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Molecular characterization of antibiotic resistance pattern among gram negative bacteria isolated from red meat in Karachi

Abstract

The present study was undertaken to determine the microbial status of red meat supplied to the citizens of Karachi and to observe the resistance pattern of bacteria isolated from these sources. The Molecular characterization of antibiotic resistance pattern among gram-negative bacteria isolated from meat was carried out. The meat samples were collected (purchased) from open air local retail shops, situated in different areas of Karachi. A total of 12 strains were selected from the previous study (Data under publication) showing resistance to more than one antibiotics of a five antibiotic regime at concentration of 500µg/mL. These antibiotics include Amoxil, Cefizox, Gentamicin, Septran and Streptomycin. All the pathogens were Gram-negative scattered rods, including *Escherichia coli*, *Salmonella spp*. and *Klesiella spp*. The resistance against these antibiotics was 50%, 8.3%, 0%, 25% and 8.3% respectively. The frequency of resistance was the highest for Amoxil followed by Septran, Cefizox, Streptomycin and Gentamicin.

Genetic determinant of antimicrobial resistance was determined by curing using physical agent (UV light and heat). The cured strains were analyzed for resistance against respective antibiotics and it was found out that the strain was no more resistant to that antibiotic following curing, thus establishing the extra chromosomal control of antibiotic resistance by plasmid borne genes. The plasmids were then isolated and visualized by performing gel electrophoresis.

Hereby, we would like to draw attention to this most important and avoidable problems associated with slaughtering practices, processing and storage of raw meat so that the development of such resistant bacteria causing nuisance in processing could be controlled. This study could serve as a guideline for preventing various zoonotic diseases via meat and meat products in community.

Keywords: red meat isolates, gram negative bacteria, unhygienic practices, marine algal compounds, industrial dyes, antibiotics, molecular characterization, antibiotic resistance, plasmid, gel electrophoresis

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Maria Moiz Ali, Subhan SA
Department of Microbiology, University of Karachi, Pakistan

Correspondence: Subhan SA, Assistant Professor, Department of Microbiology, University of Karachi, 30/2-4 Kane street, Pakistan, Tel 0478417712, Email mariamurtaza@outlook.com

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Abbreviations: MDR, multi drug resistant; DMSO, dimethyl sulfoxide;

Introduction

Food borne illnesses are caused when certain pathogens enter the food supply. About 1.8 million people in underdeveloped countries are killed annually, due to food borne pathogens, making it the leading cause of death and illnesses.

Poor hygiene practices, mas catering complexes, changes in eating habits and protocols of lengthy food supply with upgraded international movements are the most important determinants contributing to the food borne illnesses.

In a several way, microorganisms present in the environment acquire a chance to enter food product, causing disease. A heavy loss for the food industry is due to food borne pathogens.

Meat is an important source of proteins, vitamins such as vitamin B12 and other complex vitamins, phosphorus, zinc and selenium.[1] Meat is a perishable, containing wide nutritional composition, a suitable pH and sufficient amount of water that favors the growth of most microorganisms.[2]

Fresh meat is contaminated with microorganism by improper processing practices and livestock rearing, usage of unclean processing tools, environments and defective hygiene of the workers. The process of culling and transport to the slaughter house, causes shocks in animal, 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 specie on meat after sectioning and slaughtering.[3] Under cooked meat also serve as a source of spreading diseases. Antibiotics are used to control infection and as growth promoters in animals.

Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations. Antibiotics are used in animals as in humans for therapy and control of bacterial infections. In intensively reared food animals, antibiotics may be administered to whole flocks rather than individual animals. However the indiscriminate use of these antibiotics are must highlighted issues as they mediate high antibiotic resistance and propagation of the resistance to the normal flora of animals. Resistance phenomena exists since 1960s various *Salmonella spp*. Resistant to ampicilin, chloramphenicol and septran reported with increasing frequency throughout. A number of mechanisms are there through which bacteria can evolve antibiotic resistance.[4]

The phenomenon of antibiotic resistance could be attained by horizontal gene transfer resulting a new mutant in the field or it could be an innate property of an organism. 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.[5] 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.[6].

Antibiotic resistant microorganisms emerge as antibiotics are use as performance enhancers or for therapy or prevention of bacterial diseases.[7] High resistance rates among these pathogens shows 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 action is necessarily required in this regard.[8]

The purpose of this study was to determine the microbial status of meat at local retail shops located in different areas of Karachi along with the pattern of antibiotic resistance among these meat isolates and on the crude basis, algal compounds and industrial dyes were screened and molecular characterization of antibiotic resistance among these isolates was determined.

Materials and methods

Materials

Bacterial strains

A. **MDR meat isolates:** A total of 12 MDR meat isolates from previous research were used in this study.

B. ***Micrococcus luteus*:** *Micrococcus luteus* was used as a test organism, as it is sensitive to many of the antimicrobial agents.

Media: Agar technical agar (LP0013 oxoid), Nutrient broth (CM0001 oxoid), MacConkey's Agar (CM0115 oxoid).

Chemicals: Gram's reagents (crystal violet, Gram's Iodine, alcohol, safranin), distilled water, DMSO, Alcohol 70% v/v (disinfection), 1% agarose, DNA loading dye (1x), Ethidium bromide Reagents for plasmid isolation.

Antibiotics: Amoxil, Cefizox, Gentamicin, Septran and Streptomycin.

