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Isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) from *Rattus rattus* from Adhamiyah district in Baghdad governorate

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Abstract
This study focused on the isolation of methicillin resistance *Staphylococcus aureus* (MRSA) from black rats (*Rattus rattus*) from Adhamiyah district. A Total of 30 black rats (*Rattus rattus*) used in this study. Specimens from the upper respiratory tract, feces, and urine were collected in aseptic conditions for bacteriological culture. Standard diagnostic methods were used to isolate Gram-positive (+ve) bacteria. All samples were cultured on mannitol agar, a differential media for *staphylococci* (*Staph*), and incubated at 37°C for 24 hours. Gram stain was achieved to identify Staph bacteria. Also, the biochemical tests and the API Staph system were applied for identification of S*taphylococcus aureus*. The results of this study showed that twenty-eight rats out of thirty were harbored *Staphylococci*. In this study, four samples of upper pharyngeal swab were negative for *Staphylococcus aureus*, while the other 26 samples were positive. In urine samples, 11 samples were negative, and the rest (19 samples) were positive for *Staphylococcus aureus*. All examined rats showed no *Staph* growth in fecal samples. The isolated staphylococci distributed as coagulase-negative (*S. xylosus*, 2 (3.57); *S. epidermidis*, 6 (10.71); and *S. sciuri*, 3 (5.35), and coagulase positive (*S. aureus*, 45). Out of these forty-five isolates, 26 (45.42%) were from the deep pharyngeal swap and the rest 19 (33.92 %) were isolated from urine. The occurrence of MRSA was investigated by disc diffusion method. The results indicated that out of the 45 isolates, 42 (93.9%) and 3 (6.1%) were sensitive and resistance, respectively, to the Methicillin. All MRSA was obtained from urine. However, no *Staphylococci* were isolated from feces. In conclusion, this study approved that MRSA isolated from black rat (*Rattus rattus*) from Adhamiyah district could play a crucial role in spreading diseases to human and animal via dried urine, which is un-visible by the naked eyes. Consequently, the authors recommend another future studies to investigate in details the actual role of black rats in the transmission of infections to man and another animal.

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Introduction

Staphylococci are diverse ubiquitous opportunistic colonizers of human epithelia involved in nosocomial infections that cause diseases of major importance in both human and animals, ranging from minor skin infections to life-threatening bacteremia as well as septicemia (Aklilu *et al.*, 2010). Systemic infections include endocarditis, osteomyelitis, meningitis, and toxemic syndromes. It is a highly resistant non-spore forming and can survive in dryness for extended periods, rendering it impossible to eradicate from the environment. *Staphylococcus aureus* produces a highly heat-stable enterotoxins that cause gastroenteritis in humans and predominantly incriminated in staphylococcal food poisoning (Weese, 2010).

Approximately 20% and 60% of the human populations carry *S. aureus* either as long term or intermittently, respectively (Chambers, 2001). The presence of *S. aureus* not often indicates an infection. It can be found as normal commensals on the axillae, perineum, the nasopharynx and anterior nares (Paterson *et al., 2012*). In addition, *S. aureus* can also be present in many domesticated animals such as cats, dogs, horses (Chiller *et al.*, 2001; Wertheim *et al*., 2005), pigs, cows (Kluytmans *et al*., 1997; Bedirli *et al.,* 2004; Khudaier *et al.,* 2013) and rats (Kato *et al*., 1995). *Staphylococcus aureus* has the ability to developed resistance to antibiotics rapidly, mainly methicillin and many others, giving the rise for the term methicillin-resistant *Staphylococcus aureus* (MRSA) and become a worldwide public health problem (Petinaki *et al*., 2001; Maddox, 2011). Lately, a new methicillin resistance mechanism gene, mecC was incriminated in MRSA isolated from humans and animals rendering it tenacious and potentially destructive (Walther *et al*., 2012; Harrison *et al.,* 2013; Khudaier *et al*., 2013; Paterson *et al.,* 2014; Porrero *et al.,* 2014). The MRSA pose a serious health care impact due to higher mortality rates, increased antibiotic resistance and treatment costs (Roberts *et al.,* 2009).

