Isolation and Identification of Bacterial Isolates from House Flies in Sulaymaniya City

Dr. Ali Shihab Ahmed
University of technology, Applied Science-Biotechnology Branch/Bagdad
Email:plasmid20032003@yahoo.com
Karim Mohammad Ahmed
Foundation of Techical Education Sulaymaniya ; Agricultural Technical Institute of Bakrajo Department of Plant Protection.
Sabiha Sharif Salih
Hosptel Sulaymaniya

Received on: 6/2/2012 & Accepted on: 24/6/2012

ABSTRACT

The objective of this study was to isolate and identify bacteria found in natural association with adults of Musca domestica.In this study adults of Musca dometica were collected from four sites in Sulamani city,Kudistan,Iraq to isolate and identify bacteria on the external surface of house flies . Bacteria from fly samples isolated using the conventional isolation technique were E.coli , Pseudomonas aeruginosa , Staphylococcus albus , Staphylococcus aureus , Salmonella sp. and Klebsiella sp. The results of current study confirm that the houseflies are much more than nuisance and they pose potentially serious health risks. Consequently, the population of houseflies has to be controlled.

Key Words: Musca domestica , Vector, bacteria, antibiotic ,resistance.

عزل وتشخيص عزلات بكترية من ذباب المنزل في مدينة السليمانية

الخلاصة

تهدف الدراسة الى عزل وتشخيص البكتريا الموجودة في تداخل طبيعي مع الحشرة اليافعة Musca domestica . في هذه الدراسة تم جمع الحشرات اليافعة لMusca domestica من اربعة مواقع في مدينة السليمانية ، كردستان العراق لغرض عزل وتشخيص البكتريا من السطح الخارجي لذبابة المنزل . تم عزل البكتريا من عينات الحشرات باستخدام تقنية العزل التقليدية وهي , E .coli Pseudomonas aeruginosa , Staphylococcus albus , Staphylococcus aureus , Salmonella sp. and Klebsiella sp. اكدت نتائج الدراسة الحالية ان ذبابة المنزل اكثر خطورة على الصحة من الحشرات خارج المنزل . والبنتيجة نجد من الضروري السيطرة على تجمعات حشرة الذباب .

https://doi.org/10.30684/etj.31.1B.4
2412-0758/University of Technology-Iraq, Baghdad, Iraq

INTRODUCTION

The house fly, Musca domestica L. is well- known cosmopolitan pest. It has a worldwide distribution and is found throughout the country in close association with human activities. It receives the common name of house fly by virtue of being the most common fly found in and around houses in addition to being a nuisance pest [1] ., It is a vector of many pathogenesis carrier of over 100 different pathogenic organisms including organisms for diseases, typhoid ,cholera, bacillary dysentery, ,ophthalmia neonatorum and infantile diarrhea as well as parasitic worms [2,3] .

House flies Musca domestica are of medical and veterinary importance due to their capacity to act as mechanical vectors of microorganisms originating in animal manure and other decaying organic substrates [4]. How-ever, its classification as a "disease causing fly" as follows (1) its confirmed association with food-borne pathogenus Escherichia coli , Salmonella, and Shigella,(2)the fact that it is ecologically associated with humans (synanthropic),(3) its association with domestic vironments(endophilic) ,(4) its equal attraction to excrements and human food sources, and (5) its municative behavior that allows the house fly to easily move from heavily contaminated to human populated areas [5].

Houseflies Musca domestica have been shown to be carriers of several species of bacteria; this is because of their close association with decaying organic matters, garbage and faeces [6] .The hairy proboscis and feet with glandular hairs and pads that secrete sticky material enable the flies to pick up the pathogens on to their bodies. In addition the regurgitation of vomits and deposit of faecal droplets during feeding process contribute to the flies' ability to spread the pathogens [7,8] .The common house fly ,is a medically-important insect worldwide [9,10,11] . House flies have been implicated as vectors or transporters of various human pathogenus, including Vibrio cholerae, Entrobacteriaceae pathogens, Staphylococcus aureus, and Pseudomonas spp. [5, 9, and 12]. Transmission takes place when the fly makes contact with people or their food. As many as 500000 microorganisms may swarm over its body and legs [13]. A number of researchers have studied and isolated pathogens, such as Vibrio cholera [9], Escherichia coli 0157:H7 [2], Salmonella and Compylobacter [14, 15, and 16] from house flies and reported them as a potential source for transmission and spread of these pathogens. While feeding or resting, house flies often defecate, leaving fly specks and organisms passing through their digestive system [2]. This a simple mechanical transfer of microbes by vector whose behavior places the contaminants from decayed and diseased source they visit [6]. According to [17], flies can contaminate clean surfaces with approximately 0.1mg of food per landing. The

