Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(2):1103-1109. **OPEN ACCESS**

Molecular detection of *Acinetobacter baumannii* from fish meat available in market

Samiyah Tasleem[1], S.M.Moinuddin[2] Muhammad Ejazuddin[2], Attiq Ullah[3] Habib Ullah[4] and ShahKhalid[4]

[1]Department of Microbiology, University of Karachi and FUUAST-Karachi, **Pakistan**
[2] Pakistan Education, Health and Technology Innovation Consultant Group, **Pakistan**
[3]Beijing Institute of Technology, China, Lanzhou University, **China**
[4] The University of Lahore, **Pakistan**
***Correspondence: samiyahtasleem2005@yahoo.com** **Received: 15-02-2022, Revised: 29-05-2022, Accepted: 31-05-2022 e-Published: 05-06-2022**

Acinetobacter is a genus of gram-negative bacteria belonging to a class of Gammaproteobacteria. Due to intake of food contaminated with *Acinetobacter spp*, it causes many health problems. The serious health issues associated with the *Acinetobacter spp* are pulmonary diseases, hospital acquired infections, lungs infection, inflammation of meninges, gastro problems like diarrhea and bacteremia is also caused by this pathogen. 20 to 60% mortality is associated with the diseases caused by this pathogen. This disease is transmitted by the contact of one person to another person, contamination of food and water and contaminated equipment's in hospitals. Our study was directed with the aim of isolation of *Acinetobacterbaumannii* from fishes available in different markets of city area of Lahore, Pakistan. Many markets in city area of Lahore were selected for fish samples collection. *Acinetobacter baumannii* was identified by the use of microscopy, traditional biochemical identification and final verification was done by molecular techniques. From February to June, 2018, total 100 fish meat were taken from different markets in Lahore. Out of 100 fish meat samples collected from different market in Lahore 18 samples were confirmed as *Acinetobacter baumannii* by using microscopic and traditional biochemical testing techniques. These 18 samples were further processed for molecular confirmation by amplification of 16S rRNA gene. Out of 18 sample confirmed by microscopy and biochemical testing 12 samples were positive for *Acinetobacter baumannii* by PCR. Our studies conclude that *Acinetobacter baumannii* is prevalent in fish meat available in different market of Lahore. These contaminated fishes are consumed by human and it is a great public health concern. Our study also conclude that fishes should not only be examined for food-borne diseases but should also be examined for *Acinetobacter baumannii* because it is a major public health concern

Keywords: *Acinetobacter baumannii*; fishes; bacteremia; foodborne pathogen

INTRODUCTION

*Acinetobacterspp*is a genus of gram-negative bacteria belonging to a class of gammaproteobacteria. This bacterium is obligate aerobe. They have non-pigmented, pale yellowish or whitish to gray color colony (Doughari,et al. 2011). *Acinetobacter* is associated with the habitat like areas having vegetables, places that are dump and areas having hydrocarbons (Berlau J, et al. 1999). Another study finds their habitat in water, in soil, fishes of fresh water etc. (Čož-Rakovac, et al. 2002). Another study showed that *Acinetobacter* is associated with animals that produce food (Wang Y, et al. 2012) while a study done by Doughari *et al* shows their association with human (Doughari,et al. 2011). *Acinetobacter spp* have been isolated and identified from different dairy products like cheese and milk. It has also been isolated and identified from different meat products like meat of fish, chicken, beef, mutton etc. A previous study done in Lebanon by Rafei et al.2015 isolated *Acinetobacter spp*that arenon-*Acinetobacter baumannii.* They isolated the non-*Acinetobacter baumannii spp* from different meat product, milk product, cheese and vegetables. While in a current study they isolate the *Acinetobacter spp* from the vegetables that arecarbapenem resistant (Rafei R, 2015). Due to intake of food contaminated with *Acinetobacter spp*, it causes many health problems. The serious health issues associated with the *Acinetobacter spp* are pulmonary diseases, hospital acquired infections, lungs infection, inflammation of meninges, gastro problems like diarrhea and bacteremia is also caused by this pathogen. 20 to 60% mortality is associated with the diseases caused by this pathogen. This disease is transmitted by the contact of one person to another person, contamination of food and water and contaminated equipment's in hospitals (Doughari et al. 2011). Meat is most essential source for physical and mental health activities because meat is a

