Detection of methicillin or multidrug resistant *Staphylococcus aureus* (MRSA) in locally produced raw milk and soft cheese in Baghdad markets

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Accepted on:29/5/2013

Summary

In order to investigate the presence of methicillin or multidrug resistant *Staphylococcus aureus* in food-chain especially Cows raw milk and white raw soft cheese and its whey, a total of 30 samples were collected randomly from different markets in Baghdad Province during December 2012 till February 2013, in which samples were analyzed by a standard isolation protocols of food microbiology with some modification processing by new, modern and rapid technology tools such as chromogenic medium Baird-Parker agar, Electronic RapID™ Staph Plus Code Compendium Panel System (ERIC®) Dryspot Staphytect Plus and Penicillin Binding Protein (*PBP2'*) Plus assays; as well as, studying the susceptibility of isolates to different selected antibiotics. The results profile showed isolation, identification, confirmation and enumeration of 10 (33.4%) isolates of MRSA as 4 (13.4%) isolates from raw milk and 6 (20%) isolates from white raw soft cheese with its whey. These findings suggest presence of MRSA type in locally produced raw milk and soft cheeses in Baghdad markets thus recommended to monitoring these products periodically to inshore public health.

Keywords: *Staphylococcus aureus*, Methicillin, Raw milk, Soft cheese.

Introduction

Methicillin or multidrug resistant *Staphylococcus aureus* is genetically well equipped to survive food processing technology and host defense strategies. Therefore mimicking behavior of this super pathogen will reduce the risk of contamination of food producing animals and cases of food poisoning in man by verifying a new novel strategies in food chain and hospitals through modifying a hazard analysis and critical control points plans (HACCP strategies) about environmental epidemiology of MRSA populations from good management manufacturing practices to consumers with the aid of new, rapid and precise tools for isolation, differentiation and identification of MRSA (chromogenic media to genetic engineering) especially in epidemic and unknown regions by studying the geographical distribution of these interesting bacteria as a survey in man and animals especially food chain (1 and 2).

Staphylococcus aureus is one of the major bacterial pathogens which cause clinical infection and food poisoning cases that is also an emerging concern in veterinary medicine and animal agriculture (3 - 5).

Unhygienic treatment of food has to be considered as a major risk of contamination and *Staphylococcal* food poisoning is often associated with highly manually handled food (6). Asymptomatic food handlers may harbor *S. aureus* and can contaminate food during preparation (7).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged globally as a significant public health / antimicrobial resistance problem both in human and in veterinary medicine, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of food in which enterotoxigenic *Staphylococci* have grown and produced toxins (2). *Staphylococcal* enterotoxins are considered a potential biological threat because of their stability at high temperatures (100©C for 1 h) and ability to incapacitate individuals for several days to two weeks (8). *S. aureus* is present on the skin and mucosa of food-producing animal reservoirs, such as ruminants and it is frequently associated to subclinical mastitis leading to contamination of milk and dairy products (9).

S. aureus possesses many adhesion proteins on its surface, but it is not known how they interact with each other to form stable interactions with the substrate (10). Resistance to methicillin is often accompanied by resistance to other antibiotic (11). The dramatic

increase in the number of antibiotic multi-resistance *Staphylococci* coupled with the slow development of new anti-infective agents has renewed interest in the development of immunotherapeutic directed against *S. aureus*, previous vaccination attempts using killed or live attenuated bacteria or selected bacterial subunits, produced only partial immunity, may be due to antigenic variation phenomenon (12 and 13). The objective of this study was to evaluate the microbiological safety, relative to standards of food regulations, of raw milk and soft cheeses made in various places across the Baghdad markets, by testing the presence of *S. aureus* especially MRSA type in raw milk and soft cheeses.

Materials and Methods

Collection and Processing of Samples: In order to investigate the presence of *Staphylococcus aureus* (MRSA) from locally produced raw milk and white raw soft cheese with whey in Baghdad markets, a total of 30 samples were collected randomly from different regions and markets in Baghdad (College of Veterinary Medicine field, Abu-Ghraib and Al-Fudhaliyah) during December 2012 till February 2013. The samples were analyzed and processed according to standard reference food microbiological protocols (14 - 16).