https://doi.org/10.30684/etj.31.1B.4
2412-0758/University of Technology-Iraq, Baghdad, Iraq

organisms associated with house flies number in the hundreds and commonly include dysentery-causing and tissue-infecting agents such as Bacillus spp., Staphylococcus spp., Enterococcus spp.,Shigella spp., Esherichia coli, Bacillus anthracis, Chlamydiales,Corynebacterium spp., and other parasitic organisms[5,18] . Synantropic flies are major epidemiologic factors responsible for spread of acute gastroenteritis, trachoma among infants and young children in developing countries and transmission of nosocomial infections with multiple antibiotic-resistance bacteria in hospitals environment [10].

Insects, such as houseflies, that develop in decaying organic material may transmit antibiotic-resistance bacteria from the manure of animals and other decaying organic substrates to residential setting. The habitats in which it develops (e.g., manure), its dependence on a live microbial community, its feeding mechanism(regurgitation),its attraction to human food, and its ability to fly long distance make this insect a very good candidate for dissemination of fecal bacteria, including human and animal pathogens[10,19,20].

In the present study we collected house flies from residential area of four locations in Sulamani city and isolated human pathogenic bacteria from the external surfaces of this flies. Afterward, we determined the susceptibility of the bacterial strains to different antibiotics.

MATERIAL AND METHODS

Collection of flies

The study was confined to the city of Sulamani. House –flies were collected in batches of 10 flies each from four different places (i.e. slaughter houses, garbage dumps, restaurant dumps, hospital dumps).Flies were caught from the selective sites during the period of study with sterilized nets and from9:00 a.m to 1:0 p.m., when the flies are active. The collected flies were placed in to sterile 150 ml container, and flies were transferred immediately to bacteriological laboratory.

BACTERIAL ISOLATION

Culture Media

The Following media were used in culturing the bacteria, Cetrimide agar, Manitol salt agar, Chocolate agar, MacConky agar, EMB agar, API-20E system.

Isolation of bacteria from flies

Bacteria from fly sample collections were isolated using the normal isolation technique. The flies were anesthetized by keeping them at 4C^{o} for 5 minute [21]. Each flies transferred individually with sterile tweezers to agar plate, flies were kept alive on plates where they moved over the surface while feeding, walking. After 1 hour at room temperature, the flies were removed and the plates were incubated at 37C^{o} for 24 hours [6]. Bacterial colonies presenting morphological differences were picked and streak on new blood agar plate's .The cultures were then observed daily for growth and all bacteria colonies subculture on to corresponding media and further incubated until pure colonies were obtained [7]. The bacteria were identified to the genus level by

colony morphology, texture and Gram staining and species level for some of them by using API-20E system. Antibiotic susceptibility test was performed and the following antibiotics were used in this study: Tetracycline, Erythromycin, Ciprofloxacin, Gentamycin, Ampicillin, Amoxicillin, Penicillin, Nalidixic acid, Ferodantin, Amikacin, Rifadin [10].

Results

From the 40 house flies collected from slaughter house, gabage dumps, hospital dumps, and restaurant dumps at Sulamani city, house flies were found to carry several species of bacteria on the external surface. This study showed that six species of bacteria were isolated from Musca domestica. The bacteria isolated from these sites as represented in Ttable (1).

The most frequent bacteria isolated from houseflies coming from all locations of the city were Pseudomonas, Staph. aureus, Staph. albus, and E-coli while the least present bacteria were Klebsiella sp. and Salmonella s*p*.(Table - 1). A total 40 house flies collected from slaughter houses, garbage dumps, restaurant dumps , hospital dumps. In general, flies may transport agents of diseases on and within their bodies. In this work, only the exterior (external surface) transmission of microorganism was studied. As mechanical vectors, this fly transferred microorganism by means of their leg or/and mouth parts. This was also reported from many other authors [15]. All flies investigated transmitted more than microorganisms were identified and demonstrated inTable (1). Figures (1, 2), show the primary screening of isolates from flies on selective media. Antibiotic resistance were tested for six bacteria cultures obtained from house flies to determine their disc sensitivity or resistance to 11 commonly used antibiotics Table (2). Among all the isolates from slaughter house *E.coli* and Pseudomonas resistance to Tetracycline , Erythromycin , Gentamycine , Ampicilin , Amoxillin ,Penicillin , Nalidixic acid , Ferodantin ,and Rifadin), and they were sensitive to Amikacin ,Ciprofloxin respectively, while *Staph aures* resistance to Erythromycine , Penicillin , Nalidixic acid , Ferodantin , Rifadin and sensitive to Tetracycline , Cipromycine , Gentamycine , Ampicillin , Amoxilline , Amikacin .