rich source of minerals, micronutrients, macronutrients, proteins, minerals and vitamins (Jalil H, et al. 2013). In the whole world fish and chicken meat is stored in freeze conditions between 0°C to 10°C. This storage condition make it concerning because of contamination by psychrophilic bacteria (Chouliara, 2008). Early studies showed that chicken meat and fish meat is contaminated by different genus of psychrophilic bacteria like *Acinetobacter*, *Lactobacillus*, *Moraxella*, *Pseudomonas etc* (Gill C, Newton. 1978). Currently the emerging pathogen in the world that is most worrying for institutions of health care is *Acinetobacterbaumannii.* It cause different epidemics of hospital acquired infections. Certainly, According to the American infectious disease society *Acinetobacterbaumannii* is the part of the pathogenic bacteria group named as ESKAPE. To the United States health care this is instant dangers because these all pathogens cause many hospital acquired infections and these pathogens have the ability to escape the treatment due to resistance developed to the current available antibiotics (Jacobs A. C., 2014). Currently *Acinetobacterbaumannii* have been reported to cause infections in many animals like pigs, cattle, cats, turkeys,dogs, birds,horses and many other animals (Hamouda A, et al. 2011). In china many reports are publish from 2001 to 2013. In these reports they showed that *Acinetobacterbaumannii* cause infections in ducks, fishes, pigs and cattle (Hamouda A, et al. 2011, Zhang L.N. 2012, Zhong S et al. 2014). All these report shows that *Acinetobacterbaumannii* is highly associated with the infections of animals. In 1997 a study was in china on the isolation of *Acinetobacterbaumannii* from the unhealthy mandarin fishes. This isolated bacteria cause mice death through virulence regression analysis (Xia L, et al. 2008). In horses isolation of Seven *Acinetobacterbaumannii* spp have been done from jugular catheter tips and they shows that this pathogen was only associated with localized infection (Zhong S et al. 2014). In a study done in Switzerland, they found *AcinetobacterBaumannii* infection in 17 dogs and 2 cats. Amongst these infection nine dogs died in hospital intensive care unit. The symptoms of these dogs were wounds in respiratory and urinary track and blood infections (Francey T.,et al 2000). In Belgium a study was done in Ghent University disease clinics, shows the association of the antibiotic resistant strain of *Acinetobacter baumannii* with the infections in horses. Additionally, from horses and pets *Acinetobacter baumannii* have been isolated having numerous properties and the pattern of molecular drug resistance is same as in human (Endimiani A, 2011). The infections cause by *Acinetobacter baumannii* can only be prevented by the use of antimicrobials agents in immuno compromised patients. (Wisplinghoff H., Seifert H. , 2014)

Acinetobacter baumannii is major opportunistic disease causing organism that spread mainly amongst patients in hospitals and it is prevalent in the environment of hospital (Bergogne-Berezin, E. and Towner, K. J. 1996).Amongst all the infection caused by gram negative bacteria *Acinetobacter baumannii* constitute 2 to 10% of all the infection and is a major pathogen for hospital acquired infections (Richet, H. and Fournier, P. E. 2006). *Acinetobacter baumannii* is important because of its ability to grow on any surface and to get easily antibiotic resistance (Fernandez-Cuenca, F, 2004). Many in vitro studies shows that in immune compromised animals it cause dangerous infections having death rate of 75 to 100% (Rodríguez-Hernández, M. J,2000). Currently on genomic basis there are 24 species of the genus *Acinetobacter*. It is very difficult to identify some strains of the genomic species phenotypically (Rodríguez-Hernández, M. J, 2006). In order to differentiate the various strains of this bacteria in epidemiological studies, typing methods are required because *Acinetobacter spp* is spread widely in nature (Rodríguez-Hernández, M. J, 2006). From other bacteria related to *Acinetobacter* genus can be differentiated by many commercial available kits and systems and traditional biochemical testing's. While genomic level identification is difficult because the commercial available systems have no ability to differentiate them into different genomic species (Dijkshoorn, L. 2007). Currently identification of the *Acinetobacter spp* by using molecular techniques based on 16S rDNA methods is used widely due to availability of the sequence databases for comparison (Misbah, S.et al. 2005).In our country there is limited work done on the molecular level study of *Acinetobacterbaumannii* so we proposed our study of isolation and molecular identification of *Acinetobacter baumannii* from fishmeat available in different markets of Lahore.