Isolationand Identification procedure: Consult to dairy microbiological procedures; pure isolated single colonies on Baird-Parker agar with standard criteria of coagulase-positive *Staphylococcus aureus* produce black, shiny, convex colonies (2-4mm size) with entire margins and clear zones, with an opaque precipitation zone after 48 hours (i.e. double clear and opaque zones), were enumerated, picked up and recultured on double strength power trypton soya bean yeast extract broth (TSB-YE) at 35-37©C for 24 hours, then transferred to double strength power trypton soya bean yeast extract agar (TSA-YE) at 35-37©C for 24 hours, after that inoculated universal and bijous slant bottles preserved inside a refrigerator with 2-3 drops of 0.02% thiomersal solution as pure seeds or nucleus for other identification procedures. Directly milk samples were inoculated on chromogenic selective and differential Baird-Parker agar at 35-37©C for 18-48 hours, and indirectly resuscitated on TSB-YE at 35-37©C for 18-24 hours then streaked on Baird-Parker agar at 35-37©C for 18-48 hours. Directly cheese samples with whey were macerated and emulsified by 2% buffered sodium citrate inside a stomacher for 5-10 minutes then streaked with a sterile cotton swabs on Baird-Parker agar at 35-37©C for 18-48 hours, and Indirectly without sodium citrate, macerated then resuscitated on TSB-YE at 35-37©C for 18-24 hours then streaked on Baird-Parker agar at 35-37©C for 18-48 hours. Purification and Identification (Confirmation) procedures were done via Electronic RapIDTM Staph Plus Code Compendium Panel System (ERIC®) with Installation ERIC® CD and standard color differential chart and online ATCC Codes, for the biochemical identification of *Staphylococcus* species. Pigmentation on mannitol salt agar, slide and tube coagulase for rabbit and human plasma with DNase activity were done. Haemolysis Pattern on sheep and human blood agar. Antibiogram assay: antibiotic susceptibility test and penicillin binding protein (PBP2) extraction and agglutination test (14 -17).

Study isolates sensitivity to selected antibiotics were tested according to a standard method of Clinical Laboratory Standards Institute CLSI (National Committee for Clinical Laboratory Standards NCCLSs, 18) and was followed in this account of the Kirby-Bauer disc diffusion method (17 and19).

The data were analyzed according to statistical software, Statistical Package for the Social Sciences (SPSS, version 21, 2013), including Cross Tabulation, Chi-square analysis and Z values to checking significance differences among study isolates in accordance with Steel and Torri (20).

Results and Discussion

Generally there were multi-interconnected and complex factors (direct and indirect) for percentages of isolation and distribution and frequency of MRSA populations in Iraqi environment due to poor or insufficient hygienic measurements (contamination and pollution) and post processing contamination in food chain especially after 2003, all these