Test compounds

a. **Algal compounds:** Activity of 28 algal compounds of known origin (prepared in DMSO) were tested against 12 multi-drug resistant meat isolates. This work on algal compound was in collaboration with Dr. Nizamuddin, Assistant Professor, Centre of Excellence in marine biology.[9]

b. **Industrial Dyes:** 3 Industrial Dyes used for coloring the medicinal capsules were also tested against 12 multi-drug resistant meat isolates. These Dyes were pink, orange and blue color.

Glassware and equipments: Petri dishes, test tubes, test tube stand, glass slides, 1.5ml vials, 100 and 200ml beakers, 100, 200, 500 and 1000ml flasks, wire loop, 1cm borer, compound microscope (anti-mold NIKON), refrigerator, water bath, incubator, a box of tooth picks, weighing balance, microwave oven, UV ray hood, Bunsen burners, tripod stand, micropipette, micropipette tips, gel electrophoresis kit, UV trans illuminator, centrifuge machine, cold centrifuge machine, vortex machine, pH meter, filter membrane.

Methods

Staining: 12 multi-drug resistant meat isolates were stained using Gram's staining technique. After staining, strains morphological characteristics and arrangements were observed under compound microscope.

Sample processing: The given cultures were streaked on MacConkey's agar to obtain isolated colonies and to observe lactose fermentation. Using gram staining, cultures were purified. After purification, cultures were streaked on nutrient agar slants and were stored in refrigerator for further use.

(Note: all the cultures were revived after every week, using the above procedure).

Preparation of working solutions

i. **Algal Compounds:** Algal compounds extracts were prepared in 1ml 60% DMSO in 1.5ml vials containing dried algal compounds. Same procedure was followed for all algal compounds.

ii. **Preparation of antibiotic solutions**: Antibiotic solutions of 500µg/mL of five different antibiotics were used.

Industrial dyes: Two concentrations i.e. 1:2 and 1:4 of the dyes were prepared. First 0.4gm of the respective dye (powder) was added in 10ml of distilled water to make a stock concentration. Next, two concentrations, i.e. 1:2 and 1:4 were prepared from the stock. Once dilutions were prepared, the dye solutions were filtered using filter membranes and were stored in test tubes for further use.

Assay for antibiotic resistance profile

I. Agar well diffusion method

Antibiotic resistance profile of the organisms was determined by the agar well diffusion method. In this method, a loop of culture is inoculated in nutrient broth for about 3-4hours, 0.1ml of this 3-4hours old culture is added in 4ml of soft agar, shake it well and then poured on Nutrient agar plate, uniformly rotate the plate and let it solidify. Once solidified, make wells (1cm) with the help of borer. Add 100µl test compound (algal compounds, neem compounds or dyes) into the wells and incubate the plates, overnight at 37°C.[10]

II. Curing (Plasmid elimination) procedure

To determine the location of the genetic factors (Plasmid borne or chromosomal) responsible for antibiotic resistance, the curing experiments were performed using physical agents as UV light and heat. One of the isolate, which was resistant to more than one antibiotic i.e. multi drug resistant, was selected for curing. First a loop of MDR organism was added in a flask of 50ml of broth containing 0.2ml of antibiotic ampin, the flask was incubated at 37°C for overnight. Next day, 1ml from the nutrient broth containing antibiotic and MDR culture was added in 9ml of saline to make 10-2 dilution. From this dilution, about 3ml was poured in each of the three Petri plates; first Petri plate was exposed to UV light for 30seconds and second for 60seconds, remaining third serve as a control (unexposed plate). Next, from the 30second UV exposed plate transfer 1ml into 9ml of saline making dilution 10-3. Now divide this 10ml into two 5ml tubes, heat one tube at 42°C for 30minutes i.e. its UV exposed and heated tube. Inoculate,

with the help of tooth pick, form this tube, spots on plain nutrient agar plates and nutrient agar plates containing antibiotic. Second 5ml tube is the only UV exposed tube; inoculate from this tube also on both plain Nutrient agar and Nutrient agar containing antibiotic and repeat the same procedure for the 60seconds UV treated plate.

Next, for the procedure, using only heat for curing, first make dilutions, like the UV treatment and divide it into three tubes. Heat one tube at 42°C for 30minutes and other at 42°C for 45minutes. Third tube serve as control (not exposed to heat). Inoculate spots from all the three tubes into Nutrient agar and Nutrient agar plate containing antibiotic respectively.

Cured colonies were obtained were obtained on plain Nutrient agar plates without antibiotic and were absent on plates containing antibiotics due to loss of the resistant plasmid.

I. Plasmid isolation using lysozyme

Tube containing 10ml of culture (KM-96) grown in nutrient broth containing the antibiotic to which the isolate was resistant, was centrifuged at 3000rpm for 15minutes. Supernatant was discarded and the pellet was suspended in 1.4ml of TE buffer. Next, it was transferred in Eppendorf tubes and spin for 3minutes. Discard the supernatant and suspend pellet in 0.4ml of solution, mix vigorously in vortex machine and cool on ice. Add 0.1ml of freshly prepared lysozyme, mix carefully and incubate on ice for 20minutes. Add 0.3ml of precooled Triton buffer, incubate on ice for 20minutes and centrifuge at 4°C for 4minutes. Transfer the supernatant into two Eppendorf tubes having equal volumes and add -20°C ethanol in it for DNA precipitation, mix gently. Allow tubes to dry for 10-20minutes. Add 50 µl pre chilled buffer to tubes. Sample is ready for use and can be freeze and stored indefinitely.[11]

