ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Detection of Some Gram Positive bacteria from Respiratory tract and Intestine of wild mice and rats

[1]Osama Sadiq Al-edani; [2]Afaf AbdulRahman Yousif

1. College of Veterinary Medicine /University of Baghdad

2 Dept. of Internal and Preventive Vet. Medicine/ College of Veterinary Medicine /University of Baghdad/ Iraq**.** College of Veterinary Medicine /University of Baghdad

Manuscript Info

Manuscript History:

Received: 14 August 2014
Final Accepted: 25 September 2014
Published Online: October 2014

Key words:

Staphylococcus aureus,Streptococcus, mice, rats

Corresponding Author

Afaf Abdulrahman Yousif, E.mail:Afaf_a.rahman @yahoo.com

Abstract

This study was undertaken to determine Gram positive Bacteria from respiratory tracts and intestine of mice and rats trapped from Baghdad and Karballa Provinces. A total of 152rodents{70 house rat (*Rattusrattus*) and 82 house mouse (*Musmusculus*)} were caught from four types of sites included; Residential area (houses), Flour mills, Fruit/ vegetable shops and Grocery shops. These sites were randomly selected in each of the three localities for rats and mice collection.Specimens from respiratory system and digestive system after euthanatizing the rodents by ether were collected in aseptic conditions by using transport swabs for bacteriological culture. Diagnostic standard methods were used for identification of bacteria.

The results showed that *Staphylococcus aureus* and *Streptococcus* were isolated from respiratory tract (lung and Bronchus) only, negative bacterial results from intestine samples. From 82 mice and 70 rats, the percentage of*Staphylococcus aureus* was 30.5% &42.9% respectively. While the percentage of *Streptococcus* 4.9% & 5.7% respectively. The results concluded that the percentage of *Staphylococcus aureus* isolates was higher than the percentage of *Streptococcus*isolation from both mice and rats.

Introduction

Rodentia is the largest order of placental mammals, representing about 43% of the total number of mammalian species with approximately 2,050 species divided into 28 families (Huchon*et al*., 2002). Rodents can harbor and transmit pathogens that can cause severe disease in humans, companion animals and livestock. Such zoonotic pathogens comprise more than two thirds of the currently human pathogens (Jacob *et al*., 2014).

Staphylococci are Gram-positive cocci, approximately 1 µm in diameter, that tend to occur in irregular clusters resembling bunches of grapes. The name derives from the Greek words staphyle and kokkos for a 'bunch of grapes' and a 'berry' respectively. At least 30 Staphylococcus species occur as commensally on skin and mucous membranes; some may act as opportunistic pathogens causing pyogenic infections (Quinn*et al* 2011). Some species of *Staphylococci* are found only in animals and are recognized as veterinary pathogens, such as *Staphylococcus hyicus* and *Staphylococcus intermedius*. Humans may become colonized with these organisms by close contact with animals (e.g., veterinarians, farmers, zoo workers) (Koneman et al., 2006).

Staphylococcus aureus may cause a variety of infectious diseases ranging from relatively benign skin infections to life-threatening systemic illnesses skin infections include simple folliculitis and impetigo, as well as furuncles and carbuncles involving subcutaneous tissues and causing systemic symptoms, such as fever. *Staphylococcus aureus* is frequently isolated from postsurgical wound infections, which may serve as a nidus for development of systemic infections (Konemanet *al*., 2006).

The *Streptococci* is Gram-positive cocci that occur in pairs or chains of varying lengths. Each coccus is about 1µm in diameter. They are facultative anaerobes, catalase-negative, oxidase-negative and non-motile. The streptococci are fastidious and require the addition of blood or serum to media for growth (Markey *et al*., 2014).

The classification of *Streptococci* into major categories has been based on a series of observations over many years, colony morphology and hemolysis on blood agar, serologic specify of the cell wall group-specific substance and other cell wall or capsular antigens, biochemical reactions,resistance to physical and chemical factors and ecologic features (Jawetz*et al*., 2013).

The purpose of this study was to determine the prevalence of *Staphylococcus aureus* and *Streptococcus* Spp in wild rodents trapped in the two provenances in Iraq.

Materials:

Animals:

One hundred fifty two rodents, 70 house rat (*Rattusrattus*) and 82 house mouse (*Musmusculus*) were caught from Karballa and Baghdad city. Four types of sites included; Residential area (houses), Flour mills, Fruit/ vegetable shops and Grocery shops. These sites were randomly selected in each of the three localities for rats and mice collection.

Traps:

Fifty live traps were set at different sites. The traps were baited with bread soaked in cooking oil, fresh vegetables, fruits and peanuts butter as adopted by Asgari*et al*. (2007). Each live trap with captured animal was tagged indicating locality. They were then brought to laboratory and kept in separate cages. Each specimen was physically restrained as mentioned by (Jann*et al*., 2003).