complex scenarios leads to development of emerging outbreaks of multidrug resistant microbes and adaptation tropism properties of these armamentarium virulent pathogens in man and animals primarily via foods\feeds mediators resulting in a disease or asymptomatic carriers. The results in (Tables, 1 - 5) showed significant isolation of 10 isolates of MRSA populations from regions of College of Veterinary Medicine field, Abu-Ghraib and Al-Fudhaliyah, in which all isolates showed resistance to different selected antibiotics especially Methicillin especially from samples of white raw soft cheese, which may indicates unhygienic processing strategies (no-thermal treatment of raw milk, contaminated milk equipment especially milk cans, asymptomatic maid milkers' carriers, flies, insects, polluted water, retailing situations, etc.) as well as presence of MRSA food poisoning cases in Iraqi environment, low number of samples and time of collection (climatic factor), presence of chronic infectious foci (biofilm problems) in animals fomites and the role of hospitals as a nosocomial factor in a disease history. Milk and soft cheese were ideal growth media for most pathogens (21 and 22) but, cheese represents the major reservoirs of MRSA populations may be due to its sequestered tropism nature especially its whey, pre- and post-contamination and cross-contamination processing. The coincidence of MRSA isolation may reveal the selective new technology in resuscitation of sub-lethally damaged Staphylococcus aureus from samples of raw milk and white raw soft cheese and its whey, especially the vital components of Baird-Parker agar with the double strength power of TSB-YE and TSA-YE tools. From scientific and hygienic points of view, the isolation percentages were higher than expected in accordance with the similar researches in nearby countries, which may reflex high level of contamination and development of resistance in these pathogens due to partially abuse of antibiotics in therapy or as growth promoters especially in Cows. These results are unaccepted in USA, UK and Canada as restricted legislations especially in USA with zero-tolerance strategy of any MRSA cells in foods\feeds and their products, and the ratio of isolation if reached further than 5% this may indicate a redline risk with forcing banding laws about products from these epidemic countries (22 and 23).

These findings suggest presence of MRSA type in locally produced raw milk and soft cheeses in Baghdad markets thus recommended to monitoring these products periodically to inshore public health.

Table, 1: Isolation percentages of mannitol and coagulase positive *Staphylococcus aureus* isolates from selected Regions in Baghdad Markets.

Region	Samples Number	Positive *Staph. aureus* on Baird-Parker agar	Mannitol and Coagulase positive Isolates	% From 10	Total % From 30
College field	10	7^{Ca}	1^{Cb}	10^{C}	3.4^{C}
Abu-Ghraib	10	10^{Ba}	3^{Bb}	30^{B}	10^{B}
Al-Fudhaliyah	10	13^{Aa}	6^{Ab*}	60^{A}	20^{A}
Total	30	30	10		33.4

A,B,C: Indicate significant differences vertically at level (P≤0.05). a,b: Indicate significant differences horizontally at level (P≤0.05). * indicate highest isolation percent of *Staph. aureus* from Al-Fudhaliyah region.

Table, 2: Isolation percentages of Mannitol and Coagulase positive *Staphylococcus aureus* isolates from Raw Milk in Baghdad Markets.

Region	Raw Milk Samples				
	Samples Number	Positive *Staph. aureus* on Baird-Parker agar	Mannitol and Coagulase positive Isolates	% From 5	Total % From 15
College field	5	8^{Ba}	1^{Ab}	20^{B}	6.7^{B}
Abu-Ghraib	5	7^{Ba}	2^{Ab*}	40^{A}	13.4^{A}
Al-Fudhaliyah	5	11^{Aa}	1^{Ab}	20^{B}	6.7^{B}
Total	15	26	4		26.8

*** indicate highest isolation percent of *Staph. aureus* from Abu-Ghraib region.**

Table, 3: Isolation percentages of Mannitol and Coagulase positive *Staphylococcus aureus* isolates from Raw Soft Cheese in Baghdad Markets.

Region	Raw Soft Cheese Samples with its whey				
	Samples Number	Positive *Staph. aureus* on Baird-Parker agar	Mannitol and Coagulase positive Isolates	% From 5	Total % From 15
College field	5	2^{Ba}	0^{Cb}	0^{C}	0^{C}
Abu-Ghraib	5	10^{Aa}	1^{Bb}	20^{B}	6.6^{B}
Al-Fudhaliyah	5	10^{Aa}	5^{Aa*}	100^{A}	33.4^{A}
Total	15	22	6		40

A,B,C: Indicate significant differences vertically at level (P≤0.05). a,b: Indicate significant differences horizontally at level (P≤0.05). * indicate highest isolation percent of *Staph. aureus* from Al-Fudhaliyah region.

Table, 4: Isolation percentages of Mannitol and Coagulase positive *Staphylococcus aureus* isolates according to Sample Type in Baghdad Markets.