The bacteria which were isolated from garbage dump ,E.coli was resistance to Tetracycline, , Amoxcilline , Penicilline , Ferodantin , Rifadin) ,and sensitive to Cipromycine , Ampicilline Ciprofloxacin , Gentamycine , Nalidixic acid , Nalidixic acid. Staph albus resistance to all antibiotics and sensitive to Amikacin , Staph.

Aureus showed resistance to Erythromycine , Gentamycine, Ampicilline , Amoxilline , Penicilline , Ferodantin , Rifadin and sensitive to Tetracycline , Ciprofloxaxcin , Nalidixic acid , Amikacin). More ever the Klebsiella was resistance to the most antibiotics Erythromycine , Gentamycine , Ampicilline , Amoxilline , Penicilline , Ferodantin , Amikacin , Rifadin and sensitive to Tetracycline ,Ciprofloxacin , Nalidixic acid . All the bacteria that isolated from hospital dump were resistance to all antibiotics except E.coli sensitive only to Ciprofloxacin on other hand all isolated bacteria from restaurant dump show totally resistance to all antibiotics .

Figure (1): Adult house fly individually placed on agar plate for 1 hour.

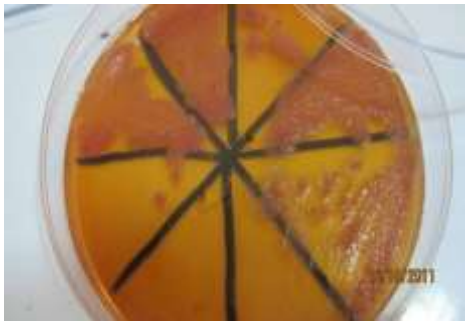

Figure (2): Bacterial colonies obtained from house fly.

Table (1): Bacteria isolated from external body surface of M. domestica collected from various sites in Sulaymaniya,Kurdistan, Iraq.

Collection sites	Means of transmission	Type of bacterial isolated and identified
Slaughter house	External surfaces	E.coli ; Pusedomonas ; Staphylococcus aureus
Garbage dump	External surfaces	E.coli ;Staph. albus ; Staph..aureus ; Klebseilla sp.
Hospital dump	External surfaces	E.coli ;Pseudomons ; Staph. aureus ; Staph. albus ; Salmonella sp .
Restaurant dump	External surfaces	E-coli 2- Pseudomonas ;Staph auerus ; Staph. albus

Collection Sites	Bacterial isolates	Types of Antibiotic*										
		Tet	**Ery**	**Cip**	**Gen**	**Amp**	**Amc**	**Pen**	**NA**	**F**	**AK**	**RF**
Slaughter house	*E.coli*	R	R	R	R	R	R	R	R	R	S	R
	Pseudo monas	R	R	S	R	R	R	R	R	R	R	R
	Staph. aureus	S	R	S	S	S	S	R	R	R	S	R
Garbage dump	*E.coli*	R	R	S	S	R	R	R	S	R	S	R
	Staph. albus	R	R	R	R	R	R	R	R	R	S	R
	Staph .aureus	S	R	S	R	R	R	R	S	R	S	R

https://doi.org/10.30684/etj.31.1B.4
2412-0758/University of Technology-Iraq, Baghdad, Iraq

	Klebsiella	S	R	S	R	R	R	R	S	R	R	R
Hospital dump	*E. coli*	R	R	S	R	R	R	R	R	R	R	R
	Pseudomonas	R	R	R	R	R	R	R	R	R	R	R
	Staph albs	R	R	R	R	R	R	R	R	R	R	R
	Staph aureus	R	R	R	R	R	R	R	R	R	R	R
	Salmonella	R	R	R	R	R	R	R	R	R	R	R
Restaurant dump	*E.coli*	R	R	R	R	R	R	R	R	R	R	R
	Psedomonas	R	R	R	R	R	R	R	R	R	R	R
	Staph. albus	R	R	R	R	R	R	R	R	R	R	R
	Staph .aureus	R	R	R	R	R	R	R	R	R	R	R

Table (2): Antibiotic susceptibility tests of identified bacteria from slaughter house, garbage dumps, restaurant dump, hospital dump at Sulaymaniya city,Kurdistan, Iraq.

