Bacteriological and molecular studies of Staphylococcus. *aureus* isolated from foods and human contact

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A B S T R A C T

A total of 50 samples were collected from different food (meat product, chicken, sandwiches, pasta, Broth, fish, Appetizer) and human contact (hand). Samples were examined microbiologically for the presence of *Staphylococcus aureus* was isolated from the samples in a ratio present (8) and identified by biochemical identification. *Staph. aureus* strains were tested for antimicrobial sensitivity and all strains showed a 100% resistant to ampicillin. The resistance to oxacillin, amoxicillin, trimethoprim, gentamicin and tetracycline was in a different ratio. However, All the strains were sensitive to levofloxacin and ciprofloxacin. Using PCR technique, amplification of some virulence gene as (*tst,ica*D, *sea*) and antibiotic resistance genes (*mec*A , *blaZ* , *van*A) was performed from the extracted DNA of *Staph. aureus* strains .All extracted DNA samples of *Staph. aureus* showed a positive results for *mec*A ,*tst* , *blaZ, ica*D and sea genes . However, all the samples did not give any PCR product on agrasoe gel. Using sequencing technique gene in two positive strains, the Phylogenetic analysis of *mec*A gene of these isolates were clustered together and little away from other published isolates of *MRSA*, Amino acid identities were 99% which had accession number MF774211 corresponding GenBank sequence.

Key work: *Staphylococcus. aureus*, mecA, *blaZ, van*A, *tst* ,*ico*D and *sea* genes ,antibiotic sensitive ,sequences *mec*A gene, human, foods

(http://www.bvmj.bu.edu.eg) (BVMJ-33(2): 38-45, DECEMBER, 2017)

1. INTRODUCTION

The most important bacterial pathogens in human foods that are responsible for food –human infection include *E.coli* ,*salmonella* and coagulase positive *S.aureus* (Edris et.al 2015) Meat and meat products are the most palatable of highly material value. foods for human being as source for protein, fat, mineral, vitamin and other nutrient (Biesalski, 2005) Poultry meat is a common vehicle of food borne illness, *S.aureus* usually being one of the causes of outbreaks involving large number of peoples (Georgnaras and Von Holy, 2001).In the twenty first century, the bacterium *Staph. aureus* continues to be a global threat to human and animal health. There is currently no vaccine for preventing *S.aureus* infections and this bacteria have developed resistance to many if not most antibiotics. Hence, the therapeutic options are rapidly disappearing. The genetic and physiological flexibility allows this commensal bacterium to become a powerful pathogen and elucidating the myriad of mechanisms its employ to avoid the host defense and/or antimicrobial agents. Theretofore, it presents an important area of research (Greg et.al. 2016) *Staph. aureus* possesses many virulence factors and the most notable are the five major classical types of staphylococcal enterotoxins (*SES: SE*A to *SEE*), the non-classical SE-like toxins (*SE*l: *SE*G *to SE*U), and other virulence genes such as toxic shock syndrome toxin 1 (*TSST*-1), exfoliative toxins and cytolytic toxins (leukocidin and hemolysins). Staphylococcal enterotoxins (*SEs*) are heat stable proteins that are mainly associated with food poisoning outbreaks (Hennekinne 2012, Argudin 2012), while *TSST*-1 is a super antigenic exotoxin that causes toxic shock syndrome (Fueyo 2005). The exfoliative toxins are responsible for staphylococcal scalded skin syndrome that typically affects infants and young children (Ladhani 2003) lukPV cytotoxin causes leukocytosis with necrotic lesions in the skin or mucosa (Lina 1999). *Staphylococcus.aureus* has developed resistance to most classes of antimicrobial agents. Penicillin was the first choice

of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase, *S.aureus* becomes resistant. More than 90% S.aureus strains were resistant to penicillin. However, methicillin, semi synthetic penicillin, was used to treat Penicillin Resistant S. *aureus* but resistance finally emerged. *MRSA* is mediated by the presence of PBP-2a which is expressed by an exogenous gene, *mec*A (Livermore, 2001). In Japan however, an *S.aureus* strain of human origin isolated from raw chicken samples appeared capable of producing enterotoxin C (Kitai. et. al., 2005). The aim of this work bacteriological and molecular studies of Staph.aureaus isolated from foods and humane

