Molecular Typing of Staph - Aureus MRSA Strains Isolated from Processed Fish in Port-Said Governorate

Mohamed E. Enany[1]; Abdelazeem M. Algammal[1]; Helal, I.M.[2] and Mohammed A. Soliman[2]

[1]Deptartment of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine Suez Canal University, Ismailia, Egypt

[2]Deptartment of Bacteriology, Animal Health Research Institute - Port Said branch.

Abstract

Fish products could be contaminated with bacterial pathogens such as ***S. aureus***, which could be transmitted to human causing severe illness. In order to throw the light on the presence of ***S. aureus*** in fish products as well as molecular typing of MRSA strains, a total of 200 samples of processed fish products (100 fish burger and 100 fish fillet) were collected from different frozen fish stores in Port-Said Governorate, Egypt. The collected samples were subjected to bacteriological examination. The prevalence of coagulase +ve ***S. aureus*** was (14%) in fish burger samples and (4%) in fish fillet samples. On the other hand, the prevalence of Coagulase –ve ***S. aureus*** was (4%) in fish burger samples and (1%) in fish fillet samples. Antimicrobial susceptibility for methicillin was carried out for detection of MRSA strains, where the prevalence of MRSA strains was 17% from the total isolated coagulase positive ***S. aureus*** strains, while none of the coagulase negative ***S. aureus*** strains proved to be MRSA. PCR protocol was used for detection of ***mecA*** gene in MRSA strains, where all the tested MRSA strains were positive for ***mecA*** gene with specific amplification size at 310 bp**.** Briefly, ***S. aureus*** considered to be one of the major bacterial agents causing food-borne illness in human due to contamination of fish products.

Introduction

Food processing industry was aimed to supply a healthy and palatable food for the human consumers. Contamination by highly virulent bacteria could take place resulting in food intoxication ***(Shena and Sanjeev 2007)***. *S. aureus* is one of the most prevalent pathogenic bacteria associated with fish, which could be transmitted to human causing severe illness ***(Sichewo et al. 2013)***. Food poisoning caused by *S.aureus* is mainly characterized by nausea, vomiting, severe abdominal pain and diarrhea ***(Llewelyn and Cohen, 2002)***.

Nosocomial infections have both public health importance and veterinary importance. MRSA is one of the most incriminated microorganism in nosocomial infections. MRSA strains are characterized by their ability to resist methicillin.The process of methicillin resistance is encoded by the presence of chromosomal *mecA* gene. The genetic information of MRSA gives it the ability to resist all penicillins, and cephalosporins ***(Walther et al. ,2006).***

Ingestion of contaminated food with MRSA strains may increase the risk of becoming carriers or infected by this m.o., So it is necessary to take the suitable measures to control or even prevent food contamination by this mo. ***(EFSA, 2010).***

So, this work was planned to study the prevalence of *S.aureus* in fish products as well as molecular typing of MRSA strains isolated from processed fish products.

Materials and Methods

Samples

A total number of 200 samples (100 fish burger samples and 100 fish fillet samples) (25g from each sample) were collected aseptically in stomacher bags from different stores in Port-Said Governorate; Samples were clearly marked and submitted to the lab. in sterile containers for bacteriological examination.

Isolation and identification of *S.aureus*

Preparation of samples:

Two hundred and twenty five 225ml peptone water (1%) was added to the stomacher bags containing 25 grams of each sample and mixed in the stomacher, centrifugated at 300 rpm for 2 minute ***(ICMSF, 1978).***

Isolation of *S.aureus*:

According to ***Quinn et al. (2002);*** processed samples were inoculated in peptone water for 24 h at 37ºC and then a loopful was taken and inoculated on nutrient agar, 5% sheep Blood agar, Mannitol salt agar and Baird parker agar media. All inoculated plates were incubated at 37ºC for 24-48hrs and examined daily for bacterial growth.

Identification of *S.aureus:*

The suspected colonies were examined for their morphological characters, hemolytic activity on 5 % Sheep blood agar, microscopical examination and biochemical characters according to ***(Quinn et al., 2002).***

Antimicrobial susceptibility Testing by disc for detection of (MRSA) strains

The susceptibility to Methicillin antibiotic was tested according to the procedures of **(NCCLS, 2007)** using disc diffusion technique. The susceptibility of the strains was determined according to the diameter of inhibition zone.