*__Te__tracycline, __Ery__thromycin, __Cip__rofloxacin, __Gen__tamycin, __Amp__icillin, __Amo__xicillin, __Pen__icillin, __Na__lidixic acid, __F__erodantin, __A__mi__k__acin, __Rif__adin. (S) = Sensitive, R=Resistance

DISCUSSION

The biology and ecology of Musca domestica make it an ideal mechanical vector of human and animal pathogens. Garbage, cattle barns, poultry houses, slaughter houses, and hospitals are sites where house flies can reproduce [22]. This study showed that six species of bacteria were isolated from Musca domestica (Table - 1).Our results are in accordance with other reports which highlight the importance of house flies in caring various pathogens [23, 24,25,26] .

Many scientists indicated that the external organs of Musca domestica legs, wings, and mouth parts) constituted a large source of bacteria they isolated [24,27]. The results of this study indicated that Musca domestica could play a great role as a mechanical carrier of bacteriain this study, most of the bacteria isolated were medically important, including E.coli, Staph. aureus, Staph. albus, Pseudeomonas aeruginosa , Klebseilla , and Salmonella. These finding agree with the results of[28, 29] which presence E.coli,P. aeruginosa , K. pneumonia ,and Proteus mirabilis on the external surface of house fly collected from slaughterhouse , zoo, and hospitals .There are more studies which confirm the role of house flies on transmission of different

bacteria as a world wide agent transmission of bacteria is using different insect species and different methods of transmission. [30] isolated Salmonella enteritids ,S. infantis and S . heidelberg from house flies over poultry houses . 18 species of entero-pathogenic bacteria from different cyclorrhaphan flies were isolated in Malaysia [31] . The isolates of E.coli , Staphylococcus aureus , Pseudomonas spp., Proteus spp.,in Ahvazc city [29] . This confirm that the housefly's body can act as a mechanical vector either bacteria or many microorganisms. We examined the antibiotic susceptibility and resistance patterns of six bacteria obtained from the houseflies by using 11 commonly used antibiotics (Table 2). The types of bacteria in general were resistance to all antibiotics. These finding are in agreement with the finding of other studies which show that the enter bacteria Salmonella, E. coli, Klebsiella , Shigella isolated from house flies collected in hospital were found to be resistant to significantly more of the commonly used antibiotics that were tested than the Enterobacteria isolated from the flies caught in the streets, and Pseudomonas isolates frequently showed resistance to multiple antibiotics [32] . Multiple resistances to antibiotics are common among P. aeruginosa isolated from different clinical sources in Iran [33] . Insects such as house flies Musca domestica L. that develop in decaying organic material may transmit antibiotic-resistant bacteria from manure of animals and other decaying organic substrates to residential setting [10,19,20]. Multidrug resistance in clinical isolates has become a serious problem due to a progressive decline in the number of antibiotics that are effective for treatment of human infections [34]. It has been suggest that there is a connection between the antibiotic resistance of food animal origin, the antibiotic resistance of clinical isolates, and community health [35, 36, 37] . However, this remains a controversial issue [38, 39] because the ecology of antibiotic resistance and virulence genes in the environment is not well understood. The present study indicates that the house fly Musca domestica poses a possible health risk to communities proved that the isolated strains of bacteria were resistance to various antibiotics. In conclusion, we report that house flies collected in slaughter house garbage dump, hospital dump, and restaurant dump may be involved in the spread of antibiotic resistant bacteria and may increase the potential for human exposure to antibiotic resistant bacteria. It recommended that suitable steps must be taken to control the flies.

REFEFRENCE

[1].Moon,R.(2002). Muscide Flies(Muscidae).In:MullenGR,Durden A.editors.Medical and Verterinary Entomology.San Diego: Academic Press.p.279-301.

[2].Sasaki, T, Kbaayashi, M,,and Agui, N. (2000) .Epidemiological potential of excretion and regurgitation by Musca domestica (Diptera:Muscidae) in the dissemination of Echrchia coli o157:H7 to food.J Med Entomol.37:945-9.

[3]. Fotedar , R. , Banerjee , U.,Singh , S.,Shriniwas.,and Verma, AK . (1992b) . The housefly (Musca domestica as a carrier of pathogenic organisms in a hospital environment Hospital Infection.20:209-15.