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 50 random samples collection from different food (meat product, chicken, sandwiches, pasta, Broth, fish, Appetizer) and human contact (hand) were examined for bacteriological samples. Samples were collected from different shops at Sixth of October City, Aussem, Boulak, Dokki, Giza, Cairo during the period from 2016 and 2017. and transferred with minimum delay to the laboratory for studying its bacteriological examination. Each sample was subjected to bacteriological status taken alone in sterile plastic bags, kept in icebox samples used were collected under aseptic condition and safety precautions. (Rodgers.et al. 1999)

2.2. Bacteriological examination.

Pre-enriched non-selective media (Buffer peptone water) inoculated with the collected samples and then inoculated at 37c for 24h under aerobic condition. A loopful from incubated nutrient broth was streaked into mannitol salt agar and Barid parker agar and incubated for 24-48h at 37c .the developed colonies were picked up and subculture for purification .the purified colonies were morphological identified by gram stain and biochemical test (Swayne 1998)

2.3. Antibiotic sensitivity test.

The disk diffusion test technique was applied according as (Koneman et al, 1979) Eight types of antibiotic from different groups (oxacillin, ampicillin, amoxicillin, trimethoprim, levofloxacin, entamicin, ciprofloxacin, tetracycline). The interpretation of inhibition zone of tested culture was according to (Nccls. 2002).

2.4. Detection of some virulence and resistance genes of isolates of S. aureus:

By using QIAamp (R) DNA minimum kitset reaction (catalogue no M50IDIoo) (Sambrook and Russall Davids. 2001) It was applied on: random isolates S.aureus for detect virulence genes as (*ico*D ,*tst* , *sea*) and resistance gene as (*mec*A ,*blaZ* ,*van*A)

2.5. Sequencing and phylogenetic analysis of (Sanger et.al.1977)

3. RESULTS

Purified and sequenced *mecA* gene of two identity *Staphylococcus aureus* isolated from two strains one of them isolated from food and other isolated from human**.** The result of sequence of *mecA* gene of S.aureus .We have provided a GenBank accession number is MF 774211 .The sequence altaned were 99% identical to the corresponding GenBank sequence .

Sample No 1

Results and Comments:

TGGCCGGTTAAAGATATAAACATTCAGGA
TCGTAAAATAAAAAAAGTATCTAAAAATA
AAAAACGAGTAGATGCTCAATATAAAATT
AAAACAAACTACGGTAACATTGATCGCAA
CGTTCAATTTAATTTTGTTAAAGAAGATGG
TATGTGGAAGTTAGATTGGGATCATAGCG
TCATTATTCCAGGAATGCAGAAAGACCAA
AGCATACATATTGAAAATTTAAAATCAGA
ACGTGGTAAAATTTTAGACCGAAACAATG
TGGTATCA.

Sample is genetically characterized as Staphylococcus aureus subsp. Aureus strain LA-MRSA ST398 isolate E154 Staphylococcus aureus strain NZ15MR0322 genome assembly, chromosome. Staphylococcus aureus strain ST93 SCCmec-Ivn genomic island, with99%identity

Sample No3

TGGCCGGTTAAAGATATAAACATTCAGGA
TCGTAAAATAAAAAAAGTATCTAAAAATA
AAAAACGAGTAGATGCTCAATATAAAATT
AAAACAAACTACGGTAACATTGATCGCAA
CGTTCAATTTAATTTTGTTAAAGAAGATGG
TATGTGGAAGTTAGATTGGGATCATAGCG
TCATTATTCCAGGAATGCAGAAAGACCAA
AGCATACATATTGAAAATTTAAAATCAGA
ACGTGGTAAAATTTTAGACCGAAACAATG
TGGTATCA