Earlier, the MRSA was considered as a human infection, until it was isolated in a dairy cow with mastitis (Devriese *et al.,* 1972) and in pigs (Stefani *et al.,* 2012). Recently, there is evidence of MRSA transmission between human-to-animals and vice versa (Umaru *et al.*, 2014). Rates of MRSA infection higher than 50% have been reported in the USA, South Korea (77.6%), Vietnam (74.1%), Taiwan (65%) and Hong Kong (56.8%) and decline below 50% in Africa, China and Europe (Meja *et al.*, 2010; Stafeni *et al.*, 2012). Outbreaks of Community-Associated MRSA is mostly seen in populations such as sports teams (Collins and O'connell, 2012), prisons (Palavecino, 2004), day care centers (Simmonds *et al.*, 2008), military quarters (Marchese *et al.*, 2000) homeless people and intravenous drug abusers (Levy *et al.*, 2013). Furthermore, MRSA was isolated from most companion animal species, e.g., horses, dogs and to a less extent cats (Morgan *et al*., 2000; Loeffler and Lloyd, 2010; Chomel and sun, 2011) as well as many captive wildlife animals (van der Mee-Marquet *et al.*, 2014). Animals may act as asymptomatic carriers. *Staphylococcus aureus* infection occurs following physiologic changes in the host such as immunosuppression and stress. Once pathogen gained entrance through mucous membranes or the digestive system, it is excreted into mucus and urine. Records refer to the isolation of MRSA from Wild rat, (Himsworth *et al*., 2013), wood mice (Gomez *et al.*, 2014) red deer, Iberian ibex, vulture, wild boar (Porrero *et al.*, 2014), cottontail rabbit and a lesser yellow migratory shore bird (Wardyn *et al.*, 2012). In abattoirs, *S. aureus* was isolated from food handlers that subsequently contaminates

carcasses (Broens *et al*., 2011). Contaminated skin, feces, infected organs and water used in meat processing are the vital sources of contamination (Gilbert *et al*., 2012).

MRSA recurrent infection is responsible for killing more people, approximately 19,000, in the USA than AIDS and its incidence is rising globally. A high infection recurrence (17%) was found in injuries. Almost 95% of *S. aureus* strains are penicillin resistant, worldwide, due to plasmid encoded penicillinase readily transferable via transduction or conjugation (CDC, 2010). There are scarce studies regarding MRSA in Iraq. So, this study intended to isolate and determine the occurrence of MRSA from black rat (*Rattus rattus*) in Capital Baghdad, in addition to show the possible route of *Staphylococci* dispersing by rats.

Materials and Methods

Thirty black rats (*Rattus rattus*) were caught from garbage and old buildings at Adhamiyah district. They were then brought to laboratory and kept in big transparent polythene cage that helped to observe the movement of animals for two hours (Asgari *et al*., 2007). Thereafter, the rats were caught from tail and IP injected with 0.1 mL of the anesthesia (9:1, Ketamine + Xylazine) per 100 gm/ BW as described by (Struck *et al*., 2011). The rats were handled according to the standardized international animal care and use. Deep pharyngeal swab, feces pellets from rectum and urine were collected aseptically for bacteriological investigation (Islam *et al.,* 2014). All samples were streaked on blood agar, mannitol salt agar, Congo-red agar and nutrient agar (Freeman *et al*., 1989; Mathur *et al*., 2006). Gram Stain and biochemical (catalase, oxidase and coagulase) tests supported by API Staph System were carried out to confirm the identification of isolates. Bacterial isolates were subjected for antimicrobial susceptibility test according to Modified Kirby-Bauer method by using Methicillin 5µg/disc (Morello *et al*., 2006). The diameter of the zone of inhibition was measured and compared values listed in a standard chart (Vandepitte *et al.,* 2003). All media and chemicals used in this study were prepared according to manufacturer guidelines.

Results and Discussion

Role of rats in transmitting diseases

Rodents, particularly rats, are widely incriminated to be the source of most human diseases and reported to have a high frequency of bacterial infections. They harbor a wide variety of microorganisms and become among the most common source of zoonotic pathogens (Himsworth *et al.,* 2014). For instances, leptospirosis (Guerra, 2009), *Klebsiella oxytoca*, *Enterococcus faecalis*, *Proteus mirabilis* and *E coli* are commonly identified in rodent urine causing UTI (Holcombe *et al*., 2006; Gibbs *et al.,* 2007). Many outbreaks etiology of rat origin have been still remained obscure. The occurrence of bacteria in rat in this study confirm that idea. Moreover, in Iraq, there is scarce knowledge about MRSA in rats.