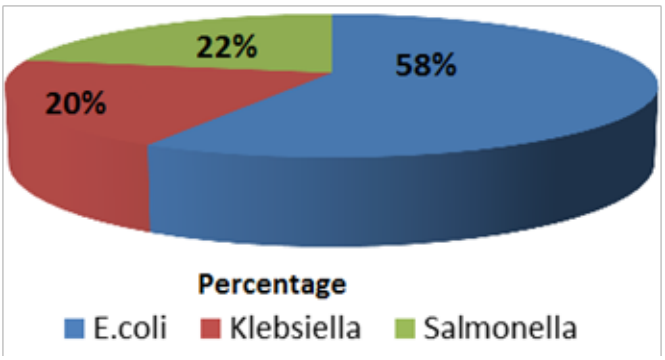
II. Gel electrophoresis

The presence or loss of plasmid was further confirmed by performing gel electrophoresis on 1% agarose gel. About 0.4gm of agarose in 40ml of TAE buffer was melted in boiling water and when temperature dropped to 45°C, about 1µl of the Ethidium Bromide (10mg/ml) was added in the gel. The melted agarose was poured after assembling the gel-casting tray with comb at one end and sealing the ends of the gel casting tray with tape, allow the gel to set. Once set, remove the tape form the ends and poured 1X TAE buffer in the tank to sub-merge the gel. Sample from the freezer was heated and briefly centrifuged. About 10µl of the respective sample was transfer into the vials containing 2µl of DNA loading dye, the vials were briefly centrifuged again and about 12µl of samples were loaded in the wells and electrophoresis was carried out at 100volts for an hour. Gel was removed and was viewed with UV illumination. Photograph the gel and examine the bands.

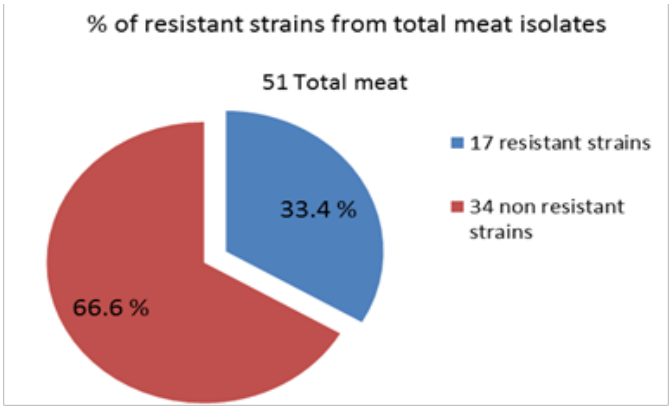
Results

Pattern of antibiotic resistance and multi drug resistance among meat isolates

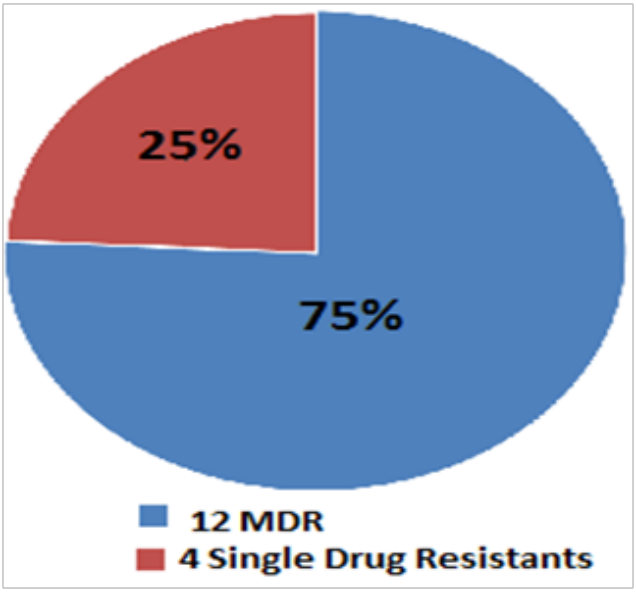
A total of 5 antibiotics were tested against 50 strains of meat. These meat strains include 3 genera of Gram-negative organisms such as *Escherichia coli* (58%), *Salmonella spp* (22%) and *Klebsiella spp* (20%), as shown in (Graph 1). These organisms were isolated from different local retail meat shops of Karachi (Table 1). 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. Among the antibiotic resistant meat isolates, 25% strains were resistant to single drug while 75% were multi-drug resistant. Results showed in (Graph 2A & Graph 2B). The antibiotics tested against meat isolates include Amoxil, Cefizox, Gentamicin, Septran and Streptomycin. Resistance to the antibiotic among meat isolates was highest for Amoxil (50%), followed by Septran (25%), Cefizox and Streptomycin (8.3%) and Gentamicin (0%), which showed no resistance at all to any of the tested isolates.



Graph 1 Percentage of different genera of gram-negative meat isolates.



Graph 2a percentage of antibiotic resistant and non resistant strains among meat isolates.



Graph 2b percentage of multidrug resistant (mdr) and single drug resistant strains among antibiotic resistant meat isolates.