Sample is genetically characterized as Staphylococcus aureus subsp. Aureus strain LA-MRSA ST398 isolate E154, Staphylococcus aureus strain ST93 SCCmec-Ivn genomic island Staphylococcus aureus strain NZ15MR0322 genome assembly. With99%identity

Table (1): Incidence of *Staphylococcus aureus* from different samples of foods and human samples

origin	Types of samples	Total number of samples	Positive samples	Negative samples
Foods	Meat product	8	1	7
	Chickens	15	0	15
	Sandwiches (aubergine, bean, sausage)	3	0	3
	Pasta	7	2	5
	Broth	3	0	3
	Fish	1	0	1
	Appetizer	4	1	3
Human	Sample from hand	9	4	5

Table (2): Antimicrobial sensitivity testing for all coagulase positive *S. aureus* isolates.

Antimicrobial disk	Antibiotic sensitivity of Coagulase positive *S. aureus*					
	Resistant		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
AX	7	87.5	-	-	1	12.5
SXT	4	50	-	-	4	50
OX	5	62.5	-	-	3	37.5
AMP	8	100	--	-	-	
LEV	-	-	-	-	8	100
GM	1	12.5	2	25	5	62.5
CIP	-		-	-	8	100
TE	6	75	1	12.5	1	12.5

{ OX (oxacillin), AMP (ampicillin), AX (amoxicillin), SXT (trimethoprim) , LEV (levofloxacin), GM gentamicin) ,CIP (ciprofloxacin), TE (tetracycline) })(8 In relation to total number of isolates of S.aureus%)

Table (3) Oligonucleotide primers sequences source. They have specific sequence and amplify a specific product

Target	Gene	Primer Sequence	Amplified product	Reference
Staph. Aureus	*mecA*	F GTA GAA ATG ACT GAA CGT CCG ATA A R CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al*., 2006
	icaD	F AAA CGT AAG AGA GGT GG R GGC AAT ATG ATC AAG ATA	381 bp	Ciftci *et al*., 2009
	blaZ	F ACTTCAACACCTGCTGCTTTC R TGACCACTTTTATCAGCAACC	173 bp	Duran *et al*., 2012
	Sea	F GGTTATCAATGTGCGGGTGG R CGGCACTTTTTTCTCTTCGG	102 bp	Mehrotra *et al*., 2000
	Tst	F ACCCCTGTTCCCTTATCATC R TTTTCAGTATTTGTAACGCC	326 bp	
	vanA	F CATGACGTATCGGTAAAATC R ACCGGGCAGRGTATTGAC	885 bp	Patel *et al*., 1997

Table (4) Amplification of fragment of resistance genes. (*mec*A , *blaZ* , *van*A) genes from the extracted DNA of all isolated positive *S.aureus* strains

Staph.aureus	Sample ID	Results		
		*mec*A	*blaZ*	*van*A
1	38	+	+	-
2	8	+	+	-
3	H4	+	+	-
4	A5	+	+	-

Sample number (1, 2) isolated from food and (3, 4) isolated from humans

Table (5) Amplification of fragment of virulence genes (tst ,*ica*D, sea) genes from the extracted DNA of all isolated positive *S.aureus* strains

Staph. aureus	Sample ID	Results		
		Tst	*ica*D	*Sea*
1	38	+	+	+
2	8	+	+	+
3	H4	+	+	+
4	A5	+	+	+