Molecular typing of MRSA strains:

1- DNA extraction from MRSA strains according to *(Van eys et al., 1989)*.

2- **Estimation of purity and**

concentration of DNA according to *Sambrook and Russel, (2001)*

- Spectrophotometer was used to determine the concentration and purity of the extracted DNA by estimating the optical density at wave lengths of 260 and 280 nm.

-The concentration was calculated as follows: one OD at 260nm = 50 ug /ml.

3-polymerase chain reaction (PCR):

Samples of DNA were tested [in 50 µl. Reaction volume in a 0.2 ml PCR tube , containing PCR buffer] (50 mMKCl , 10 mMtris - HCl , 1mM $Mgcl_2$) each dNTPS 200 uM each (dATP , dGTP , dCTP and dTTP) , [Two pairs of primers each at 50 picomol / reaction] and 0.5 of taq DNA polymerase . After layering 40ul of mineral oil, thermal cycling was done in a programmed heating block (Coy vorporation, Grasslake, Michan, USA). A control negative PCR reaction was included with no template in this assay.

- PCR Protocol of *mec* gene according to (McClure et al., 2006)

Step 1: Initial Denaturation at 94 ^{o}C for5 min.

Step 2: Denaturation at 94 ^{o}C for 45 sec.

Step 3: Annealing at 50 ^{o}C 45 sec.

Step 4: Extension at 72 ^{o}C for 45 sec.

- Cycles repeated for 35 times and proceeded by initial denaturation at 95 ^{o}C for 5 min. and followed by final extension at 72 for 10 min.

4- Agarose gel electrophoresis:

Ten µl of amplified PCR products were analyzed by electrophoresis on 2% agarose gel stained with 0.5 µg of ethedium bromide / ml. Electrophoresis was made in 1X TAE buffer at 80 volt for 1 hour. Gels were visualized under UV transilluminator (UVP, UK) then photographed.

Table (1): *list of primers used for PCR assay*

Primer	Primer sequence (5'-3')	Size of amplified product (bp)
mecA-F.	GTA GAA ATG ACT GAA CGT CCG ATA A	310 bp
mecA-R.	CCA ATT CCA CAT TGT TTC GGT CTA A	

Results

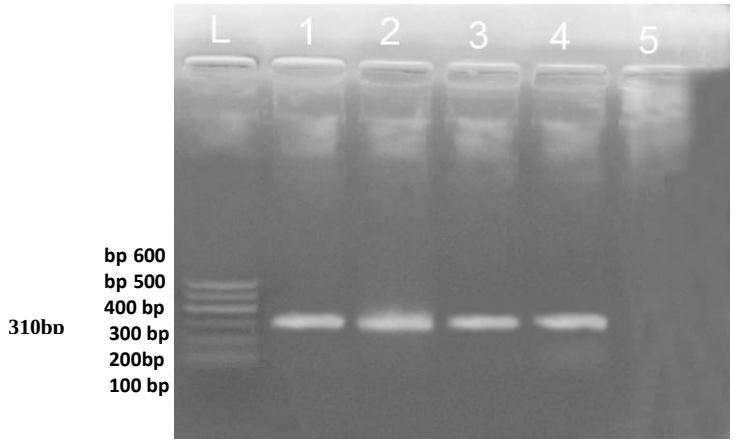
Table (2): *Number and percentage of S. aureus strains isolated from fish products:*

Type of samples	No of examined samples	No. & percentage of *S. aureus*					
		No of Coagulase +ve *S. aureus*	%	No of Coagulase –ve *S. aureus*	%	Total No of *S.aureus* strains	%
Fish burger	100	14	14	4	4	18	18
Fish fillet	100	4	4	1	1	5	5

Total	**200**	**18**	**9**	**5**	**2.5**	**23**	**11.5**

Table (3): *Number and percentage of MRSA strains isolated from fish products (based on antibiotic sensitivity):*