Table 1 Diameters of zones of inhibition around both concentrations of antibiotics

Isolate	Diameter of zones (In mm)									
	Amoxil		Cefizox		Gentamicin		Septran		Streptomycin	
	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml
KM01	14	17	16	21	24	30	23	28	29	30
KM02	17	20	18	23	28	30	28	20	20	28
KM03	R	14	11	11	26	30	15	19	30	33
KM04	R	13	24	29	25	29	15	17	29	38
KM05	17	27	30	33	20	28	25	37	20	32
KM06	R	22	30	31	15	19	22	26	15	18
KM 07	20	22	24	31	21	24	25	30	24	32
KM 08	22	24	23	25	30	35	23	35	32	45
KM 09	14	27	28	37	17	19	21	26	R	15
KM 10	R	24	29	36	R	19	21	34	R	18
KM 11	22	27	23	30	28	35	22	35	30	40
KM 12	32	-	34	-	-	-	22	-	-	-
KM 13	R	R	24	29	R	23	19	37	R	17
KM 14	19	37	25	33	27	30	22	33	22	24
KM 15	15	-	31	-	-	-	26	-	-	30
KM 16	22	27	25	32	25	30	25	33	34	40
KM 17	30	35	23	28	R	25	20	25	R	R
KM18	-	-	R	31	-	-	-	-	-	-
KM 19	R	R	25	28	22	29	R	R	R	26
KM 20	25	40	25	31	20	24	25	35	20	28
KM 21	20	28	28	30	22	25	25	30	16	27
KM 22	R	R	30	32	24	28	21	30	30	32
KM 23	R	-	32	-	-	-	30	-	-	30
KM 24	R	R	R	R	23	25	R	R	R	19
KM 25	-	-	35	40	-	-	-	-	-	-
KM 26	20	22	25	27	21	26	25	25	27	32

Citation: Ali MM, Subhan SA. Molecular characterization of antibiotic resistance pattern among gram negative bacteria isolated from red meat in Karachi. *J Bacteriol Mycol Open Access.* 2016;2(5):127–138. DOI: 10.15406/jbmoa.2016.02.00039

Table Continued....

Isolate	Diameter of zones (In mm)									
	Amoxil		Cefizox		Gentamicin		Septran		Streptomycin	
	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml
KM 27	-	20	-	30	20	24	-	31	25	-
KM 28	R	R	24	28	22	24	R	R	R	17
KM 29	35	38	32	40	23	25	28	30	20	25
KM 30	R	R	25	35	23	25	20	30	25	30
KM 31	R	R	27	32	23	27	20	26	24	30
KM 32	20	38	25	31	20	32	R	14	R	13
KM 33	-	-	29	33	-	-	-	-	-	-
KM 34	-	19	-	23	22	29	-	26	24	-
KM 35	R	20	22	33	26	26	25	32	28	33
KM 36	-	R	-	27	R	21	-	35	R	-
KM 37	20	31	25	30	20	25	25	33	22	32
KM 38	30	41	16	25	27	30	30	35	20	25
KM 39	R	-	32	-	-		25			37
KM 40	20	24	26	30	21	23	R	R	19	29
KM 41	10	-	31	-	-	-	24	-	-	30
KM 42	-	-	33	41	-	21	-	31	-	28
KM 43	21	23	23	24	18	24	26	23	20	R
KM 44	28	33	21	26	R	36	18	R	R	20
KM 45	R	20	27	42	27	18	R	33	15	19
KM 46	R	25	27	35	R	20	20	32	R	17
KM 47	R	26	28	35	R	-	19	-	R	-
KM 48	-	18	-	22	21	25	-	26	23	-
KM 49	13	27	28	37	15	31	20	34	R	25
KM 50	-	39	-	25	26	21	-	31	21	28
TOTAL	41	41	45	45	41	41	41	41	41	41

The resistance of each isolate and their diameters of zones of inhibition around 100µg/Ml and 500µg/Ml concentrations of antibiotics is shown in (Table 2). A total of 6 isolates showed resistance to different antibiotics at a concentration of 500µg/Ml. Results are summarized in (Table 3).

Table 2 Area wise charecterisation of isolates

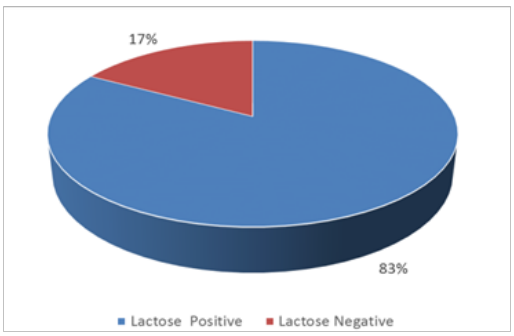
S.no	Location	Isolate collected	Percentage(%)
1	Orangi Town	KM01	12
		KM02	
		KM 03	
		KM 04	
		KM 05	
		KM 06	
		KM 07	
		KM 08	
		KM 09	
2	North Karachi	KM 10	14
		KM 11	
		KM 12	
		KM 13	
		KM 14	
		KM 15	
		KM 16	
3	Surjani Town	KM 17	12
		KM 18	
		KM 19	
		KM 20	
		KM 21	
4(a)	Manghopir	KM 22	16
		KM 23	
		KM24	
		KM25	
4(b)	Manghopir	KM 26	
		KM27	
		KM 28	
		KM 29	
		KM 30	
5	Gulshan-e-Iqbal	KM 31	14
		KM 32	
		KM 33	
		KM 34	
		KM 35	
		KM 36	
6	New Karachi	KM 37	10
		KM 38	
		KM 39	
		KM 40	
		KM 41	
		KM 42	
7	U.P	KM 43	12
		KM 44	
		KM 45	
		KM 46	
		KM 47	
8	New Karachi	KM 48	10
		KM 49	
		KM 50	

Table 3 Resistance of isolates to antibiotic at 500µg/ml

S.No	Isolates	Resistance to various antibiotics at 500µg/mL
1	KM – 63	Amoxil
2	KM – 67	Streptomycin
3	KM –69	Amoxil, Septran
4	KM –74	Amoxil, Cefizox, Septran
5	KM –78	Amoxil
6	KM –86	Amoxil
7	KM –96	Amoxil, septran, cefizox

Culture characteristic analysis

Growth pattern on MacConkey's agar confirm the isolates accordingly after Gram staining. Results of the lactose fermentation of total meat isolates are shown in (Graph 3).