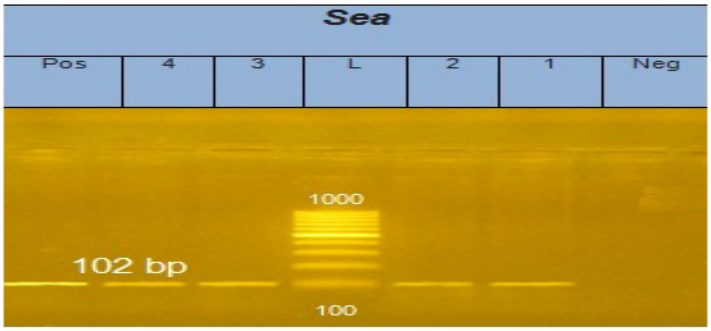

photo (1): showed the agrose gel electrophoresis with positive PCR amplification of (102bp) fragment of virulance *sea* gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

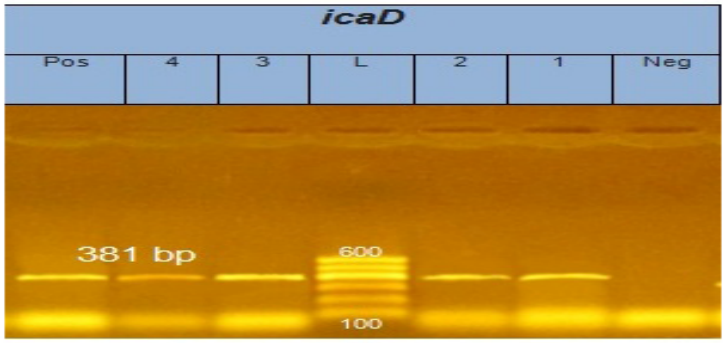

photo (2): showed the agrose gel electrophoresis with positive PCR amplification of (381bp) fragment of virulance *ica*D gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

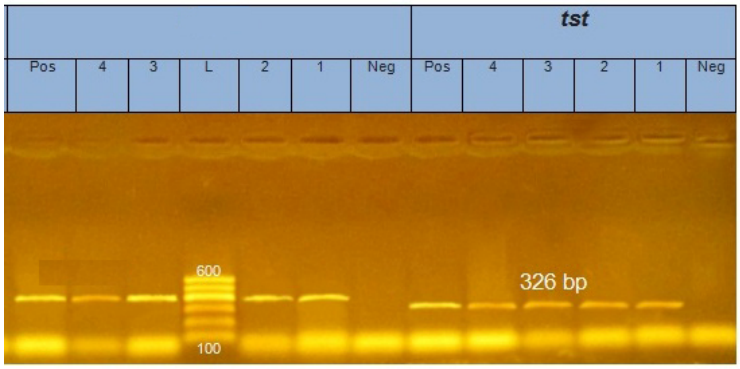

photo (3): showed the agrose gel electrophoresis with positive PCR amplification of (326bp) fragment of virulance *tst* gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

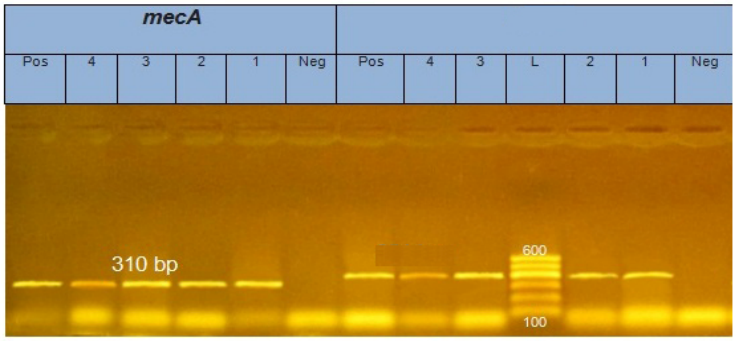

photo (4): showed the agrose gel electrophoresis with positive PCR amplification of (310bp) fragment of resistance *mec*A gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human