MATERIALS AND METHODS

Over all100 fish meat samples collection was done randomly from different markets of Lahore city. Zip bag that was irradiated was with UV was used for samples collection. The collected samples were then transported in aseptic condition in cold chain of 4^0C to UDL, university of veterinary and animal sciences Lahore where the samples were processed.

In the biosafety cabinet all the collected samples were cut and chopped into pieces by using sterile scalpel and blades. Three times the samples were washed with sterile distilled water and the samples were then washed with a 3% bleach for surface sterilization (Antwi-Agyei P, Maalekuu B. 2014). First all the chopped samples were enriched in a selective enrichment medium like baumannii medium. After enrichment of 24hour all the samples were cultured on *Leeds* Acinetobactermedium. This culturing was done in aseptic condition under biosafety cabinet. All the cultured plates were then incubated for 24hour at 37^0C.Leeds Acinetobactermedium is differential that mainly allow the growth of *Acinetobacter* species while inhibiting the growth of others (Jawad A, et al 1994).This medium contains antibiotics like cefsulodin and cephradine that cause growth inhibition of Gram-negative

bacteria and also contain vancomycin that inhibit the growth of Gram-positive. *Acinetobacter* species cause no fermentation of fructose and glucose in the Leeds Acinetobactermedium and give pink color upon growth. To obtain pure colonies sub culturing was done. To identify the pathogen various standard microbiological identification tests mainly colony morphology and traditional biochemical tests were performed. Samples were assumed positive having colony color of violet pink. Composition of the Leeds Acinetobactermedium is given in table 1.

Table 1: Composition of Leeds Acinetobactermedium

Serial number	Ingredients	Concentration
1	Agar	10 g/liter
2	acid casein hydrolysate	15 g/liter
3	neutralized soy peptone	5 g/liter
4	sodium chloride	5 g/liter
5	D-fructose	5 g/liter
6	sucrose	5 g/liter
7	D-mannitol	5 g/liter
8	L-phenylalanine	1 g/liter
9	ferric ammonium citrate	0.4 g/liter
10	phenol red	0.02 g/liter
11	vancomycin	10mg/liter
12	cefsulodin	15 mg/liter
13	cephradine	50 mg/liter

For morphological identification the colony were gram stained and then observed under microscope. Pair coccobacilli having red color were assumed as *Acinetobacter species.* For biochemical identification of *Acinetobacter species* various biochemical tests were performed. These biochemical tests include oxidase, catalase, TSI, Motility, indole, methyl red, vogues prousker, citrate utilization test and urease. As a reference manual for identification Bergey's Manual of Systemic Bacteriology was used (Vos P wad A, et al. 2011). All the positive assumed samples were confirmed by polymerase chain reaction. *GF-1*vivantis kit was used for the extraction of the DNA. The sequence of the 16-S rRNA gene primer is given in the table 2.

Table 2: Sequence of Primer used

Serial number	primer	Primer sequence
1.	Forward primer	AGAGTTTGATCCTGGCTCAG
2.	Reverse primer	TACCAGGGTATCTAATCCTGTT

The amplification mixture 25 µl composition is given in table 3.The cycling program of PCR are given in table 4

Table 3: Composition of Master Mix

Serial number	Component of master mix	Concentration
1	GoTaq® Green master mix 2X	12.5 µl
2	Forward primer AGAGTTTGATC CTGGCTCAG	2.5 µl (10 µM)
3	Reverse primer TACCAGGGTATC TAATCCTGTT	2.5 µl (10 µM)
4	DNA template	5 µl
5	PCR grad water	2.5 µl

To confirm the presence of amplified DNA by PCR, amplified DNA was run on agarose gel electrophoresis according to protocol used by Lee *et al* (28).The different fragments of DNA were separated by gel electrophoresis by operating it at 120V for 30minute. The band visualization was done by usingby UV Trans illuminator.

