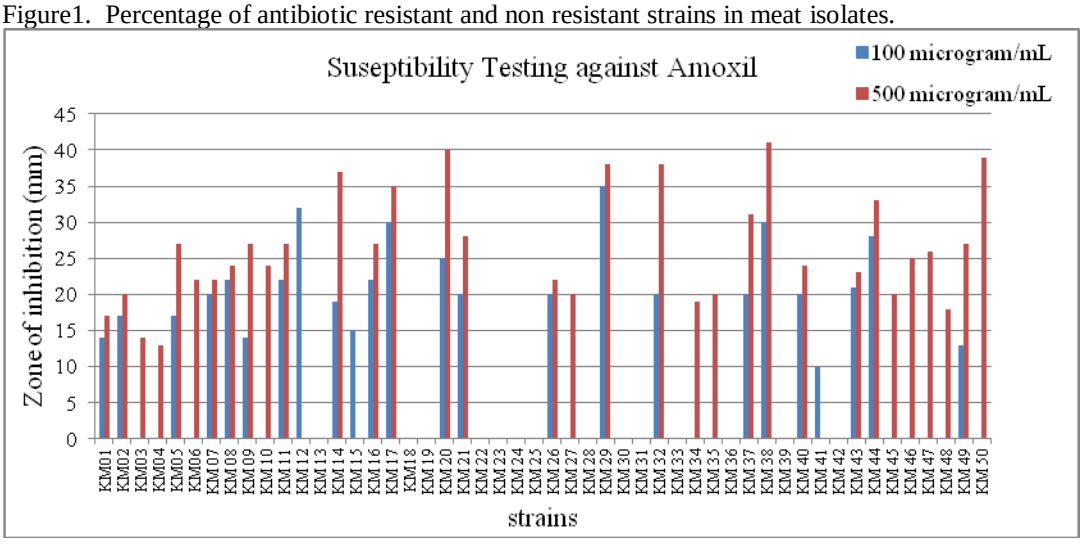


Figure 2. Susceptibility testing against Amoxil

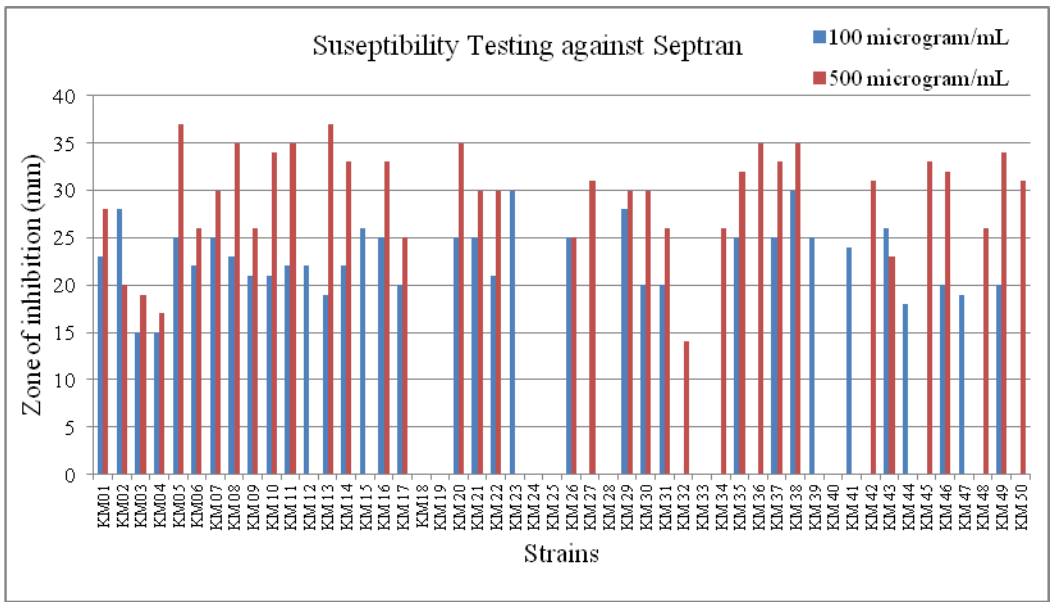


Figure 3. Susceptibility testing against Septran

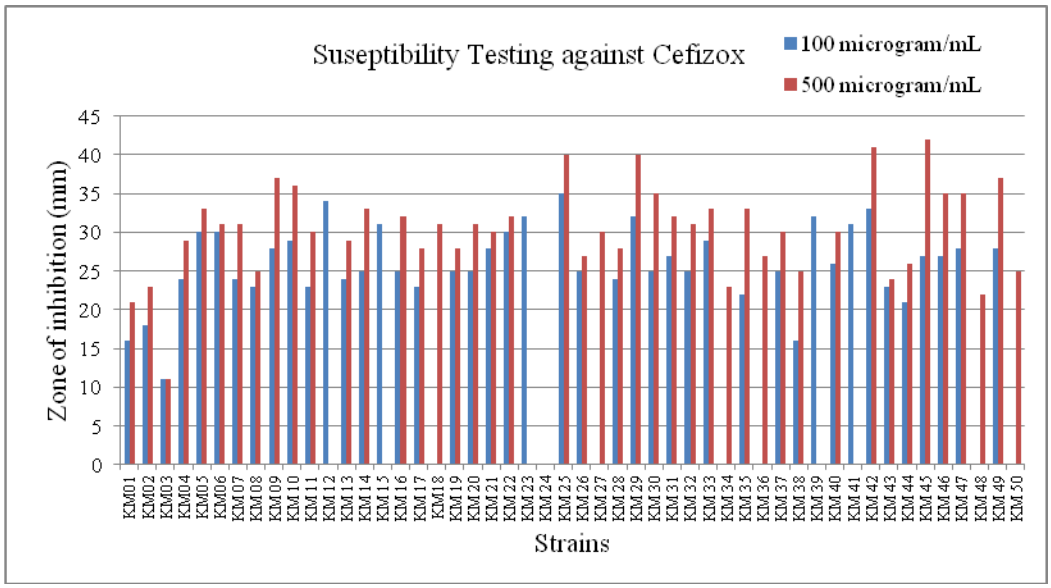


Figure 4. Susceptibility testing against Cefizox

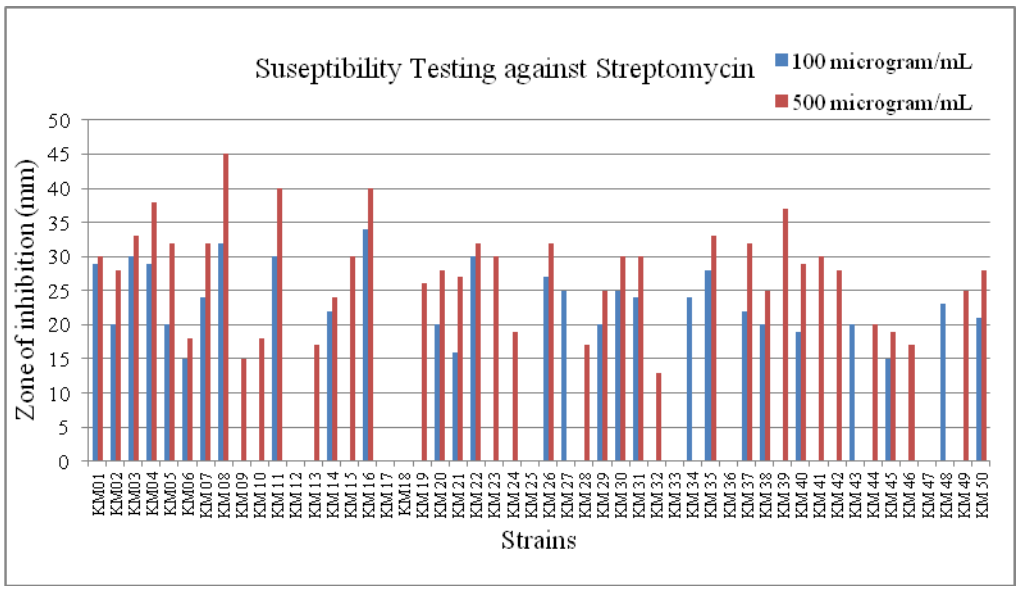
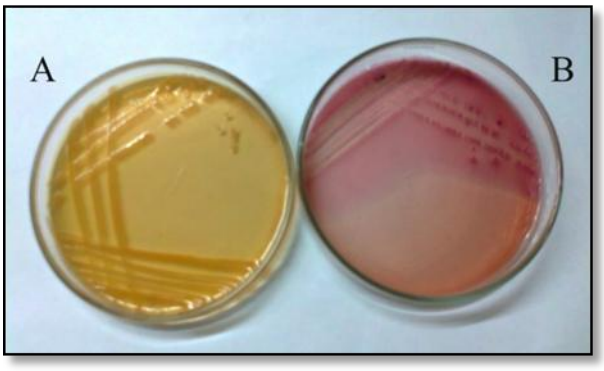


Figure 5. Susceptibility testing against Streptomycin

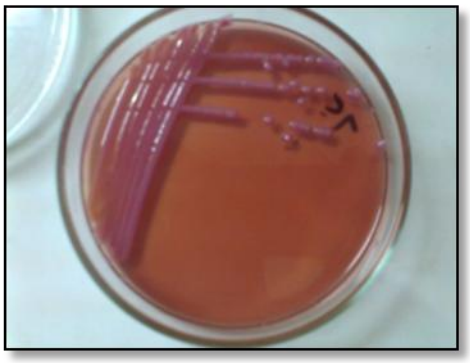
Table 2. Resistance of isolates to antibiotics at 500 µg/ml

S.No	Isolates	Resistance to various antibiotics at 500 µg/ml
1	KMS –14	Amoxil
2	KMS – 21	Streptomycin
3	KM S– 28	Amoxil, Septran
4	KM S–34	Cefizox, Streptomycin
5	KM S–37	Amoxil
6	KM S– 42	Amoxil
7	KM S– 47	Septran, Cefizox, Streptomycin, Amoxil



A: Lactose Non- Fermenting Colonies
B: Lactose fermenting colonies.