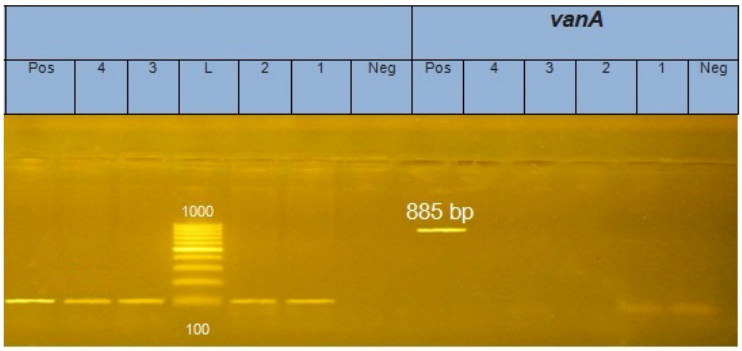

photo (5): showed the agrose gel electrophoresis with negative PCR amplification of (885 bp) fragment of resistance *van*A gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 huma

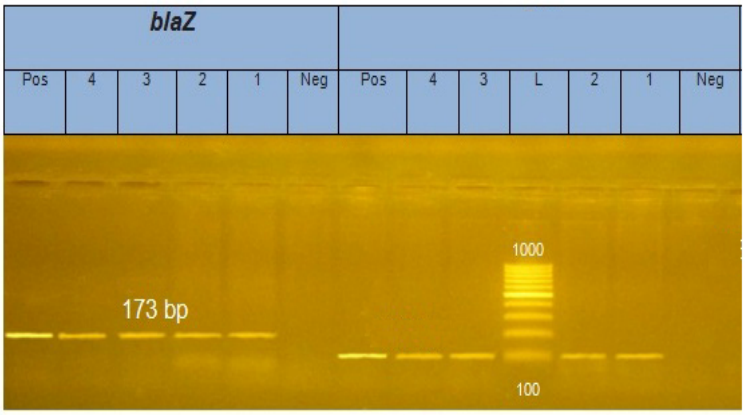

photo (6): showed the agrose gel electrophoresis with positive PCR amplification of (173 bp) fragment of resistance *blaZ* gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

4. DISCUSSION

Staphylococcus aureus is an important food borne pathogen, a major cause of food poisoning cases and out breaks worldwide (Wang et al. 2012) poultry meat industry has become the predominant source of protein from meat in the diet of the population of most developing countries (Robert, 1990). But during conventional slaughter procedures and further processing, microorganisms are introduced into and onto carcasses (Holder et al., 1997). In this study, Table (1) showed that the total prevalence of positive S.aureus from food and human contacts samples were (8/50) of the samples, while,(42/50).were negative Staphylococci. Out of 15 chickens samples, 15 samples were negative with the percentage of 100%, that near agree with the results of (Diaz-lopez et al 2011) the 70 samples, 27 were from retail outlets and 43 from street vendors. All

specimens were negative by both microbiological and molecular methods for S.aureas bacteria Regarding to the current study 9 cloacal swabs subjected for isolation of S.aureus, The overall isolated positive S.aureus was 4 with On the other(Wang et al.2017) which isolation and identification of staphylococcus aureus were performed totally 67 s.aureus strains were isolated .32 s.aureus strains were isolated from patient samples. Studying of 8 strains of coagulase positive S.aureus against 8 antimicrobial discs revealed different dgree of sensitivity. Those results coincide with many authors as (Gardini et al. 2003) who found that Staphylococci were generally susceptible to beta –lactams, 8 were resistant to oxacillin, while (Aarestrup et al. 2000) showed antimicrobial susceptibility to chosen antimicrobial agents among 118 Staphylococcal isolates in Denmark . High frequencies of S.aureus (47%) were resistant to tetracycline ,30% were resistant to ciprofloxacin

Abd El-Salam (2014) reported that all S.aureus isolates tested were susceptible to ciprofloxacin which could be a good choice for treatment . 100% of S.aureus isolates were resistant to methicillin and,more than half of isolates resistant to amoxicillin while the isolates showed a variable presentage of resistanes to trimethoprim and gentamicin.