Table 4: Cycling program of polymerase chain reaction

Serial number	Cycling condition	Temperature	Time
1	Initial denaturation	95^0C	3minute
2	Denaturation	95^0C	1minute
3	Annealing	55^0C	1minute
4	Extension	72^0C	1minute
5	Final extension	72^0C	5minute

RESULTS

Our study was directed with the aim of isolation of *Acinetobacter baumannii* from fishes available in different markets of city area of Lahore, Pakistan. Many markets in city area of Lahore were selected for fish samples collection. *Acinetobacter baumannii* was identified by the use of microscopy, traditional biochemical identification and final verification was done by molecular techniques. From February to June, 2018, total 100 fish meat was taken from different markets in Lahore. Leeds Acinetobacter Medium was used for the isolation of *Acinetobacter species* from meat samples of fish. Pink colonies on Leeds Acinetobacter Medium were assumed to be *Acinetobacter species.* For morphological identification the all the purified pink colony were gram stained and then observed under microscope. Pair coccobacilli having red color were observed under microscope when gram staining was done. The result of gram stain is given in figure 1. All the positive assumed samples were confirmed by polymerase chain reaction. Out of 100 fish meat samples collected from different market in Lahore 18 samples (18%) were confirmed as *Acinetobacter baumannii* by using microscopic and traditional biochemical testing techniques while 82 samples (82%) were negative. (Table 6) The colonies of the bacteria on Leeds Acinetobacter Medium is given in

figure 2.These 18 samples were further processed for molecular confirmation by amplification of 16S rRNA gene. Out of 18 sample confirmed by microscopy and biochemical testing 12 (12%) samples were positive for *Acinetobacter baumannii* by PCR. (Table 6) The results of the PCR bands are given in figure 3. The results of the biochemical tests are given in the table 5.

The comparative result of the traditional biochemical test and PCR is given in the figure 4.

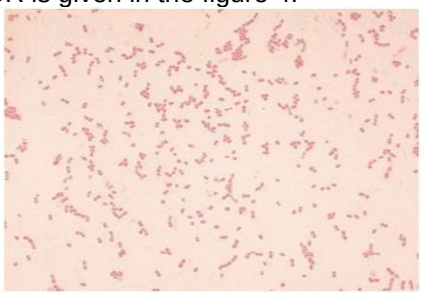

Figure 1: Gram staining of the isolate

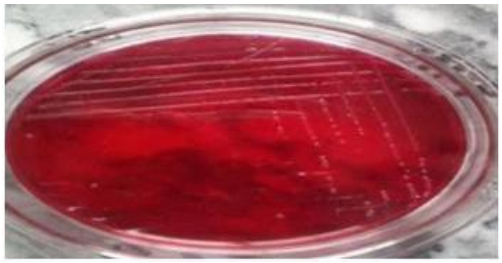

Figure 2: Colonies of the isolate on the Leeds Acinetobacter Medium

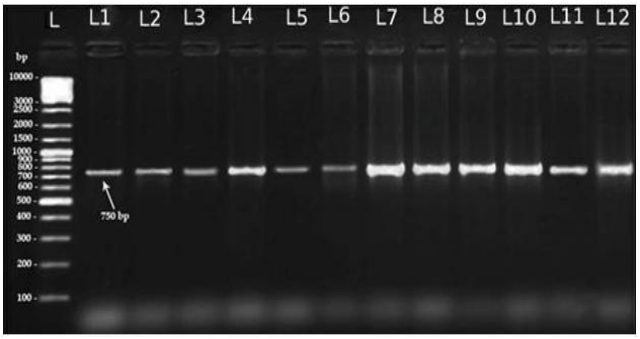

Figure 3: Bands of the amplicon on the gel electrophoresis

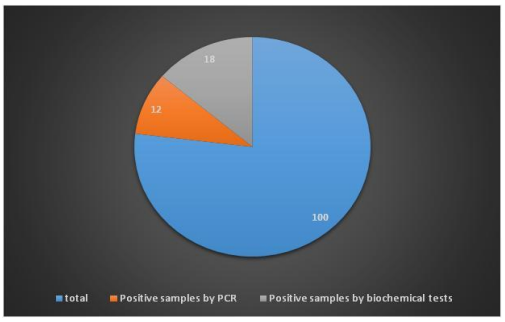

Figure 4: Comparative prevalence by PCR and Traditional biochemical testing

Table 5: Results of biochemical tests

Serial Number	Biochemical Test	Result
1.	Oxidase,	negative
2.	Catalase	positive
3.	TSI	No H_2S production, non-glucose fermenter, fructose fermenter
4.	Motility	negative
5.	Indole	negative
6.	Methyl red,	negative
7.	Vogues Prousker,	positive
8.	Citrate utilization test	positive
9.	Urease.	negative