[4]. Chakrabarti , S., Kambhampati , S., and Zurek , l . (2010). Assessment of House fly dispersal between rural and urban habitats in Kansas,USA.Journal of the Kansas Entomological Society .83 (20:172188.

[5]. Olsen, A.R. (1998). Regulatory action criteria for filth and other extraneous material III.Review of flies and food borne enteric isease.Regul.Toxicol.Pharm.28:199-211.

[6]. Holt, PS., Geden, C. J., Moor, R.W., and Gast, R.K. (2007). Isolation of Salmonella enterica serovar Enteritidis from houseflies (Musca domestica) found in rooms containing Salmonella serovar enteritidis-challenged hens.Applied and Environmental Microbiology 73:6030-6035.

[7] .Rosef, O. and Kapperud , G. (1983) .House flies (*Musca domestica*) as possible vectors of Campylobacter fetus subp.jejuni.Applied and Environmental Microbiology 45:381-383.

[8]Nazni, W.A., Seleena, B., Lee, H.L., Jeffery, J., T. Rogayah,T.A.R. and Sofian, M.A. (2005) . Bacteria fauna from the house fly, Musca domestica(L.). Tropical Biomedicine 22:225-231.

[9]. Fotedar , R. (2001) .Vector potential of house flies. (Musca domestica) in transmission of *Vibrio cholerae* in India.Acta Tropica.78; 31-34.

[10]. Graczyk , T.K. , Knight , R . , Gilman, R., and Cranfield , M . (2001) . The role of non-biting flies in the epidemiology of human infection diseases. Microbes and infection.3:231-235.

[11]. Kabkaew, L. , Manasanant , B., Banyong , K. , Somsak , P. ,Yupha , R., and Kom, S.(2007) .Comparison between east Asian .J Trop Med Public Health .38(1):38-44.

[12]. Rajjendran, J.,and Pandian, R.(2003).Microbial flora isolated from an urban population of non-biting vector Musca domestica and their susceptibility to antibiotics.Asian Microbial Biotechnol and Environ Sc.5:381-385.

[13]. Thirumalai, V., Immanual, G.,and Selvaraj, P. (2008).Vector competence of Musca domestica Linn. With reference to the virulent strains of Salmonella typhi in bus stands and markets at Madurai.Tamil Nadu. Current Biotica.2 (2):154-160.

[14]. Olsen, A.R.and Hammack, T.S.(2000).Isolation of Salmonella spp.from the ouse fly,Musca domestica L., and the dump fly, Hydrotaea aenescens(Wiedemsnn)(Diptera:Muscidae), at caged-layer house.Journal of Food Protection .63:958-960.

[15]. Nichols, G.L. (2005).Fly transmission of Campylobacter. Emerging Infectious Disease.11 (3):361-364.

[16]. Wales, A.D., Carrique-Mas, J.J., Rankin, M., Bell, B., Thind, B.B.and Davies, R.H.(2010).Review of the carriage of zoonotic bacteria by arthropods, with special reference to *Salmonella* in mites,flies and litter beetles.Zoonoses and public Health 57:299-314.

[17]. De Jesus, A.J., Olsen, A. A., Bryce, J.R., Whiting ,R.C.(2004).Quantitative contamination and transfer of Esherichia *coli* from foods by house flies,Musca

domestica L.(Diptera:Muscidae).International Journal of food Microbiology .93(2):259-262.

[18]. Nmorsi, O.P., Agbozele. G, and Ukwandu, N.C. (2007).Some aspects of epidemiology of filth flies;Musca doestica, Musca domestica vicina,Drosophilia melanogaster and associated bacteria pathogens in Ekpoma,Nigeria. Vectore-Borne Zoonotic Diseases .7:107-117.

[19]. Alam, M. J.,and. Zurek, L.(2004).Association of Echerichia coli o157:17 with houseflies on a cattle farm.Aplly.Enviromental.Microbiology.70:7578-7580.

[20]. Zurek, L., Sehal, C., and D. W. Watson (2000).Diversity and contribution of intestinal bacterial community to the development of Musca domestica (Diptera:Muscidae) larvae. Journal Med. Entomol.37:942-928.

[21]. Peter, G.,James, S0.H., Frank, K., Chong, N. A., Burstein ,C. M., and David ,R.C.(1997). Vector potential of house flies (Musca domestica) for Heilobacter pylori.Journal of Clinical Microbiology.Vol.35, No.6.