Out of 30 feral rats trapped in this study, 28 rats were positive to staphylococci and the rest showed negative results (Table.1). Despite whether the two apparently free rat included, all rats could carry mixed infection besides *staphylococci*. The data reported in

this study is in agreement with previous study (Al-Edany, 2015). The specimens were collected from upper respiratory tract, intestine and urine. *Staphylococcus* coagulase positive recorded 45 (53, 57%), while 11 (13, 09%) were *Staphylococcus* coagulase negative. *Staphylococcus* coagulase positive, referring to *Staphylococcus aureus*, were isolated from upper respiratory tract (57.77 %), urine (42.22` %) with no any evidence in feces (Table.1). This finding was compatible with Baker, (1998), who found that infection with *Staphylococcus aureus* occurs in urinary tract and in the upper airways most commonly. In 1991, the NRC refer to a gram-positive, Coagulase-positive coccus that commonly colonizes the upper respiratory tract and can be found in urine and causes disease (NRC, 1991). In 2008, Van den Broek and his colleagues demonstrated MRSA in rats living on pig farms in the Netherlands, a years after identifying MRSA in black rats trapped in downtown-Tokyo (Kato *et al.,* 1995). Thereafter, methicillin-resistant *Staphylococcus sp was reported* in Canada (Himsworth *et al*., 2014) and, later on, the isolation of MRSA from wild rat and wood mice was confirmed (Himsworth *et al.,* 2013 Gomez *et al.,* 2014).

Bacterial isolation and identification

The specimens collected from (pharyngeal swab, urine and feces) showed a wide variety for staph growth. Forty nine isolates have the ability to grow on the Mannitol salt agar, which is considered as a selective and differential media for genus *Staphylococcus*. Unlike the non-mannitol-fermenters, *S. aureus* had the ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones and turned the color of the medium from pink to yellow (Figure.1). The bacterial morphology was observed microscopically as Gram-positive cocci arranged in grape-like irregular clusters (Figure.2) (Benson, 2001). On nutrient agar, the colonies revealed round, smooth, and raised, mucoid and glistening appearance (Figure.3), which was similar to that described by Benson, (2001). Moreover, the isolated bacteria revealed β haemolytic zone on blood agar (Figure.4).

Table 1. Shows the isolation of MRSA and other Staphylococci from urine, feces and deep Pharyngeal swab of trapped rats.

Black Rats (n)	Type of samples	*Staphylococcus*				MRSA (%)
		Epidermidis	*sciuri*	*xylosus*	*Aureus*	
28	Urine	2	0	2	19	3(6.1)
	Feces	0	0	0	0	0
	Deep pharyngeal	4	3	0	26	0
2	Urine, Feces, Deep pharyngeal	-ve	-ve	-ve	-ve	0
Total= 30		6	3	2	45	3

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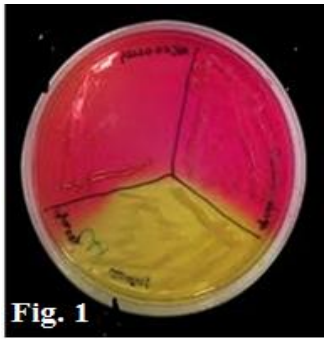


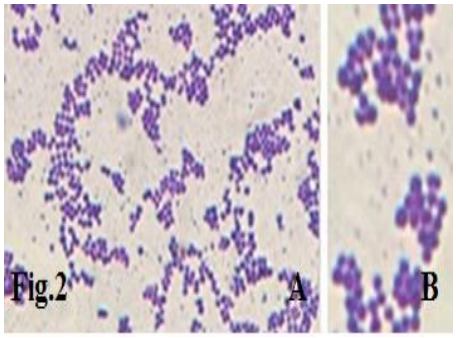
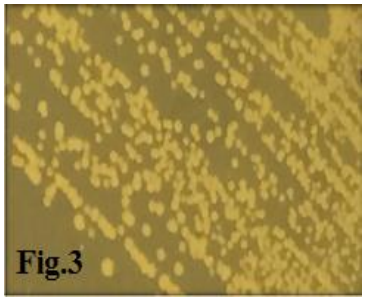