Graph 3 percentage of lactose fermenter and non fermenter among meat isolates

Screening of algal compounds against meat isolates

28 algal compounds of known origin were tested against meat isolates. The codes of these algal compounds and their activity against meat isolates are summarized in (Table 4). All the meat isolates in this study were found to be resistant against all tested algal compounds shown in (Figure 1). This 100% resistance may be due to the method used for preparation and testing of these compounds.

Table 4 Activity of algal compounds against isolates

S.no	Code of algal compounds	Activity of algal compounds against isolates
1	MT-P-5	-
2	MT-P-6	-
3	MT-P-7	-
4	MT-P-8	-
5	MT-P-9	-
6	MT-P-10	-
7	MT-P-11	-
8	MT-P-12	-
9	MT-P-13	-
10	MT-P-14	-
11	MT-P-15	-
12	MT-P-16	-
13	MT-P-17	-
14	MT-P-18	-
15	MT-P-19	-
16	MT-P-20	-
17	MT-P-21	-
18	MT-P-22	-
19	MT-P-23	-
20	MT-P-24	-
21	MT-P-25	-
22	MT-P-26	-
23	MT-P-27	-
24	MT-P-28	-
25	MT-P-29	-
26	MT-P-30	-
27	MT-P-31	-
28	MT-P-32	-

Key

-(Negative)=No activity

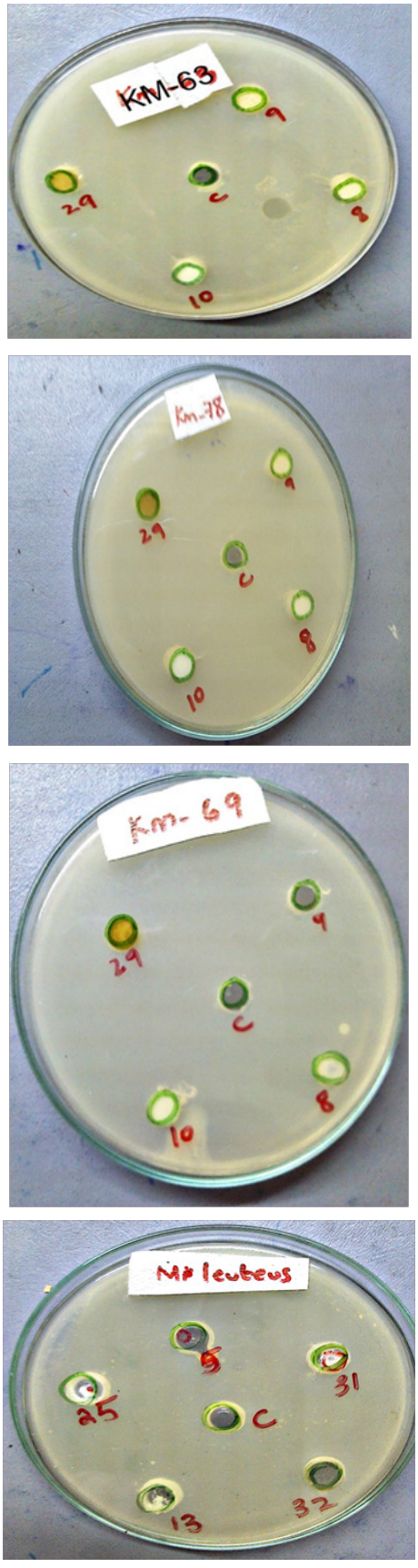


Figure 1 Activity of algal compounds against isolates.

*All the algal compounds were dissolved in DMSO and then tested against meat isolates. Since these compounds were extract in ethanol, so it would be likely to be tested in ethanol, however, ethanol itself possess antibacterial property, so keeping in view the antibacterial property of ethanol, DMSO was selected as a dissolving solvent for each algal extract. 100% DMSO was also found to possess antibacterial activity against tested isolate, as shown in (Figure 2), so 60% DMSO was selected for the preparation of these algal compounds.

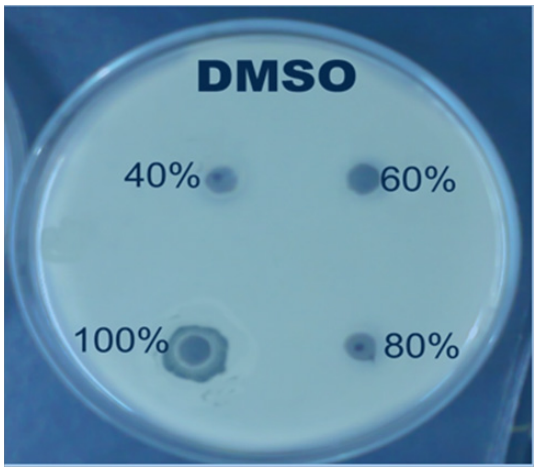


Figure 2 Dmso activity at different concentrations

Screening of industrial dyes against meat isolates

3 industrial dyes, Dye 1 (pink), Dye 2 (orange) and Dye 3(blue) from a known pharmaceutical company were also screened against tested isolates of meat. These dyes were prepared by dissolving dye powder in sterilized distilled water.

Initially dyes were itself tested to check any contamination. To see, if presence of any microbe in dyes results shown in (Figure 4). Once it was screened that dyes were free of any contamination, these dyes were tested against the meat isolates.

Dye 2 (orange) and Dye 3(blue) failed to give any antimicrobial activity against tested isolates while Dye 1 (pink) in two concentration (1:2) and (1:4) exert its antibacterial effect against 4 tested isolates and control organism *Micrococcus luteus* results summarized in (Table 6, Figure 4A-4B).

