

## Prevalence and Antimicrobial Resistance Profiles of *Staph. Aureus* Isolated from Animal Origin

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### Abstract

*S. aureus* which causes food poisoning, suppurative lesions and mastitis is one of the most serious bacteria causing community acquired infection. A total of 191 random samples were collected as follow 52 samples from raw meat, 51 samples from raw milk, 45 samples from poultry and 43 samples from lesion swabs (abscess, wounds and sputum of animals) for isolation of *S. aureus* and detection of its sensitivity to various antimicrobial drugs. One sample of raw meat was positive for *S. aureus*, two samples of raw milk one of them was from mastitis case were positive for *S. aureus*, sputum sample from dog with respiratory manifestation was positive for *S. aureus*, abscess sample from ear of cat was positive for *S. aureus*, with total of five *S. aureus* isolates (9.5%). All this isolate was positive to slide coagulase test. According to antimicrobial resistance patterns, *S. aureus* isolates were extremely resistant to tetracycline, ciprofloxacin, erythromycin, ceftiofur, norfloxacin, Penicillin G, oxacillin, but very sensitive to Sulphamethoxazole trimethoprim, rifampicin, chloramphenicol, moxifloxacin, gentamicin, ofloxacin, vancomycin, clindamycin.

**Key words:** *S. aureus*, food poisoning, Prevalence, Antimicrobial resistance.

### Introduction

The *staphylococci* are important bacterial pathogens that can infect both animals and humans and are responsible for numerous hospital- and community-acquired infections yearly. *Staphylococcal* infections result in a significant burden both economically and clinically due to several factors, including increasing antibiotic resistance and lack of

effective vaccines. (*Asante et al 2020*)

Multidrug resistant strains, like methicillin-resistant *Staphylococcus aureus* (MRSA) are ubiquitous, being isolated from humans, pets, food, other animals and the environment. (*Kock et al 2013*)

Given the increasing recognition of the importance of previously overlooked *Staphylococcus* species, the goal of this review was to

present evidences that put these bacteria in the front row of resistance dissemination and highlight their potential threat to human and animal health. (*Rossi et al 2019*) So the aim of this investigation was the detection of the prevalence and antimicrobial resistance pattern of *S. aureus* isolated from different animal sources.

## Materials and methods

### Samples:

(191) random samples were collected as follow 52 swabs from raw meat and 51 samples from raw milk and 45 samples from poultry and 43 samples from lesion swabs (abscess & wounds and sputum of animals). All samples were collected aseptically in polyethylene bags and transported to the laboratory in an isothermal box for subsequent analysis according to (*Bennette and Lancette, 2001*), collected swabs were placed in peptone saline and transported to the laboratory for analysis in a cool box with ice packs.

Twenty-five g from each sample of raw meat and poultry were collected from different sources; Samples were clearly marked, so that it is easily identifiable and submitted to Lab for bacteriological examination in sterile containers in "stomacher bag"

Twenty-five ml from each sample of raw milk were randomly collected from different sources in

clean, dry and sterilized sampling bottles.

Collected samples were transferred to lab in an insulated ice box without delay to be subjected for bacteriological examination.

dry swabs of lesions from animals were collected from cases of Veterinary clinics were put in 10 ml *Staphylococcus* broth (*Difco, 2009*) and transferred to lab in an insulated ice box without delay.

### Bacteriological isolation and identification of *S. aureus*:

According to *Koneman et al. (1996)* and *Quinn et al. (2002)*; samples were cultured onto pepton water for 24hr at 37<sup>0</sup>C and then a loopfull was taken and cultured onto 5% sheep blood agar, mannitol salt agar. All inoculated plates were incubated at 37<sup>0</sup>C for 24-48hr then colonies were identified.

Suspected colonies of *S. aureus* were examined morphologically, biochemically according to (*FDA, 2001*) and microscopically according to (*Ryan and Ray, 2004*)

### Gram stain:

A loopfull from isolated colonies were examined with Gram's stain microscopically for characteristic cellular morphology.

### Slide coagulase test (Clumping factor) (Bound coagulase):

A drop of sterile distilled water or physiological saline was placed on a glass slide. Then a suspension of the organism to be tested was added to the drop of water, using an inoculating loop or applicator stick then a drop of plasma was placed

immediately adjacent to the drop of bacterial suspension and was thoroughly mixed. In positive result immediate formation of a granular precipitate of white clumping within 15 to 20 second was observed.

**Antimicrobial susceptibility testing of *S. aureus* using the disc diffusion method: (Fingold and Martin, 1982)**

**a. Preparation of bacterial suspension:**

Four similar colonies in morphology were transferred using a sterile loop to 5-ml sterile Muller Hinton broth, and incubated at 37°C for 2 hours until becomes turbid. (McFland's tube No.5)

**b. Inoculation of plates:**

1-A sterile cotton swab was dipped into the bacterial suspension.