Restraining of rats and mice

Rodents in the traps were putting in a thick transparent polythene bag that helped to observe the movement of animals. A cotton swab soaked in ether were putting in the bag to anaesthetize the animal as described by (Singla*et al*. 2008).

Dissection of Captured rodents

After euthanatizing the rodents by using ether, each rodent was disinfected by 70% ethyl alcohol and dissected individually, the abdominal cavity was opened by using sterile disposable plate under sterile hood.

Specimens from respiratory system and digestive system were collected in aseptic conditions by using transport swabs for bacteriological culture (Markey *et al*., 2014).

Bacteriological culture

Two types of media used in this study, blood agar and Mannitol salt agar. These media prepeared in laboratory according to manufacture instructions,

The biochemical tests used in identification of *Staphylococcus aureus* KOH test, Catalase, Oxidase, O/F, Coagulase, Mannitol fermentation, Bacitracin susceptibility, Hemolysis on blood agar.

The biochemical tests used in identification of *Streptococcus* spp. were KOH test, Catalase, Oxidase, O/F, Bacitracin susceptibility, Hemolysis on blood agar and Optochin susceptibility

API staph

This system is used to confirm the results of biochemical test for presemptive diagnosis of *Staphylococcus aureus.*

Ethics committee approval:- The research was approved by ethics committee of College of Veterinary Medicine/ University of Baghdad.

Results and Discussion

Isolation and identification of *Staphylococcus aureus*.

Cultural characteristics on blood agar showed round, smooth, white to yellow color and glistening. Colonies tend to appear substantial and opaque with double zone of hemolysis.Microscopically the isolated bacteria appeared as gram-positive cocci in arrangement of bunches of grapes.OnMannitol salt agar the colonies were appeared round, smooth, yellow in color, Figure (1). Biochemical results listed in table (1).

Table (1) Results of biochemical test for *Staph. aureus*

Test	*Staphylococcus* spp.
KOH test	+
Catalase	+
Oxidase	-
O/F	Fermentative

Coagulase	+
Mannitol fermentation	+
Maltose fermentation	+
Bacitracin susceptibility	Resistant
Hemolysis on blood agar	+ (-)

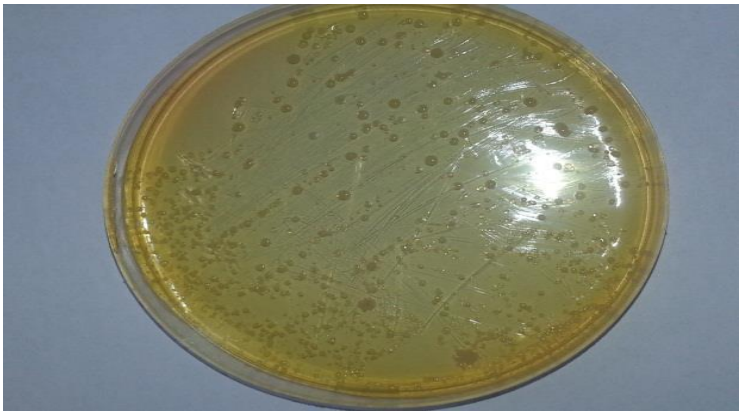
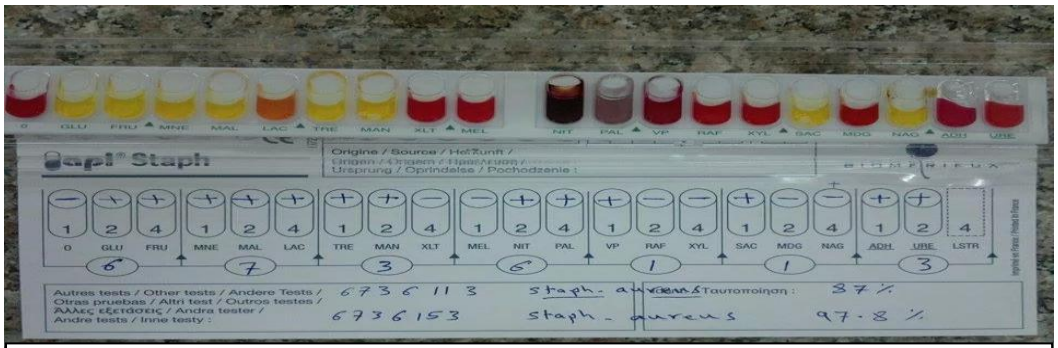


Figure 1:*Staphyllococcusaureus* on mannitol salt agar

Results of API tests to confirm *Staphylococcus aureus*in addition to primary identification by other tests, table (2), figure (2)