Curing of plasmid

Plasmid was cured using physical agent as UV light and heat (temperature). An individual as well as combined effect of both of these physical agents was used to cure plasmid. Loss of the ability of the multi-drug resistant strain KM-96 to resist the action of the antibiotic after curing indicated the presence of drug resistance marker on plasmid. Table 6 shows the pre-curing and post-curing status of the isolate KM-96 with reference to its ability to grown on the media supplemented with antibiotic.

Figure 3 Antibiotic resistance pattern

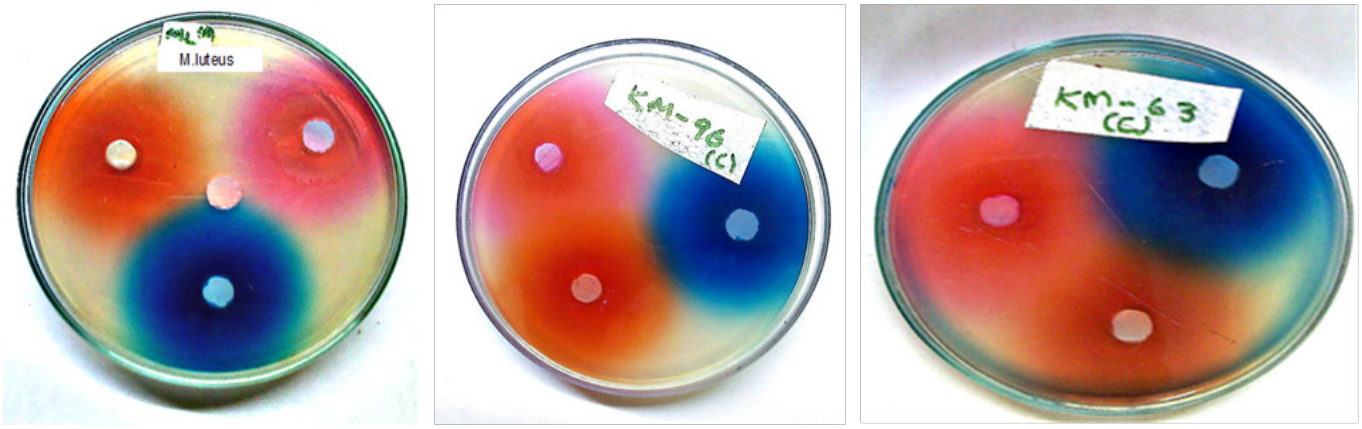


Figure 4 Activity of industrial dyes against isolates

Table 5 Activity of dyes on meat isolates

S.no	Isolates	Dyes			
		Orange	Pink		Blue
			1:2 (C)	1:4 (D)	
1	KM-96	-	+	-	-
2	KM-69	-	+	-	-
3	KM-74	-	+	-	-
4	KM-63	-	+	-	-
5	KM-78	-	+	-	-
6	MI	-	+	-	-

Key

C=Concentrated

D=Diluted+(Positive)=Growth Inhibited

-(Negative)=No Growth Inhibited

Table 6 Location determination by plasmid curing

S. no	Antibiotic resistance meat isolate	Presence of growth on antibiotic supplemented medium	
		Pre- curing	Post-curing
1	KM-96	+	-

Key

+=Growth

- = No Growth

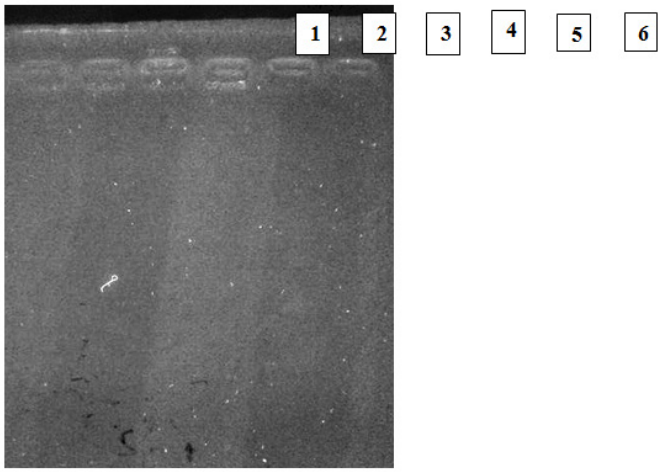


Figure 4a Gel picture

1. Cured sample 2. Uncured sample 3. Uncured sample

4. Uncured sample 5. Cured sample 6. Cured sample

Isolation of plasmid and gel electrophoresis

Plasmid was isolated using lysozyme method for various Gram-negative bacteria[11] from the isolates used for curing and was visualized using UV trans illuminator after performing gel electrophoresis. The samples were run in duplicate in the gel. Well 1, 5 and 6 were loaded with cured sample while wells 2, 3 & 4 and were loaded with uncured sample, shown in (Figure 4A). The uncured sample was again subjected to gel electrophoresis, this time a DNA ladder of 1kb was also run as shown in (Figure 4B). The glow in the well number five, indicated the presence of plasmid but due to its larger size, it failed to move outside the wells.