Table 6: Prevalence of *Acinetobacter baumannii* in fish meat by PCR and Traditional biochemical testing

Serial No	Total sample	No of Positive sample by traditional biochemical Tests (%)	No of Negative sample by traditional bioch emical tests (%)	NO of Positive samples by PCR (%)	NO of negatives amples by PCR (%)
1.	100	18 (18%)	82 (82%)	12(12%)	88(88%)

DISCUSSION

Acinetobacter spp is a genus of gram-negative bacteria belonging to a class of gammaproteobacteria. This bacteria is obligate aerobe. They have non-pigmented, pale yellowish or whitish to gray color colony (Doughari et al. 2011). *Acinetobacter* is associated with the habitat like areas having vegetables, places that are dump and areas having hydrocarbons (Berlau J, et al. 1999). Another study find their habitat in water, in soil, fishes of fresh water etc. (Čož-Rakovac R, et al. 2002). Another study showed that *Acinetobacter* is associated with animals that produce food (Wang et al. 2012) while a study done by Doughari *et al* shows their association with human (Doughari ,et al. 2011). *Acinetobacter spp* have been isolated and identified from different dairy products like cheese and milk. It has also been isolated and identified from different meat products like meat of fish, chicken, beef, mutton etc. A previous study done in Lebanon by Rafei *et al* isolated *Acinetobacter spp* that arenon-*Acinetobacter baumannii.* They isolated the non-*Acinetobacter baumannii spp* from different meat product, milk product, cheese and vegetables. While in a current study they isolate the *Acinetobacter spp* from the vegetables that are carbapenem resistant (Rafei R, 2015). *Acinetobacter spp*is a group of heterogeneous microorganism in literature which is distributed mostly in the environment and can easily be found. These have been isolated from birds, animals and fishes in different studies (Jung J, Park W (2015) 。Very few researchers have evaluated the infections caused by *Acinetobacter baumannii* in animals. Amongst early research studies

Acinetobacter baumannii have been isolated from ducks, pigeons, chickens, donkeys, rabbits, pets, cattle, sheep, goats etc (Endimiani et al. 2011, Al Atrouni et al. 2016)

Our study was directed with the aim of isolation of *Acinetobacter baumannii* from fishes available in different markets of city area of Lahore, Pakistan. Many markets in city area of Lahore were selected for fish samples collection. *Acinetobacter baumannii* was identified by the use of microscopy, traditional biochemical identification and final verification was done by molecular techniques.From February to June, 2018, total 100 fish meat were taken from different markets in Lahore. Out of 100 fish meat samples collected from different market in Lahore 18 samples (18%) were confirmed as *Acinetobacter baumannii* by using microscopic and traditional biochemical testing techniques while 82 samples (82%) were negative. These 18 samples were further processed for molecular confirmation by amplification of 16S rRNA gene. Out of 18 sample confirmed by microscopy and biochemical testing 12 (12%) samples were positive for *Acinetobacter baumannii* by PCR.

It is very difficult to identify some strains of the genomic species phenotypically (Towner, K. 2006). In order to differentiate the various strains of this bacterium in epidemiological studies, typing methods are required because *Acinetobacter spp* is spread widely in nature (Towner, K. 2006). Currently, more accurate identification needsmethods that are DNA-based which are increasingly used. However our study used both PCR and traditional biochemical testing methods and PCR give 12 positive bands in 18 samples confirmed by traditional biochemical methods.Gu et al reported *Acinetobacter spp* forthe first time from mandarin fish. During his study he identify the bacteria by using biochemical testing (Gu, T.Z, 1997). Xia et al. conducted a study in which they showed that *Acinetobacter baumannii* cause infection inchannel catfish. This bacteria was identified by using biochemical and 16S rRNA gene sequence analysis (Xia, L.,et al. 2008)