Table (2) API staph results for isolated *Staphylococcus aureus*

Tests	Substrate	*Staphylococcus aureus*
GLU	D-Glucose	+
FRU	D-Fructose	+
MNE	D-Mannose	+
MAL	D-Maltose	+
LAC	D-Lactose	+
TRE	D-Trehalose	+
MAN	D-Mannitol	+
XLT	Xylitol	-
MEL	D-melibiose	-
NIT	Potassium nitrate	+
PAL	B-naphthyl phosphate	+
VP	Sodium pyruvate	+
RAF	D-raffinose	-
XYL	D-xylose	-
SAC	D-saccharose	+
MDG	Methyl-aD-glucopyranoside	-
NAG	N-acetyl-glucosamine	+
ADH	L-arginine	+
URE	urea	+



Figure, (2): API staph. for identification of *Staphylococcus aureua*

4.1.2: Isolation and identification of *Streptococcus Spp*.

The colonies of *Streptococcus* spp. appeared as small (about 1mm) round, smooth, pale-white color and opaque or translucent with alpha, bata or gamma hemolysis**.** Under the microscope the bacteria appeared as gram-positive cocci round or oval shaped arrangement in single, pair or chain, and then the isolated colonies give different results by biochemical tests. Positive result to KOH and negative to catalase and oxidase, fermentative to O/F test. Bacitracin and Optochin susceptibility give different results which indicated different spp. of streptococci, table (3), and figure (3,4).

Table (3) Biochemical tests for *Streptococcus spp*.

Test	*Streptococcusspp.*
KOH test	+
Catalase	-
Oxidase	-
O/F	fermentative
Bacitracin susceptibility	+ (-)
Hemolysis on blood agar	+
Optochin susceptibility	+ (-)

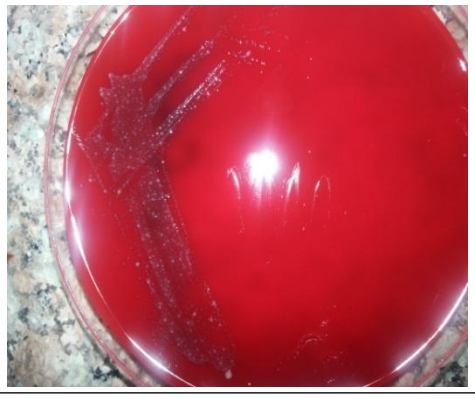


Figure (3) *Streptococcus Spp*. on blood agar

Figure (4) Bacitracin test (inhibition)Optochin (resistant) above

Samples from respiratory tract of mice and rats revealed that 25 isolates (30.5%) of *Staphylococcus aureus*were isolated from mice and 30 isolates (42.9%) in rats. While *Streptococcus spp.* was isolated from rats was

(4) isolates (5.7%) and in mice (4) isolates (4.9) (Table, 4). All samples from intestine gives negative results to Gram positive bacteria. Table (5) shows the distribution of *Staphylococcus aureus*&*Streptococcus spp.* in respiratory tracts of mice and table (6) showed the distribution of these bacteria in respiratory tracts of rats.

Table (4) Numbers of isolated gram positive bacteria in Baghdad and Karbala provinces.

organs	Bacteria	Mice (82)	%	Rat (70)	%	Total
Respiratory system (lung and bronchus	*Staphylococcus aureus*	25	30.5	30	42.9	55 (36.18%)
	Streptococcus spp.	4	4.9	4	5.7	8 (5.2%)

Table (5) Distribution of G+bacteria in mice in Baghdad and Karaballa Provinces

Organs	Bacteria	Mice in Baghdad(24)	%	Mice in Karballa(58)	%	Total
Respiratory system (lung and bronchus	*Staphylococcus aureus*	8	33.3	17	29.3	25
	Streptococcus spp.	0	0	4	6.9	4

Table (6) Distribution of G+bacteria in rats in Baghdad and Karballa Provinces

Organs	Bacteria	Rats in Baghdad(24)	%	Rats in Karballa (58)	%	Total
Respiratory system (lung and bronchus	*Staphylococcus aureus*	21	58.3	9	26.5	30
	Streptococcus spp.	3	8.3	1	2.9	4

The bacterial isolation of respiratory tract gives the high incidence of *Staphylococcus aureus* in rats and mice (42.9%, 30.5%) respectively, and the total percentage of *Staph. aureus* in our study was (36.1%) which was agreement with Himsworth*et al.* (2014) which was found that the percentage of carrier of *Staph. aureus* in rats (Rattus spp.) was (40.1%), also our study agreement with Sequin *et al*. (1986) who found that the proportion of carrier of *Staph. aureus* in rats (Rattusnorvegicus) was (53%).