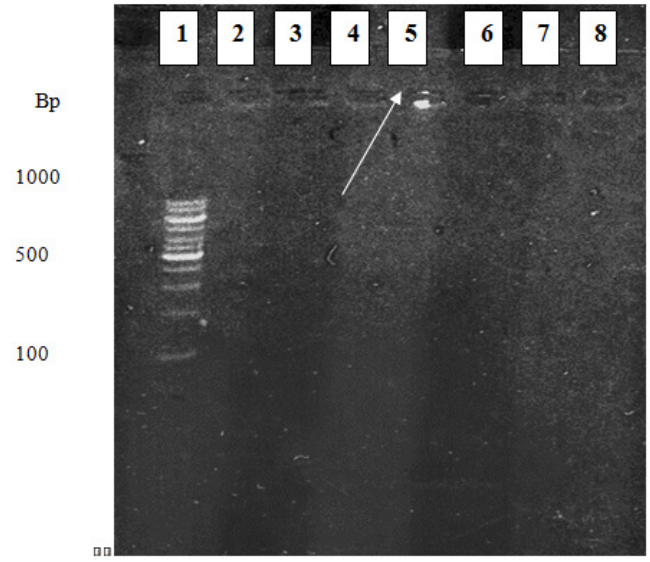


Figure 4b Gel picture

Cured and uncured samples of MDR strain KM-96 was run on gel, along with 1 Kb DNA ladder. Due to high molecular weight of plasmid it remained in the well.

Discussion

The research was conducted to screen out the presence of Gram-negative bacteria in meat samples from various meat shops located in Karachi. Further, we planned to screen such isolates for antibiotic resistance, resistance towards natural compounds as algal compounds.

Gram-negative bacteria were isolated from meat using selective media i.e., MacConkey agar. MacConkey agar contains bile salts, methylene blue and others that make it selective for Gram-negative bacteria.[12] In meat, the presence of bacteria has been documented widely from different parts of the world. Dissemination of food-borne pathogens from contaminated meat to different surfaces can result in spread of infections in the community.[13] The bacterial contaminants of meat sample in this study include *Escherichia coli, Salmonella spp*. and *Klebsiella spp.* Among different genera of Gram-negative isolates, *E.coli* wasthe most dominant one, followed by *Salmonella* spp and then *Klebsiella spp.*

Poor hygiene as backyard slaughtering and street meat sales practices (which is very common in Pakistan), results in higher incidence of *Escherichia coli* in meat. Moreover, inappropriate hygienic practices in butcher shops also increase the incidence of these organisms.[14] In this study, about 33.4% strains were resistant to antibiotics out of total meat isolates. Clinical isolates of meat, giving growth up to concentration of (500µg/mL) i.e. the highest concentration used in the media are considered as resistant. Among the resistant ones, about 75% of the strains were Multi-drug resistant (MDR). Multi-drug resistant gram negative *bacilli* have been known to cause infections, with significant mortality and morbidity.[15] Such findings reflect high magnitude of contamination at slaughter houses and food establishments which may contribute to the incidence of

food associated diseases. Therefore a strong controlling system of educating food handlers about the basic concepts of processing and producing a safer food and personal hygiene is necessarily required.[16]

The antibiotics used for studying resistance pattern among meat isolates include, Amoxil, Cefizox, Gentamicin, Septran and Streptomycin. The percentage of isolates, resistant to antibiotic at 500µg/mL concentration was highest against b-lactam antibiotic Amoxil (50%), followed by Septran (25%), both Streptomycin and Cefizox (8%) and Gentamicin (0%). Thus, Gentamicin turns out to be the most effective and Amoxil as least effective antibiotic among the antibiotic regime used against meat isolates at 500µg/mL concentration. B-lactams and Tetracyclines were the most widely used antimicrobial agents on dairy herds, according to a study by.[17] Wide use of these antibiotics has lead to increased emergence of resistance to these antibiotics. 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 0% resistance and 100% sensitivity. On other hand, Cefizox, an antibiotic of class Cephalosporins also showed high activity against meat isolates. This increased activity may be due to its less veterinary application according to World Organization for Animal Health (OIE), 2007 and World Health Organization (WHO), 2007, while in humans, it is largely used to treat serious infections.[18]

The lack of written plans 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 antimicrobial agents and emergence of antimicrobial resistant bacteria.[17]

The emergence, selection and dissemination of antibiotic resistant microorganisms in both human and veterinary medicine are possibly due to use of antibiotics. Pathogenic bacteria along with endogenous flora of exposed individuals (humans and animals) population can acquire this resistance such as resistant *E.coli*, which can then be transfer to humans via food chain or direct contact with animals. Aminoglycoside-modifying enzymes encoded on transmissible plasmids facilitate the Aminoglycoside resistance in *E.coli*.[19]

In Gram-negative bacteria, dissemination of antimicrobial resistance has been largely attributed to inter and intra-specific DNA exchange, among which the horizontal transfer of plasmid- mediated resistance genes is the most dominant mechanism of origin of acquisition of resistance in bacterial pathogens causing hospital or community acquired infections.[6]

Rapid development of resistance to b-lactams by related plasmids present in unrelated *Salmonella* strains have been reported. Bacteria of nosocomial origin can be responsible for horizontal transfer of resistance and presence of ESBL genes in *Salmonella.* Since *Salmonella* carriage of such transmissible plasmids may facilitate the spread of variety of resistance determinants to other community acquired harmful bacteria, this phenomenon is a threat to public health. Therapeutic choices for severe *Salmonella* infections may decrease due to further spread of such strains in a community. This shows that plasmid-mediated antimicrobial resistance is a global problem having strong capacity to be transmitted horizontally irrespective of any boundaries, either humans and animals or bacterial species or genera. Thus, role of many current resistance plasmids genes in bacterial virulence could be suspected.[4]

Resistant bacteria to antibiotics, used in humans may result from usage of antibiotics in food animals, selecting that resistant bacterium. Hence, using antibiotics as growth-promoters should be ban, as these resistant bacteria may spread via food to humans, causing some serious human infections. Antibiotics in low dosage used for growth promotion are an un quantified hazard. Despite the fact that some of the antibiotics are implicated for both, human and animal use, in humans, most of the resistance problems arise from human use only. Selection of resistance can be in food animals and these resistant bacteria can contaminate the food from animal origin but proper cooking of food destroys them.[20] Most of the domestic cooking, nowadays, is done using pressure cooker including the meat dishes, in order to decrease their cooking time. As the pressure cooker works on the principle of autoclave, the survival of pathogens in such harsh condition is difficult. It seems that our population is safe from hazards of food borne illnesses, but in actual, risk factors are still associated with the improper hygiene status of the sellers and buyers of meat.