Archer and Niemeyer (1994) determined that The S. aureus had acquired a gene (*mec*A) coding for the altered penicillin-binding protein 2A, allowing the organism to grow in the presence not only of methicillin but also all new β-lactams. While Strommenger et al. (2006) confirmed that all isolated S.aureus that carrying the *mec*A gene mediated resistance to β-lactam antibiotics.

when used PCR technique of positive S.aureus strains to detection of some genes the result was positive for resistance genes (*mec*A,*bla*Z) and negative for (*van*A) gene this result agree with (Anna 2011) and Few studies were planned for detection of *mec*A among chickens (Perez-Roth et al. 2001). In present study 4 from 4 samples were containing *mec*A gene which are more than that recorded by (Lee et.al 2003) who found only three (10%) from chickens (6%).

And positive for virulence genes (*tst, icaD, sea*) in 4 positive S.aureus isolated which it agree with (Klotz et.al. 2003) who detection of *sea* gene as well as the *mec*A gene coding methicillin resistance and (Manfredi 2010) who detection *sea* gene from the food while (Lidia piechowicz.et.al 2008) detect (*tst*)gene were in most of the strains and (Mottola et.al. 2016) which of *(ica*D) gene in more strain and one strain positive for (*tst*) gene .There are also concerns about *MRSA* as a possible zoonosis.

Both human-to-animal and animal-to-human transmission are known to be possible; however, it has not yet been determined whether animals are an important primary source of MRSA infections for humans, or if most animals are colonized after contact with human carriers (Baptiste et al. 2005, Duquette and Nuttall. 2004, Weese et al., 2006). In contrary, some authors conclude that, currently the risk to human health from-+ zoonotic MRSA seems to be very small (Duquette and Nuttall, 2004). Amino acids of two isolates with other reference staph isolates showed that Sequenced part of the *mecA* gene showing partial homology to other Staphylococcus aureus strains in 99%. this result is identical to the results obtained by Salwa (2015)

5. REFERENCES

Argudin M.A., Mendoza M.C., Gonzalez-Hevia M.A., Bances M., Guerra B., Rodicio M.R.(2012) Genotypes, exotoxin gene content, and antimicrobial resistance of Staphylococcus aureus strains recovered from foods and food handlers. Appl. Environ. Microbiol.;78:2930–2935. Doi: 10.1128/AEM.07487-11. [PMC free article]

Anna c. shore. Emily C. Deasy, Peter Slickers, Grainne Brennan, Brian O'Connell , Stefan Monecke, Ralf Ehricht and David C.Coleman(2011) :Detection of Staphylococcal Cassette Chromosome mec Type XI Carrying Highly Divergent mecA, mecI, mecR1, blaZ, and ccr Genes in Human Clinical Isolates of Clonal Complex 130 Methicillin-Resistant Staphylococcus aureus]; , Antimicrob. Agents Chemother. August vol. 55 no. 8 3765-3773

Aarestrup; F.M. Agers. L.y Ahrens. p.J (2000): Antimicrobial susceptibility and presence of resistance genes in staphylococci from poultry.vet.Microbial.74(4):353-364

AbdEl-salam. Azzas. (2014): Molecular detection of antimicrobial resistance for some food borne pathogens : Ph.D. Thesis (Bacteriology, Mycology ,Immunology) Fac. vet. med. Zagazig Uni.

Archer, G.L. and Niemeyer, D.M. (1994): Origin and evolution of DNA associated with resistance to methicillin in Staphylococci. Trends. Microbiol., 2: 389–392.

Baptiste, K. E.; Williams, K.; Willams, N. J.; Wattret, A.; and Clegg, P. D.; Dawson, S.; Corkill, J. E.; O'Neill, T. and Hart, C. A. (2005): Methicillin-resistant Staphylococci in companion animals. Emerg. Infect. Dis., 11(12):1942-1944.

