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. A total of 12 strains were selected from the previous study (Data under publication) showing resistance to more than one antibiotic of a five antibiotic regime at concentration of 500µg/mL. These antibiotics include Amoxil, Cefixox, Gentamicin, Septran and Streptomycin. All the pathogens were Gram-negative scattered rods, including Escherichia coli, Salmonella spp. and Klebsiella 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, Cefixox, 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

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 ampicillin, chloramphenicol and septan 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, betalactams, 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, 1.5 ml vials, 100 and 200 ml beakers, 100, 200, 500 and 1000 ml flasks, wire loop, 1 cm 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 1 ml 60% DMSO in 1.5 ml 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 0.4 gm of the respective dye (powder) was added in 10 ml 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 3-4 hours, 0.1 ml of this 3-4 hours old culture is added in 4 ml 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 (1 cm) with the help of borer. Add 100 µl 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 50 ml of broth containing 0.2 ml of antibiotic ampin, the flask was incubated at 37°C for overnight. Next day, 1 ml from the nutrient broth containing antibiotic and MDR culture was added in 9 ml of saline to make 10-2 dilution. From this dilution, about 3 ml was poured in each of the three Petri plates; first Petri plate was exposed to UV light for 30 seconds and second for 60 seconds, remaining third serve as a control (unexposed plate). Next, from the 30 second UV exposed plate transfer 1 ml into 9 ml of saline making dilution 10-3. Now divide this 10 ml into two 5 ml tubes, heat one tube at 42°C for 30 minutes 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 5 ml 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 60 seconds 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 30 minutes and other at 42°C for 45 minutes. 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.

III. Plasmid isolation using lysozyme

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

IV. Gel electrophoresis

The presence or loss of plasmid was further confirmed by performing gel electrophoresis on 1% agarose gel. About 0.4 gm of agarose in 40 ml 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 10 µl of the respective sample was transfer into the vials containing 2 µl of DNA loading dye, the vials were briefly centrifuged again and about 12 µl of samples were loaded in the wells and electrophoresis was carried out at 100 volts 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.

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 50µg/Ml. Results are summarized in (Table 3).

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).

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.
*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.

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 6-8, Graph 5-10 and Figure 4A-4C).

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 grow on the media supplemented with antibiotic.

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 1 kb 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.

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.
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 β-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 un cured plasmid sample while no band was present in control lane (2, 3, 4, 6, 7 and 8), having cured plasmid sample of test organism. 1 Kb 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 cefoxitin 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.

References

1. Pereira PM, Vicente AF (2012) Meat nutritional composition and nutritive role in the human diet. Meat Sci 93(3): 586-592.
2. De Oliveira TL, Das Graças Cardoso M, De Araújo Soares R, Ramos EM, Piccoli RH, et al. (2013) Inhibitory activity of Syzygium aromaticum and Cymbopogon citratus (DC.) Stapf. essential oils against Listeria monocytogenes inoculated in bovine ground meat. Braz J Microbiol 44(2): 357-365.
3. Del Serrone P, Nicoletti M (2013) Antimicrobial activity of a neem cake extract in a broth model meat system. Int J Environ Res Public Health 10(8): 3282-3295.
4. Carattoli A (2003) Plasmid-mediated antimicrobial resistance in salmonella enterica. Curr Issues Mol Biol 5(4): 113-122.
5. Sáenz Y, Britñas L, Domínguez E, Ruiz J, Zarazaga M, et al. (2004) Mechanisms of resistance in multiple-antibiotic-resistant escherichia coli strains of human, animal and food origins. Antimicrob Agents Chemother 48(10): 3996-4001.
6. Carattoli A (2013) Mini review Plasmids and the spread of resistance. Int J Med Microbiol 303(6-7): 298-304.
7. Van den Bogaard AE, Stobberingh EE (1999) Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 58(4): 589-607.
8. Nguyen KV, Thi Do NT, Chanda A, Nguyen TV, Pham CV, et al. (2013) Antibiotic use and resistance in emerging economies: a situation analysis for VietNam. BMC Public Health 13: 1158.
9. Nizamuddin M (2001) Genus enormous stockhouse from northern coast of the Arabian Sea (Pakistan). Pakistan Journal of Marine Biology 7: 147-232.
10. Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A, et al. (2007) Antibacterial, antisecretory and anthelmintic activity of Azadirachta indica used to treat cholera and diarrhea in India. J Ethnopharmacol 111(3): 607-612.
11. Rohde C, Henze B (2011) Plasmid Isolation from Bacteria some fast step-by-step procedures tried out at the DSMZ.
12. Czyeska FJ, Seiter JA, Marks SN, Jay JM (1981) Culture medium for selective isolation and enumeration of gram-negative bacteria from ground meats. Appl Environ Microbiol 42(2): 303-307.
13. Hassan AN, Farooqui A, Khan A, Khan AKY, Kazmi SI (2010) Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J Infect Dev Ctries 4(6): 382-388.
14. Hâleselassie M, Taddele H, Adhana K, Kalagou S (2013) Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle city, Ethiopia. Asian Pac J Trop Biomed 3(5): 407-412.
15. Garbati MA, Al Godhairi AI (2013) The Growing Resistance of Klebsiella pneumoniae; the Need to Expand Our Antibiogram: Case Report and Review of the Literature. Afr J Infect Dis 7(1): 8-10.
16. Tassew H, Abdissa A, Beyene G, Gebre Selassie S (2011) Microbial flora and food borne pathogens on minced meat and their susceptibility to antimicrobial agents. Ethiop J Health Sci 20(3): 137-143.
17. Savant AA, Hegde NV, Straley BA, Donaldson SC, Love BC, et al. (2006) Antimicrobial-resistant enteric bacteria from dairy cattle. Appl Environ Microbiol 73(1): 156-163.
18. Szomolka A, Nagy B (2013) Multi drug resistant commensal Escherichia coli in animals and its impact for public health. Front Microbiol 4: 258.
19. Miles TD, Laughlin WM, Brown PD (2006) Antimicrobial resistance of escherichia coli isolates from broiler chickens and humans. BMC Vet Res 2: 7.
20. Phillips I, Casewell M, Cox T, De Groot B, Fris C, et al. (2004) Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data J Antimicrob Chemother 53(1): 28-52.

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21. Suffredini IB, Paciencia ML, Nepomuceno DC, Younes RN, Varella AD (2006) Antibacterial and cytotoxic activity of Brazilian plant extracts - Clusiaceae. Mem Inst Oswaldo Cruz 101(3): 287-290.

22. Cabrita MT, Vale C, Rauter A (2010) Halogenated compounds from marine algae. Mar Drugs 8(8): 2301-2317.

23. Marder HP, Kayser FH (1977) Transferable plasmids mediating multiple-antibiotic resistance in *streptococcus faecalis* subsp. *liquefaciens*. Antimicrob Agents Chemother 12(2): 261-269.