Figure .1. Shows mannitol salt agar inoculated with *Staphylococcus aureus* showing mannitol fermentation (yellow) and *Staphylococcus epidermidis* showing growth but no fermentation of mannitol (pink)
Figure .2. Shows Gram stain of *Staphylococcus aureus* Cells typically occur in clusters. The cell wall readily absorbs the crystal violet stain (A, 100x; B, 400x).



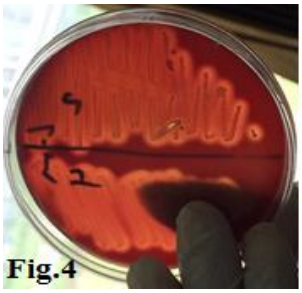
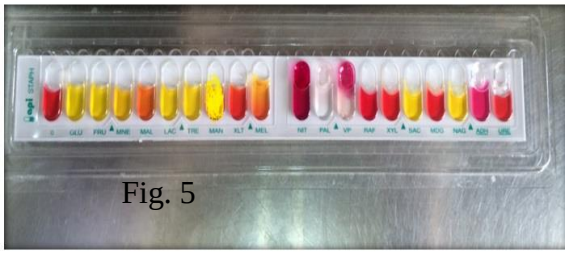


Figure .3. Shows *Staphylococcus sp*., the colonies appeared round, smooth, raised, mucoid and glistening on nutrient agar.
Figure .4. Shows the β haemolysin production of *Staphylococcus aureus* on the human Blood agar.

All the 45 isolates gave positive results and this differentiates Staphylococcus from genus *Streptococcus* which gives negative results (Laurent *et al*., 2012). Moreover, the 45 isolates gave negative results to the oxidase test which preformed to differentiate Staphylococcus from genus Micrococcus that usually gives positive result (Collee *et al*., 1996). The bacterial isolates were confirmed, by the API Staph system (Figure.5), which

was used for a precise and accurate species identification as *S. aureus* (n=5), S. *epidermidis* (n=6), *S. sciuri* (n=3) and *S. xylasue* (n=2).



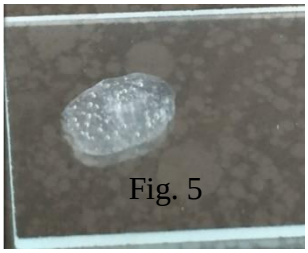


Figure .5. Shows the Api Staph system for the identification of *Staphylococcus aureus.*
Figure .6. Positive catalase reaction. The Catalase Test is used to detect whether or not an unknown bacterium has the enzyme, catalase. All members of the genus *Staphylococcus* have the catalase enzyme. The catalase test is the differential test between the genera *Staphylococcus* and *Streptococcus*.

After isolates identification at generic level, the coagulase test was performed to identify the bacterial isolates at the species level. From the total 90 isolates, 45 isolates (50%) showed the ability to produce coagulase enzyme (coagulase +ve), and 11 isolates (8.8%) were coagulase-negative. Coagulase production was detected by using the tube coagulase test which is employed for the detection of secreted extracellular free coagulase that reacts with coagulase reacting factor (CRF) in plasma to form a Complex, which is thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma. While slide coagulase test uses to detect cell wall associated coagulase (bound coagulase) or also known as clumping factor. It cross-links α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual coccus sticks to each other and clumping is observed (Kateete *et al.,* 2011). Formation of fibrin is a potent pathogenic criteria for *S. aureus* (Dominique *et al.,* 2012). Slime layer production by most *S. aureus* isolates in this study approved by Congo red agar. This result is in agreement with the previous study (Akiyama *et al.,* 1997), who found that 50% of *S. aureus* isolates showed strong slime layer on the Congo red agar and 40% was indeterminate producer and only 10% showed negative result (pink colonies). Also, this result is in agreement with Turkyilmaz and Kaya, (2006), who showed that 77.8% of coagulase positive *staphylococcal* isolates were slime producer. *Staphylococcus aureus* has wide array of virulence factors. Of these, extracellular toxins and surface structures that facilitate tissue colonization, immune evasion, and tissue destruction (O'Neill *et al.*, 2007). *Staphylococcus* protein A inhibits the phagocytosis of the organism (Schallenberger *et al.,* 2008) and the biochemical properties including carotenoid (Liu *et al.*, 2005; Clauditz *et al.*, 2006). Besides, catalase production (Figure 6) confirmed in this study, is an important survival factors for detoxifying ROS, and thereby protecting the bacteria against desiccation (Sanz *et al.,* 2000; Horsburgh *et al.,* 2001).