Maintaining proper hygiene conditions and intelligent use of antibiotics in animal husbandry is important to control resistance emergence and dissemination.[13] The aim of this study was to present the overall microbial status of different local retail meat shops in Karachi and its surrounding environment and the pattern of antibiotic resistance among these pathogens. Besides antibiotics, algal compounds were also tested to see their effect on meat isolates. The genetic determinant of the resistant strains was also determined to see whether the antibiotic resistant genes are plasmid borne or located on chromosomes.

Increased use of antibiotics as a feed supplement could lead to increase incidence of antibiotic resistant bacteria in humans causing bacterial infections. Therefore, a need to develop new natural antimicrobial agents has arisen due to increased incidence of food borne illnesses along with social and economic implications due to usage of antibiotics.[3]

Naturally derive products are considered as an important source of exploiting new antibacterial agents. During 1983-1994, an estimated 78% of the new drugs approved by FDA were those that were derived from unmodified natural products or semi-synthetically obtained from sources of natural origin. This reflects and supports the importance of screening natural compounds.[21] Besides antibiotics, several marine algal compounds were also tested and screened for antimicrobial effect. About 28 marine algal compounds were tested but unfortunately none of the compounds showed inhibitory effect to the growth of meat isolates and all the MDR bacteria showed 100 % resistance to these compounds. These compounds were isolated from different parts of algal structure. This work is in collaboration with Dr. Nizamuddin, Assistant Professor, Centre of Excellence in marine biology.[9]

Possibility is there that compound instability due to fractionation and isolation from the natural cellular environment or these compounds may act synergistically or more than one compound may be linked to a specific property may be responsible for difficulties in perception of the bioactivity of these compounds. Many microalgae and macroalgae are complex compound matrices which require interaction of more than one compound associated to a specific property.[22]

Moreover, originally the algae extract were prepared in ethanol and it would be likely to be tested in ethanol but knowing the fact that ethanol itself possess antibacterial activity, therefore instead

of ethanol, DMSO was selected as a dissolving agent for the algal compounds. However, 100% DMSO itself was exerting antibacterial effect so instead of 100%, 60% DMSO was used for preparation of all algal compounds in this study.

This initial screening has been followed by the determination of the genetic locus of the resistance among the meat isolates. For this purpose plasmid curing was performed. Addition of intercalating agents, used at sub inhibitory concentration to bacterial growth results in blocking of plasmid replication, this phenomenon is known as curing.[23] Curing in this research was done using physical agents as heat (temperature) and ultraviolet (UV) light.

The plasmid was visualized after gel electrophoresis. Presence of plasmids was revealed by gel electrophoresis, a large size band was observed in test lane (5) having uncured plasmid sample while no band was present in control lane (2, 3, 4, 6, 7 and 8), having cured plasmid sample of test organism. 1Kb DNA ladder was run in the lane 1. Due to high molecular weight of the plasmid, it did not move out of the well.

In humans, it is still under much debate that how much extent of antibiotic usage will contribute towards the antibiotic resistance. Use of drugs as antimicrobial growth promoters in veterinary purposes, influences the prevalence of resistance in animal origin bacteria and is likely a risk factor for the emergence of antibiotic resistance in human pathogens causing serious infection Therefore, to avoid such incidence of antibiotic resistance in humans, Proper hygiene practices, avoiding extensive use of antibiotics and prevention of cross contamination is necessary.[7]

About 33.4% antibiotic resistant strains were isolated from meat and out of the total antibiotic resistant meat isolates 75% strains were multi-drug resistant (MDR). Such increased incidence of these pathogens in meat, which is commonly consumed in our population, is an alarming situation. Regular use of antibiotic has lead to emergence of antibiotic resistance in humans and animals. Therefore exploitation of new natural compounds is necessarily required.

Conclusion

Among Gram-negative bacterial strains isolated from the meat, highest percentage of *Escherichia coli* (58%) followed by *Salmonella spp.* (22%) and *Klebsiella spp*. (20%) as a contaminant was identified. About 33.4% strains were antibiotic resistant strains out of total meat isolates and out of this resistant strains, 75% strains were Multi-drug resistant (MDR) strains, which were resistant to more than one antibiotic used out of total 5 antibiotics.

Among the five classes of different antibiotics used, gentamicin emerged as most effective antibiotic showing 0% resistance to MDR strains, followed by cefizox and streptomycin (8.3%), septran (25%) and amoxil (50%) as least effective antibiotic at a concentration of 500µg/mL, against MDR meat isolates.

Algal compounds, did not exhibit any kind of antimicrobial activity against meat isolates. Curing of the MDR meat isolate (via UV light and heat treatment) proved the extra chromosomal nature of the antibiotic resistance i.e. the genes for antibiotic resistance are harbored on plasmids. Once plasmid was isolated, it was visualized by performing gel electrophoresis.

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None.

Conflict of interest

The author declares no conflict of interest.

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