Sample Type	Samples Number	Positive *Staph. aureus* on Baird-Parker agar	Mannitol and Coagulase positive Isolates	% From 15	Total % From 30
Raw Milk	15	26^{Aa}	4^{Bb}	26.7^{B}	13.4^{B}
Soft Cheese	15	22^{Ba}	6^{Ab*}	40^{A}	20^{A}
Total	30	48	10		33.4

A,B,C: Indicate significant differences vertically at level (P≤0.05). a,b: Indicate significant differences horizontally at level (P≤0.05). * indicate highest isolation percent of *Staph. aureus* from Al-Fudhaliyah region.

Table, 5: Pooled percentages of resistance, intermediate and susceptible isolates for selected antibiotics.

Antibiotics	Resistant %	Intermediate %	Susceptible %
Methicillin	$10\ (100)^{Aa}$	None $(0)^{Cc}$	None $(0)^{Dc}$
Oxicillin	$10\ (100)^{Aa}$	None $(0)^{Cc}$	None $(0)^{Dc}$
Penicillin	$10\ (100)^{Aa}$	None $(0)^{Cc}$	None $(0)^{Dc}$
Ampicillin	$10\ (100)^{Aa}$	None $(0)^{Cc}$	None $(0)^{Dc}$
Cephalexin	$6\ (60)^{Ba}$	$1\ (10)^{Ab}$	$3\ (30)^{Ab}$
Augmentin	$10\ (100)^{Aa}$	None $(0)^{Cc}$	None $(0)^{Dc}$
Aztreonam	$4\ (40)^{Ba}$	$1\ (10)^{Ab}$	$5\ (50)^{Cc}$
Fusidic acid	$8\ (80)^{ABa}$	$1\ (10)^{Bb}$	$1\ (10)^{Cb}$
Vancomycin	$6\ (60)^{Ba}$	$3\ (30)^{Bc}$	$6\ (60)^{Ab}$
Total %	66^{A} (75.86)	6^{C} (6.89)	15^{B} (17.24)

A,B,C: Indicate significant differences vertically at level (P≤0.05). a,b: Indicate significant differences horizontally at level (P≤0.05).

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التحري عن العنقوديات الذهبية المقاومة للمثيسيلين أو المضادات الحيوية في الحليب الخام والجبن الطري المنتج محليا في أسواق بغداد

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الخلاصة

لغرض التحري عن وجود المكورات العنقودية الذهبية المقاومة للميثيسيلين أو المضادات الحيوية في السلسلة الغذائية لاسيما الحليب الخام والجبن الطري للأبقار ، جمعت 30 أنموذج عشوائيا من مختلف الأسواق في محافظة بغداد خلال كانون الأول 2012 وحتى شباط 2013 ، حيث تم تحليل النماذج حسب الطرائق القياسية لميكروبيولوجيا الأغذية مع بعض التحويرات بأستخدام أدوات التكنولوجيا الجديدة والحديثة والسريعة مثل الوسط الصباغي المتخصص بيرد باركر أجار،العدة الصباغية الالكترونية السريعة للعنقوديات الذهبية و أختبار التلازن الشريحي المتخصص مع أختبار تلازن جين المقاومة للبنيسيلين والمضادات الحيوية المتخصص، ودراسة قابلية العزلات للمضادات الحيوية المنتخبة. أظهرت النتائج عزل وتشخيص المكورات العنقودية الذهبية بنسبة 10 (33.4٪) عزلات: 4 (13.4٪) عزلات من الحليب الخام و 6 (20٪) عزلات من الجبن الطري الخام. هذه النتائج تشير الى تلوث الحليب الخام والأجبان الطرية المنتجة محليا في أسواق بغداد بجرثومة العنقوديات الذهبية المقاومة للمثيسيلين والمضادات الحيوية وبالتالي توصي الدراسة ألى مراقبة هذه المنتجات بشكل دوري للحفاظ على الصحة العامة للمستهلك.

الكلمات المفتاحية: العنقوديات الذهبية، المثيسيلين، الحليب الخام، الجبن الطري.