2-Excess fluid was removed by rotating the swabs with firm pressure against the inside of the tube above fluid level.

3-swab was used to streak the dried surface of Mueller-Hinton plat in three different planes by rotating the plat with approximately 60°c angel each time to ensure an even distribution of the inoculation.

4-Inoculated plates were allowed to remain undistributed for 3-5 minutes to allow the absorption of excess moisture.

**c. Placement of discs:**

1. With fine pointed sterile forceps, selected antibiotic discs were placed on the inoculated plates and passed firmly onto the media to ensure complete contact.

2. Discs were distributed evenly in a manner to ensure a distance of 15 mm from the edge of the petri dish and a distance of 24 mm between each other. The plates were inverted and placed in the incubator at 37°C for 24 hours.

After incubation at 37°C for 24 hours, degree of sensitivity was determined by measuring the clear zone around each disc and interpreted according to Laboratory standards (Oxoid)

**Results:**

From total of 191 samples from animal origin, five isolates were *S. aureus* as follow: One sample of raw meat was positive for *S. aureus*, two samples of raw milk one of them was from mastitis case were positive for *S. aureus*, sputum sample from dog with respiratory manifestation was positive for *S. aureus*, abscess sample from ear of cat was positive for *S. aureus*, with total of five *S. aureus* isolates (9.5%) as shown in table( 1) . All five *S. aureus* isolates were positive for slide coagulase test. According to antimicrobial resistance patterns, *S. aureus* isolates were extremely resistant to tetracycline, ciprofloxin, erythromycin, cefoxitin, norfloxacin, Penicillin G, oxacillin, but very sensitive to Sulphmethoxazole trimethoprim, rifampicin, chloramphenicol, moxifloxacin, gentamicin, ofloxacin, vancomycin, clindamycin as shown in table (2).

**Table (1):** The prevalence of *S.aureus* isolated from different animal origin

sample	No of samples	positive sample of <i>Staph.aureus</i>		
		No	Result of slide coagulase test	%
Raw meat	52	1	+	%1.9
Raw milk	51	2	+	%3.9
Animal lesions	43	2	+	%4.6
Raw poultry	45	0	0	0
total	191	5		10.4 %

**Table (2): Antibiotic sensitivity test of *S.aureus***

Antimicrobial agent	<i>S.aureus</i> of animal origin					
	No.= 5					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Tetracycline (30 µg)	0	0.0	0	0	5	100
chloramphenicol (30 µg)	5	100	0	0	0	0
vancomycin (30 µg)	4	90	1	10	0	0
rifampicin (30µg)	5	100	0	0	0	0
Cefotaxime(30 µg)	0	0.0	0	0	5	100
norfloxacin (30µg)	0	0.0	0	0	5	100
Penicillin G(10u)	0	0.0	0	0	5	100
Erythromycin(15 µg)	0	0.0	0	0	5	100
sulfa-trimethoprim (STX)	5	100	0	0	0	0
oxacillin (30µg)	0	0	0	0	5	100
gentamicin (30µg)	4	90	1	10	0	0.0
Moxifloxacin (30 µg)	5	100	0	0	0	0
Ofloxacin(30 µg)	5	100	0	0	0	0
Clindamycin(30gm µg)	5	100	0	0	0	0

## Discussion

The detection of *S. aureus* in raw milk and raw meat may be due to unclean worker hands, inferior quality of water supplied for washing utensils and carcasses, unhygienic transportation, bad storage and may be returned to milking process from subclinical mastitic cows. The present results are in line with the finding of (Noormanno et al.,2007, Boer et al.,2009, Kluytmans, 2009, Sarah, 2014).

Concerning the used culture media, selective agars like mannitol salt agar have been used successfully for the detection of negative and

positive coagulase *Staphylococcus* (Association of Official Analytical Chemists, 2012). However, such media may not allow the detection of other microorganisms and can be used only for a targeted search of *Staphylococcus*.

After 24 to 48 hours of incubation at 37<sup>0</sup>c, the colonies of *S.aureus* will appear golden yellow, convex, and shiny and 1-1.5 mm in diameter, the golden yellow colonies formed by *S.aures* indicate to the fermentation of mannitol in the medium.

The characteristic of the suspected growing colonies on the all mentioned culture media as well as

the most important biochemical reaction were the basis for *S. aureus* identification.

Traditional culture techniques for *S. aureus* detection in clinical swab samples include inoculation on blood agar plates and selective agar media followed by confirmation tests of suspected colonies. The use of an enrichment broth increases the detection rate of *S. aureus* in these samples (*Wertheim et al., 2001 and Nahimana et al., 2006*).

In the coagulase tube test, firm and complete clot that stays in place when tube is tilted or inverted is considered positive for *S. aureus* while partial clotting, formerly + and ++ coagulase reactions, must be tested further (*Sperber and Tatini, 1975*). If coagulation occurs (clots when the tube is tilted or inverted) or coagulation volume is larger than 50% original volume, it is judged as positive *S. aureus*. Meanwhile, broth culture for plasma coagulase test, containing both positive and negative *Staphylococcus* strains, is used as control (*National standard of the people's republic of china 2010*).