Biesalski. H.-K. (2005) Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet? Meat Science. Volume 70, Issue 3, July, Pages 509-524

Duquette, R. A. and Nuttall, T. J. (2004): Methicillin-resistant Staphylococcus aureus in dogs and cats: an emerging problem? J. Small Ani. Pract., 45(12): 591-597

Edris. A.M; Reham, A. Amin; Marionet Z. Nassif and Marwa Z (2015):.Bacterial Status of Fresh Marketed Chicken Meat cuts-up Mahmoud 2Food control Dept., Fac. Vet. Med., Benha University. Animal Health Search Institute, Benha branch. Benha veterinary medical journal, vol 28, no.2:52-57,

Fueyo J.M., Mendoza M.C., Martin M.C. (2005): Enterotoxins and toxic shock syndrome toxin in Staphylococcus aureus recovered from human nasal carriers and manually handled foods: Epidemiological and genetic findings. Microbes Infect.;7:187–194. Doi: 10.1016/j.micinf.2004.10.009

Gardini, F.; Tofalo, R. and Suzzi, G. (2003): A survey of 5-antibiotic resistance in Micrococcaceae isolated from Italian dry fermented sausages. J. Food Prot., 66(6): 937-945.

Georgnaras, I. and Von Holy, A. (2001): Antimicrobial susceptibilities of isolates of Staphylococcus aureus, Listeria species and Salmonella serotypes associated with poultry processing. Int. J. Food Microbiol., 70(1/2): 29-35.

Greg A. Somervilleb, Julien S. Gradnigoa, Michael J. Huetherc, Richard J. Kemmyc, Craig M. Johnsonc, Michael G. Oliverc, Etsuko N. Moriyamaa, d (2016): Genome Sequence of Streptomyces aureofaciens ATCC Strain 10762: doi: 10.1128/genomeA.00615-16 Genome Announc. May/June vol. 4 no. 3 e00615-16

Holder, J. S.; Corry, J. E. L. and Hinton, M. H. (1997): Microbial status of chicken portions and portioning equipment, British Poultry Sci., 38: 505-511

Hennekinne J.A., de Buyser M.L., Dragacci S (2012): Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiol. Rev.; 36:815–836

Klotz M, Opper S, Heeg K, Zimmermann S. J Clin Microbiol. (2003): Detection of Staphylococcus aureus enterotoxins A to D by real-time fluorescence PCR assayOct;41(10):4683-7.

Koneman, E. W.; Allen, S. D.; Sowell, V. R. and Sommers, H. M. (1979): Color atlas and textbook of diagnostic microbiology. J.B. Lippincott company, Philadelphia. Toronto. J. Infect. Dis., 123: 97-98.

Kitai, S.; Shimizu, A.; Kawano, J.; Sato, E.; Nakano, C.; Uji, T. and Kitagawa, H. (2005): Characterization of methicillin-resistant Staphylococcus aureus isolated from retail raw chicken meat in Japan. J. Vet. Med. Sci., 67(1): 107-110.

Ladhani S. (2003) Understanding the mechanism of action of the exfoliative toxins of Staphylococcus aureus. FEMS Immunol. Med. Microbiol.;39:181–189. Doi: 10.1016/S0928-8244(03)00225-6

Lina G., Piemont Y., Godail-Gamot F., Bes M., Peter M.O., Gauduchon V., Vandenesch F., Etienne J. (1999): Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin. Infect. Dis.; 29:1128–1132. Doi: 10.1086/313461

Lidia Piechowicz . Katarzyna Garbacz..Janusz Galiński (2008) ; Staphylococcus aureus of phage type 187 isolated from people occurred to be a genes carrier of eneterotoxin C and toxic shock syndrome toxin-1 (TSST-1); international Journal of Hygiene and Environmental Health;Volume 211, Issues 3–4, 15 July, Pages 273-282

Lee, J. H. (2003): Methicillin (Oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans.Appl.Environ.Microbiol.,69(11): 6489-6494.