CONCLUSION

Our study concludes that *Acinetobacter baumannii* is prevalent in fish meat available in different market of Lahore. These contaminated fishes are consumed by human and it is a great public health concern. Our study also concludes that fishes should not only be examined for food-borne diseases but should also be examined for *Acinetobacter baumannii* because it is a major public health concern. Nonetheless, stringent sanitation and HACCP must be applied alongside the complete production chain of meat until it reach the retail setting for selling, to find out the main point for identification of the main source to stop the pathogen from contamination of the product and then stop the spread to the consumer level.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

Department of Microbiology, University of Karachi and FUUAST-Karachi, Pakistan Education, Health and Technology Innovation Consultant Group, Beijing Institute of Technology, China, Lanzhou University China, The University of Lahore, Pakistan for provide resources and support.

AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example Dr. Samiyah Tasleem designed and performleed the experiments and also wrote the manuscript. Dr. S. M. Moinuddin, Muhammad Ejazuddin, Attiq Ullah, Habib Ullah and ShahKhalid was work as assistant researcher during experiment. All authors read and approved the final version.

REFERENCES

Al Atrouni A, Joly-Guillou ML, Hamze M, et al. (2016) Reservoirs of non-baumannii acinetobacter species. *Front Microbiol* 7: 49.

Antwi-Agyei P, Maalekuu B. 2014. Determination of microbial contamination in meat and fish products sold in the Kumasi metropolis (A Case Study of Kumasi central market and the Bantama market). MRJASSS 2, 38-46.

Bergogne-Berezin, E. and Towner, K. J. (1996). *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev., 9: 148–165.

Berlau J, Aucken H, Houang E, Pitt T. 1999. Isolation of *Acinetobacter spp* including A. baumannii from vegetables: implications for hospital-acquired infections. Journal of Hospital Infection 42,201-204.

Chouliara E, Badeka A, Savvaidis I, Kontominas MG. 2008. Combined effect of irradiation and modified atmosphere packaging on shelf-life extension of chicken breast meat: microbiological, chemical and sensory changes. E. Food Research Technology 226, 877-888.

Čož-Rakovac R, Strunjak-Perović I, Popović NT, Hacmanjek M, Šimpraga B, Teskeredžić E. 2002. Health status of wild and cultured sea bass in the

northern Adriatic Sea. Veterinary Med-Czech. 4, 7, 222-226.

Dijkshoorn, L.; Nemec, A. and Seifert, H.(2007). An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii.* Nature Rev. Microbiol., 5:939- 951.

Doughari HJ, Ndakidemi PA, Human IS, Benade S. 2011. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes and environment 26, 101-112.

Endimiani A, Hujer KM, Hujer AM, et al. (2011) *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. *J Antimicrob Chemother* 66: 2248–2254.

Endimiani A., Hujer K. M., Hujer A. M., Bertschy I., Rossano A., Koch C., Gerber V., Francey T., Bonomo R. A., Perreten V. (2011) *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. *J Antimicrob Chemother* 66: 2248-2254.

Fernandez-Cuenca, F.; Pascual, A.; Ribera, A.; Vila, J.; Bou, G.; Cisneros, J. M.; Rodriguez-Bano, J.; Pachon, J. and Martinez-Martinez, L.(2004). Clonal diversity and antimicrobial susceptibility of *Acinetobacter baumannii* isolated in Spain. A nationwide multicenter study: GEIH-Ab project (2000). Enferm. Infecc. Microbiol. Clin., 22:267-271.

Francey T., Gaschen F., Nicolet J., Burnens A.P. (2000) The role of *Acinetobacter baumannii* as a nosocomial pathogen for dogs and cats in an intensive care unit. *J Vet Intern Med* 14: 177-183.

Gill C, Newton K. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. Meat Science 2, 207-217.

Gu, T.Z., Lu, C.P., Chen, H.Q., 1997. Acinetobacter baumannii, a novel pathogen of acute epidemic in mandarin fish (Siniperca chuatsi). Microbiol. 2, 104–106

Hamouda A., Findlay J., Al Hassan L., Amyes S. G. (2011) Epidemiology of *Acinetobacter baumannii* of animal origin. *Intn J Antimicrob Agents* 38: 314-318.