(*Boynukara et al., 1999*) reported the importance of coagulase test in relation to latex agglutination test for identifying of coagulase *staphylococci*.

In this concern, the prevalence of *S. aureus* isolates from frozen raw chickens was low, similar to the results of (*Boer et al., 2009*) who found that the imported chicken products as frozen filets showed a

relatively low prevalence of *S. aureus*.

In other hand, our results were different than (*Manal, 2018*) who isolated *S. aureus* from fresh raw chicken by percent of 2.6%.

*Results of this study also, differ from results of other study conducted by (Abou-Khadra et al., 2020)* found that 20% of raw milk samples from Sharkia Governorate were found contaminated with *S. aureus*.

In this study, the prevalence of *S. aureus* isolated from raw milk considers less than the *S. aureus* isolated by (*Helena et al., 2009*) that was 7.3% who confirmed from potential transmission of *S. aureus* to consumers via milk of cows affected by subclinical mastitis

On the other hand, this result was nearly to (*Aseel et al., 2010, Goja et al., 2013 and Sarah, 2014*) who isolated *S. aureus* from fresh meat (beef) in percent of 5.55%, 12%, 10% respectively and less than the results of (*Kitai et al., 2005, Van et al., 2007 and Pu et al., 2009*) that was 65%, 46%, 39.2% respectively.

The present investigation was completely different to that mentioned by (*Sarah, 2014*) who found the profile of antibiotic resistance of *S. aureus* isolates from food of animal origin were (97.7%) resist Methicillin, (41.9%) erythromycin and (34.9%) trimethoprim-Sulphmethoxazole, this difference is attributed to the

study in the different geographical area.

The profile of antibiotic resistance in this study agree with the profile of MRSA isolates from other countries that found all MRSA isolates were resistant to penicillin and ampicillin, were less susceptible to erythromycin, gentamycin, and kanamycin (Saroglou et al., 1980, Voss and Doebbeling, 1995, Aires et al., 1998, Hsueh et al., 1999, Melter et al., 1999 and Seguin et al., 1999). Also (Mandelle et al., 1995) mentioned that methicillin (oxacillin)-resistant *S.aureus* are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol. In addition to (NCCLS, 1997) said that MRSA strains should be considered to be resistant to all cephalosporins, cepheids, and other  $\beta$ -lactams (such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam, and the carbapenems)

### Conclusions:

The high prevalence rate of *S.aureus* was from raw milk (mastitis case), raw meat and suppurative lesions from animal origin. According to antimicrobial resistance patterns, isolates were extremely resistant to tetracyclin, ciprofloxin, erythromycin, ceftiofur, norfloxacin, Penicillin G,

oxacillin (100%). The isolated multidrug resistant *S.aureus* should be followed up to control the disease in food industry and veterinary practice.

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## انتشار ونمط مقاومة مضادات الميكروبات لميكروب المكورات العنقودية الذهبية المعزولة من مصادر حيوانية

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### الملخص العربي

المكورات العنقودية الذهبية تعد من أسباب التسمم الغذائي والتقرحات والتهاب الضرع من أكثر الميكروبات خطورة وتسبب عدوى مكتسبة في المجتمع. اجمالي 191 عينة عشوائية تم جمعها كالاتي 52 عينة من اللحوم النية و 51 عينة من اللبن الخام و45 عينة من الدواجن و 43 عينة من مسحات الأفات(خراج-جروح-بصاق من الحيوانات) لعزل المكورات العنقودية والكشف عن حساسيتها لمختلف الأدوية المضادة للميكروبات. عينة واحدة من اللحم النيئ كانت ايجابية للمكورات العنقودية الذهبية وعينتان من اللبن الخام احدهما من حالة التهاب ضرع كانوا ايجابين للمكورات العنقودية الذهبية وعينة بصاق من كلب كان به أعراض تنفسية كانت ايجابية للمكورات العنقودية الذهبية وعينة خراج من أذن قطة كانت ايجابية للمكورات العنقودية الذهبية . باجمالي عدد معزولات من المكورات الذهبية العنقودية (9.5%). كل المعزولات كانت ايجابية لاختبار الكواجيليز. وفقا لأنماط مقاومة مضادات الميكروبات كانت عزلات المكورات العنقودية شديدة المقاومة للتيتراسيكلين والسيبروفلوكساسين و الاريثرومايسين والسيفوكسيتين والنورفولوكساسين والبنسيلين والأوكساسلين ولكن شديدة الحساسية للسالفات تريميثوبريم والريفاميسين والكلورامفينيكول والموكسيفلوكساسين والجينتاميسين والوفلوكساسين والفانكوميسين والكلينداميسين.