Livermore D.M. (2001): Antibiotic resistance in staphylococci. Int J Antimicrob Agents, 16: 3–10.

Manfredi E.A, Leotta GA (2010): Multiplex PCR for the detection of sea, seb, sec, sed and see genes of Staphylococcus aureus. Characterization of isolates from food]. Revista Argentina de Microbiologia [01 Jul 2010, 42(3):212-215]

Mottola.C, Teresa Semedo-Lemsaddek, João J. Mendes, José Melo-Cristino, Luís Tavares, Patrícia Cavaco-Silva and Manuela Oliveira (2016): Molecular typing, virulence traits and antimicrobial resistance of diabetic foot staphylococci Journal of

Biomedical Science 23:33 doi org/10.1186/s12929-016-250-

Nccls. (2002); Diagnostic Microbiology and Infectious Disease. Volume 42, Issue 2, February 2002, Pages 137-139.

Perez-Roth, E.; Claverie-Martin, F.; Villar, J. and Mendez-Alvarez, S. (2001): Multiplex PCR for simultaneous identification of Staphylococcus aureus and detection of methicillin and mupirocin resistance. J. Clin. Microbiol., 39(11): 4037-4041.

Rodgers, J. D.; McCullagh, J. J.; McNamee, P. T.; Smyth, J. A. and Ball, H. J. (1999): Comparison of Staphylococcus aureus recovered from personnel in a poultry hatchery and in broiler parent farms with those isolated from skeletal disease in broilers. Vet. Microbiol., 69: 189-198.

Robert, E. C. (1990): Poultry science in the year 2000. Poultry Sci., 69: 2102-2105.

Strommenger, B.; Kehremberg, C.; Kettlitz, C.; Cuny, C.; Verspohl, L.; Witt, W. and Schearz, S. (2006): Molecular characterization of Methicillin-resistant Staphylococcus aureus strains from pet animals and their relationship to human isolates. J. of antimicrob. Chemother., 57:461-465.

Salwa m. helmy (2015): isolation and identification of methicillin-resistant staphylococcus aureus (mrsa) from poultry meat and poultry products. soad a. nasef, etab.m. aboremela, ahmed h. ramadan bacteriology, mycology and immunology dept. fac. of vet. med. Kafr elsheikh univ., Egypt.

Sanger. F,S. Nicklen, and A. R. Coulson (1977):DNA sequencing with chain-terminating inhibitors . Current Issue > vol. 74no. 12 5463–5467 - Molecular cloning : a laboratory manual

Sambrook. Joseph, Russell. Davids W (2001): Includes bibliographical references and index. ISBN 978-1-936113-41-5 (cloth) – ISBN 978-1-936113-42-2

Swayne, David E (1998): laboratory manual for the isolation and identification of avian pathogens. American Association of Avian Pathologists [Corporate Author]

Weese, J. S.; Caldwell, F.; Willey, B. M.; Kreiswirth, B. N.; McGeer, A.; Rousseau, J. and Low, D. E. (2006): An outbreak of methicillin-resistant Staphylococcus aureus skin infections resulting from horse to human transmission in a veterinary hospital. Vet. Microbiol., 114 (1-2):160-164.

Wang, X.; Meng, J.; Zhang, J.; Zhou, T.; Zhang , Y. ;Yang , B. Xi, M. and Xia, X. (2012):Characterization of S.aureus isolated from powdered infant formula milk and infant rice cereal in China. Int.J. Food...Microbiol.,1; 153 (1-2): 142-147.

Wang X, Ouyang L, Luo L, Liu J, Song C, Li C, Yan H, Wang P. Oncotarget (2017): Methicillin-resistant staphylococcus aureus isolates in a hospital of shanghai.; Jan 24;8(4):6079-6084. doi: 10.18632/oncotarget.14036.