Our study was compatable with other study which responsible for distribution of bacteria in wild mice and rats and with laboratory mice and rats, Baker (1998) found that most commonly, infection with *Staphylococcus aureus* occurs in the skin and subcutaneous tissues, but it may also be found in the upper airways, lungs, conjunctiva, and other tissues.National Research Council*(1991) mentioned that*Staphylococcusaureus is a gram-positive, coagulase-positive coccus that commonly inhabits the skin, upper respiratory tract, and lower digestive tract of many animals, including laboratory rodents, in which it occasionally causes disease. Disease frequently occurs following physiologic changes in the host such asimmunosuppression and stress. A variety of clinical presentations have been reported in rats and mice.

Our study we were not comparative the percentage of isolation of *Streptococcus spp.* in mice and rats with other studies because we were not found any references about this percentage and the isolated streptococcus didn't typed or grouped by Lancefield group system but the National Research Council(1991) mentioned that *Streptococcus pneumoniae* is a gram-positive diplococcus commonly found in laboratory rodent colonies. More than 80 strains, grouped by capsular type, have been reported. Transmission is primarily via aerosol from infected humans.

The organism is considered a commensal under most conditions, although host strain susceptibility differences have been reported by Takashima et al(1996).

Also the National Research Council(1991) found a carrier state is established in the nasal passages and middle ears. Infection begins in a bronchopulmonary segment and spreads centrifugally. The infection spreads from the lung to the pleura, pericardium, and, via septicemic spread, to the rest of the body. The affected lung is first edematous, then becomes consolidated and eventually is cleared of cellular debris.

This study concluded that the Gram positive bacteria especially *Staph. aureus* distributed in a high percentage in rodentswhich are responsible for transmitting bacterialdiseases to Human.

References:

Asgari, Q., M. H. Motazedian, D. Mehrabani, A. Oryan, G. R. Hatam, S. M. Owji and H.Paykari (2007). Zoonotic cutaneous leishmaniasis in Shiraz, Sourthern Iran: A molecular, isoenzyme and morphologic approach. J. Res. Med. Sci., 12(1): 7-15.

Baker, D.G (1998) Natural Pathogens of Laboratory Mice, Rats, and Rabbits and Their Effects on Research.ClinMicrobiol Rev. Apr 1998; 11(2): 231–266

Himsworth CG, Miller RR, Montoya V, Hoang L, Romney MG, et al. (2014) Carriage of Methicillin-Resistant Staphylococcus aureus by Wild Urban Norway Rats (Rattusnorvegicus). PLoS ONE 9(2): e87983. doi:10.1371/journal.pone.0087983.

Huchon D.; Madsen O.; Sibbald M. J. J. B.; Ament K.; Stanhope M. J.Catzeflis F.; De Jong W. W.; and Douzery E. J. P. (2002), Rodent phylogeny and a Timescale for the Evolution of Glires: Evidence from an Extensive Taxon Sampling using Three Nuclear Genes, Mol. Biol. Evol., 19, 1053.

Jacob J.; Ulrich R. G.; Freise J.; schmolz E. (2014),Monitoring populations of rodent reservoirs of zoonotic diseases. Projects, aims and results, BundesgesundheitsblattGesundheitsforschungGesundheitsschutz, 57(5):511-8.

Jann H., L. Gerald and H. Van (2003).Handbook of Laboratory Animal Science. 2nd Ed. Vol. 1, CRC Press, LLC. 379-381.

Jawetz, (2013) Melnick, &Adelberg's, Medical microbiology, McGraw-Hill, 2013

Koneman (2006) color atlas and textbook of diagnostic microbiology, Lippincott Williams & Wilkins, Sixth edition, 2006.

Markey, B.; Leonard, F.; Archambault, M.; Cullinane, A. and Maguire, D. (2014):"Clinical veterinary microbiology", second ed. Mosby Elsevier, china.

National Research Council.(1991) Infectious diseases of mice and rats: a report of the Institute of Laboratory Animal Resources Committee on Infectious Diseases of Mice and Rats. Washington, D.C: National Academy Press.

Quinn P. J., Markey B. K., Leonard F. C., FitzPatrick E. S., Fanning S., Hartigan P. J., Veterinary Microbiology and microbial disease, second edition, Wiley-Blackwell, 2011.

Sequin B.;Boucaud-Maitre Y.; Quenin P.; and Lorque G. (1986), Epidemiologic evaluation of a sample of 91 rats (*Rattusnorvegicus*) captured in the sewers of Lyon, ZentralblBakteriolMikrobiolHyg A, 26 (4): 539-46.

Singla L. D.;Singla N.; Parshad V.R.; Juyal P.D.; and Sood N.K. (2008), Rodents as reservoirs of parasites in India, Integrative Zoology, 3(1), 21.

Takashima, K., Tateda, K; Matsumoto, T; Ito, T; Iizawa,Y; Nakao, M and Yamaguchi. K. (1996).Establishment of a model of penicillin-resistant *Streptococcus pneumonia*e pneumonia in healthy CBA/J mice. J. Med. Microbiol.45:319–322.