Jacobs A. C., Blanchard C. E., Catherman S. C., Dunman P. M., Murata, Y. (2014) An ribonuclease T2 family protein modulates *Acinetobacter baumannii* abiotic surface colonization. *PloS one* 9: e85729.

Jalil H, Hussain SS, Siddiqi AF. 2013. An empirical study of meat supply chain and prices pattern in Lahore (Pakistan): A case study. Journal of Supply Chain Management System (JSCMS),Vol. 2(4) October 2013, p. 44-52

Jawad A., Hawkey P. M., Heritage J., Snelling A. M. 1994. Description of Leeds Acinetobacter Medium, a new selective and differential medium for isolation of clinically important Acinetobacter spp., and comparison with Herellea agar and Holton's agar. J. Clin. Microbiol. 32: 2353–2358

Jung J, Park W (2015) Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. *Appl Microbiol Biotechnol* 99: 2533–2548.

Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT,2012. —Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 21(380), 219-29.284, 285–288.

Misbah, S.; Hassan, H.; Yusof, M. Y.; Hanifah, Y. A. and AbuBakar, S. (2005). Genomic species identification of *Acinetobacter* of clinical isolates by 16S rDNA sequencing Singapore Med. J., 46(9) : 461.

Rafei R, Hamze M, Pailhoriès H, Eveillard M, Marsollier L, Joly-Guillou M-L, Dabboussi F, Kempf M. 2015.Extrahuman epidemiology of *Acinetobacter baumannii* in Lebanon. Applied Environmental microbiology 81, 2359-2367.

Richet, H. and Fournier, P. E.(2006). Nosocomial infections caused by *Acinetobacter:* a major threat worldwide. Infect. Control Hosp. Epidemiol. 27:645-646.

Rodríguez-Hernández, M. J.; Pachón, J.; Pichardo, C.; Cuberos, L.; Ibáñez- Martínez, J.; García-Curiel, A.; Caballero, F.; Moreno, I. and Jiménez-Mejías, M. (2000). Imipenem, doxycycline and amikacin in monotherapy and in combination *Acinetobacter baumannii* experimental pneumonia. J. Antimicrob. Chemother. 45:493-501.

Rosa R., Arheart K. L., Depascale D., Cleary T., Kett D. H., Namias. N., Pizano L., Fajardo-Aquin Y., Munoz-Price L. S. (2014) Environmental exposure to carbapenem-resistant *Acinetobacter baumannii* as a risk factor for patient acquisition of *A. baumannii*. *Infect Control Hosp Epidemiol* 35: 430-433.

Towner, K. (2006). The Genus *Acinetobacter*. Prokaryotes,6:746-758.

Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB.2011.Bergey's manual of systematic bacteriology: 3, TheFirmicutes3, (Springer Science & Business Media).

Wang Y, Wu C, Zhang Q, Qi J, Liu H, Wang Y, He T, Ma L, Lai J, Shen Z. 2012. Identification of New Delhi metallo-β-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PloS one 7, e37152.

Wisplinghoff H., Seifert H. (2014) Epidemiology and clinical features of *Acinetobacter baumannii* infections in humans. Berl Munch Tierarztl Wochenschr. 127:11-12.

Xia L., Xiong D., Gu Z., Xu Z., Chen C., Xie J., Xu P. (2008) Recovery of *Acinetobacter baumannii* from diseased channel catfish (*Ictalurus punctatus*) in China. *Aquaculture* 284: 285-288.

Xia, L., Xiong, D., Gu, Z., Xu, Z., Chen, C., Xie, J., Xu, P., 2008. Recovery of Acinetobacter baumannii from diseased channel catfish (Ictalurus punctatus) in

China. Aquaculture

Zhang L.N. (2012) Isolation and Identification of One Strain of *Acinetobacter baumannii* in Infected Beef Cattle. *Anhui Agric Sci* 40: 2717-2719.

Zhong S., Li B., Wang D., Zhang Y. b., Zhu F.j., Yang Y., Yang S., Chi S., Zhu, R. (2014) Isolation and identification of *Acinetobacter baumannii* isolated from Capra hircus. *Chin J Prev Vet Med* 4: 170-175.