[22] Peter. S., Christopher. J., Randle. W., Moor. S.,and Richard K.(2007).Isolation of Salmonella enterica Serovar Enteritidis from Houseflies(Musca domestica)found in room containing Salmonella Serovar Enteridis-Challenged Hens. Applied Environmental Microbiology.73 (19)6030-6035.

[23]. Gurbel, JP.,Hoffman, S., Chong, FK., Burstein, NA., Mepani, C.,and Cave, Dr.(1997).Vector potential of house flies(*Musca domestica*) for *Helicobacter pylori*.Journal Clinical Microbiology.35:1300-1303.

[24]. Kobayashi, M., Sasaki, T., Saito, N., Tamura, K., Suzuk, K., Watanabe,and H., Agui, N.(1999).Houseflies not simple mechanical vectors of enter hemorrhage Eschirichia coli 0157:H7.Am .J. Trop.Med.Hyg.61:625-629.

[25]. Koura, EA.,and Kamel EG.(1990).A study of the protozoa associated with some harmful insects in the local environment.J.Egypt Soc.Parasito.20:105-115.

[26]. Pai, HH., Chen, WC.,and Peng, CF. (2003).Isolation of nontuberculous mycobacterial from nosocomial cockroaches .Journal.Hos. Infect.53:224-228.

[27]. Graczyk, T., Cranfield, R.,and Fayer R,Bixler H.(1999).House flies (Musca domestica)as transport hosts of Cryptosporidiun parvum.Am .J. Trop.Med.Hyg.61:500-504.

[28]. Vazirianzadeh, B., Setareh, S., Mahmoud, R., Hajhossien, R. , and Manijh, M. (2008).Identification of bacteria which possible transmitted by Musca domestica(Diptera:Muscide) in the region Ahvaz,SW Iran.Jundishapur Journal Microbiology.1(1):28-31.

[29]. Moosa-Kazemi, SH., Zahirnia. A., Kalantar. E., and Davari, B. (2010).Frequency of resistance and susceptible bacteria isolated from house flies. Iranian of journal of Arthropods-borne Diseases.4 (2):50--

[30]. Olsen, AR.,and Hammack, TS.(2000).Isolation of Salmonella spp.from the house fly,Musca domestica L., and dump fly,Hydrotaea aenescens (Wiedemann)(Diptera: Muscidae), at caged-layer houses.Journal of food Protection.63:958-960.

[31]. Sulaiman, S., Othman, MZ.,and Aziz AH. (2000).Isolation of enteric pathogens from synanthropic flies trapped in downtown Kula Lumpur.Journal of Vector Ecology.25:90-93.

[32]. Rahuma, N., Ghenghesh, KS., Ben Asssa, R., and Elamaari, A.(2005).Carriage by the housefly(Musca domestica)of multiple-antibiotic resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata,Libya.Annual Tropical Medicine Parasitological .99(8)795-802.

[33]. Kalantar ,E .,Motlagh, M., Lordnejad, H.,and Reshamansh, N.(2008).Prevalence of urinary tract pathogens and antimicrobial susceptibility patterns in children at 55 hospitals in Iran.Iranian J Clin Infect Dis.3(3):149-154.

[34]. Witte, W. (1999).Antibiotic resistance in gram-positive bacteria: epidemiological aspects. Journal Antimicrobial Chemotherapy.44:1-9.

[35]. Eaton, T.J., & Gasson,M.J.(2000).Molecular screening of enterococcus virulence determinants and potential for genetic exchange between food and medical isolates.Appl.Environ.Microbiol.67;1628-1635.

[36]. Salyers, A. A. (2002).An overview of the genetic basis of antibiotic resistance in bacteria and its implications for agriculture.Anim.Biotectnnol.13:1-5.

[37]. Smith,D .L., Harris, A.D.,Johnson, J.A., Silberged, E.K., and Morris, J.G.(2002).Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria .Porc.Nalt.Aca.Sci.USA 99:6434-6439.

[38]. Philips, I., Casewell, M., Cox, T., De Groot, B., Friis, C, Jones, R., Nightingale, C.,Preston,R., and Waddell, J.(2004). Antibiotic use in animals. Journal.Antimicrob.Chemother.53:28-52.

[39]. Turnidge, J. (2004).Antibiotic use in animals-prejudices, perception and realities. Journal of Antimicrobial Chemotherapy. 53:26-27.