Picture 1(a): Isolated colonies from meat samples



Lactose fermenting, mucoid colonies.

Picture 1 (b): Isolated colonies from meat samples

Picture 2: KMS-14: All antibiotics showing a clear zone of inhibition at 500µg/ml.

Picture 3: KMS-28: No Zone against Amoxil (100µg/ml) and Septran (100µg/ml) while a Clear Zone against Cefizox (100µg/ml) and Streptomycin (100µg/ml)

Table 3 Location Determination by Plasmid Curing

S. NO	Antibiotic resistance meat isolate	Presence of growth on antibiotic supplemented medium	
		Pre- curing	Post-curing
1	KMS-47	+	-

Key:
+ = growth
- = no growth

Curing of plasmid

The presence of plasmid was determined by curing experiment (loss of plasmids) using physical agent as UV light and heat. Combined effects of both of these physical agents were used to cure plasmid. Only one of the multi-drug resistant isolate was used for curing experiment. Cured isolate KMS-47 was unable to resist amoxil at concentration 100 µg/mL, suggesting the presence of resistance marker on plasmid. However, further studies are required to confirm all the strains.

Discussion

The hygienic status of meat shops in various locations of Karachi indicated that there are infact the source for the prevalence of various gastric and other infections in the city.

The research was conducted to screen the presence of gram negative bacteria in meat samples from various shops located in Karachi. Further, we tested and screened antibiotic resistance pattern among these isolates. The bacterial isolates of meat sample in this study included Escherichia coli, Salmonella spp. and Klebsiella spp. Among different genera of Gram-negative isolates, E.coli was the most dominant one, followed by Salmonella spp and then Klebsiella spp. Gram negative bacteria were isolated from meat using selective media i.e., MacConkey agar (Cyzeska et al., 1981).

A high percentage of meat isolates showed resistance at 500 µg/mL concentration to different antibiotics suggests that may contribute to the incidence of food associated diseases (Tassew et al., 2010). The isolates showed high resistance to β-lactam antibiotic amoxil (50%), followed by septran (25%), both streptomycin and cefizox (8%) and while no resistance seen against gentamicin. Thus gentamycin proved to be most effective and amoxil as least effective antibiotic against meat isolates at 500 µg/mL concentration. β-lactams and tetracyclines are the most widely used antimicrobial agents on dairy herds, according to a study by Sawant et al., 2006. Although, streptomycin and gentamicin, both belong to Aminoglycoside group of antibiotics but gentamicin at concentration of 500µg/mL was more effective than streptomycin. All the strains tested against gentamicin showed no resistance. On other hand, Cefizox, an antibiotic of class cephalosporins also showed low resistance pattern. This increased activity may be due to its less veterinary application according to World Organization for Animal Health (OIE), 2007 and WHO, while in humans, it is largely used to treat serious infections (Szmolka et al., 2013).
The lack of standard official protocols (SOPs) for treatment of sick animals, failure to complete an antimicrobial treatment course, absence of antimicrobial treatment records, and failure to consult a veterinarian for treatment of sick animals lead to inappropriate use of antimicrobial agents and emergence of antimicrobial resistant bacteria (Sawant et al., 2006).
This initial screening has been followed by the determination of the genetic locus of the resistance among the meat isolates. In gram negative bacteria, dissemination of antimicrobial resistance has been largely attributed to inter and intra-species DNA exchange. The horizontal transfer of plasmid- mediated resistance genes is the most dominant mechanism of acquisition of resistance in bacterial pathogens causing hospital or community acquired infections (Carattoli, 2013).
Drug resistant microorganisms emerge as antibiotics are used as performance enhancers or for therapy or prevention of bacterial diseases (Bogaard and Stobberingh 1999). High resistance rates among these pathogens show that many antibiotic regimens in current treatment guidelines are already ineffective against a wide range of pathogens of clinical significance. Drug resistance, like other emerging infections, can quickly disseminate from one country to another, therefore, national and an international strategy is necessary in this regard (Nguyen et al., 2013).

Conclusion:

Among gram negative bacterial strains isolated from meat, E. coli was the major contaminant. The 75% of the total antibiotic resistant meat isolates were multi drug resistant (MDR) strains. Aminoglycoside antibiotic gentamycin found to be the most effective against the MDR strain while β lactam antibiotic Amoxil was least effective at a concentration of 500 µg/ml. Curing of the MDR isolates using UV light and heat treatment suggests the extra chromosomal nature of the antibiotic resistance (plasmid borne).
Further studies are quite important in order to find out the link between prevalence of such antibiotic resistance isolates in meat and emergence of gastric and other infections. It is quite possible that zoonotic infections, like bacterial or viral became epidemic due to unhygienic practices at retail shops.

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