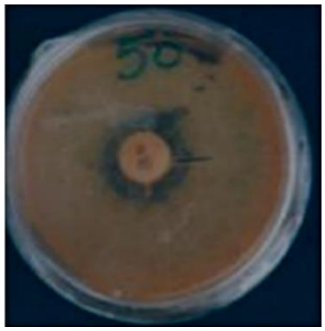


Figure 7. Shows the detection of Methicillin Resistance *S. aureus* (MRSA) by antibiotic disc diffusion method using Methicillin disc (ME 5µg/disc)

The conformation of MRSA (Figure. 7) among these isolates recorded only 3 (6.1%), while the rest 42 were methicillin sensitive. Also, the results showed that the highest rate of MRSA was obtained from rat urine followed by pharyngeal swab. These results agreed with previous study (Almeida and Jorgensen, 1984) and supported by Köck *et al.,* (2013), who reported MRSA from screening samples at a varying proportion among isolates from clinical specimens ranging between 0% in cerebrospinal fluid, 8% in blood cultures and 14% in deep respiratory fluids. Rat saliva carry many pathogens, of these *Streptobacillus moniliformis*, and can infect humans causing fatal rat bite fever (Banerjee *et al.,* 2011). There are many pathogens spread from one host to another via several routes, including direct transmission, blood-sucking arthropods, ingestion, or sexual contact. In this study, rat urine and pharyngeal swab were the more predictable way of spreading *staphylococci* rendering dried rat urine or rat sharing us our food the most dangerous way for spreading zoonotic diseases. This result agreed with the results of Al-Edany, (2015). He recovered *Staphylococcus aureus* from respiratory tract as the most prevalent gram positive aerobic bacteria from the trapped rats and found negative results for Gram +ve bacteria in the intestine. Moreover, Khudaier *et al.,* (2013) isolated staphylococci from cow milk and human pharyngeal swab in nosocomial infection among which many multi drug resistant *Staphylococcus aureus*. In addition, this study isolated MRSA from 3 urine samples (1.6 %) from total *Staphylococcus aureus* isolates (Table.1). These results are in agreement with the previous studies Netherlands, downtown-Tokyo and downtown-Vancouver, Canada (Kato et *al.,* 1995; Van den Broek *et al*., 2008; Himsworth *et al.,* 2013). The potential for pest-to-human MRSA transmission, is a particularly concerning in impoverished, inner-city neighborhoods, where factors associated with poverty may promote pest infestations (Himsworth *et al.,* 2013). Rats carry many pathogens because of their close contact with garbage and sewage. In developed countries like Sweden and USA, the outlet of Wastewater Treatment Plants still produce the *staphylococcal* mecA genes as high as 5×10^2 per 100 mL (Börjesson *et al.*, 2010; Goldstein *et al.*, 2012). Moreover, many pathogens are transmitted through rat urine (*Leptospira interrogans, Seoul Hantavirus),* saliva (*Streptobacillus moniliformis*), while rat feces act as a major source of infectious organisms for humans, including resistant strains of *Salmonella spp.* and *E. coli*, causing intestinal disease in humans and are associated with a significant global health burden (Meerburg *et al*., 2009; Duquette and Nuttall, 2012; Himsworth *et al*., 2013).

It is concluded that *Staphylococcus aureus* is the most prevalent pathogen in the respiratory tract and urine of the rats, which could be a reservoir and may transmit MRSA to human and animals. Further detailed molecular and epidemiological studies are needed to confirm the relation between pathogens harbored by black rat and human.

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