Types of samples	Number of Coagulase +ve *S. aureus*	MRSA strains		Number of Coagulase -ve *S. aureus*	MRSA strains	
		No.	%		No.	%
Fish burger	14	3	21%	4	0	0
Fish fillet	4	0	0%	1	0	0
Total	**18**	**3**	**17%**	**5**	**0**	**0**



sing PCR assay

L: (100 bp DNA ladder)
Lane 1: Control positive *S.aureus* for *mecA gene*
Lanes 2, 3, 4: showed positive *S.aureus* strains for *mecA gene* (310 bp)
Lane 5: control negative (E. coli)

Discussion

S. aureus is believed to be the third main causative pathogen of foodborne diseases transmitted by fish and fish products in the European Union ***(EFSA, 2009)***.

In the present study, the prevalence of coagulase +ve *S. aureus* was (14%) in fish burger samples and (4%) in fish fillet samples. On the other hand, the prevalence of Coagulase –ve *S. aureus* was (4%) in fish burger samples and (1%) in fish fillet samples as shown in Table (2) .These results agreed with that obtained by ***Daniel et al., (2012) and Sergelidis et al. , (2014).*** The higher prevalence of ***S. aureus*** in fish burger was due to the human handling during processing

of fish products. Contamination may occur due to poor sanitary conditions, or bad storage conditions ***(Normanno et al., 2007).*** As regards to the results of antibiotic susceptibility testing as shown in Tables (3), the prevalence of MRSA strains was 17% from the total isolated coagulase positive *S. aureus* strains, while none of the coagulase negative *S. aureus* strains proved to be MRSA**.** Resistance to methicillin occurred mostly due to the presence of *mecA* gene on *S. aureus* chromosome which is responsible for the production of Penicillin binding protein $PBP_{2a.}$ ***(Ito et al., 2003).***

In this work**,** PCR protocol was used for amplification of 310 bp fragment of *mecA* gene in the isolated MRSA strains, as shown in Fig.(1), all the tested MRSA strains were positive for *mecA* gene. These results agreed with those obtained by ***John (2003; Sajith et al. (2012); Rania et al. (2013)***.

In conclusion, *S. aureus* is a very important bacterial agents causing food-borne illness in human due to contamination of fish products. The combination of phenotypic and genotypic characterization of *S. aureus* is an effective epidemiological tool for identification of the isolates. PCR is a fast and accurate technique can be used for the detection of MRSA strains as compared with traditional methods.

References

Daniel, V.S.; Marta, L.C.; Paula, S. and Juan, J. R. (2012): Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). International Journal of Food Microbiology. 157 (2): 286–296.

EFSA, European Centre for Disease Prevention and Control (2009): European Centre for Disease Prevention and Control. The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. EFSA Journal, 271.

EFSA, European Centre for Disease Prevention and Control (2010): The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. EFSA Journal 8 (1), 1496.

International Commission on the Microbiological Specification for food. "ICMSF" (1978)*:* Microorganism in Foods, Their Significance and Method of Enumeration .2nd Ed Univ. of Toronto Press. Toronto Canada.

ItO, M.; Itou, T. and Shoji, Y. (2003): Discrimination between dog-related and vampire bat-related rabies viruses in Brazil by strain-specific reverse transcriptase polymerase chain reaction and restriction fragment length polymorphism analysis. J. Clin. Virol, 26 : 317-330.

John, H. L. (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.

Llewelyn, M. and Cohen, J. (2002): Superantigens: microbial agents that corrupt immunity. Lancet Infect. Dis. 2, 156–162.

McClure, J. A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie, T.; Hutchins, W. and Zhang, K. (2006): Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin susceptible from-resistant Staphylococci. J. Clin. Microbiol. 44: 1141-1145.

NCCLS (National Committee for Clinical Standards) (2007): Performance Standards for Antimicrobial Susceptibility Testing; FifteenthInformational Supplement According to CLSI. CLSI document M100 -S15. Clinical and Laboratory Standards Institute, Wayne, PA.

Normanno, G.; La Salandra, G.; Dambrosio, A.; Quaglia, N.C. ; Corrente , M. Parisi, A.; Santagada, G.; Firinu, A.; Crisetti, E. and Celano, G.V.(2007): Occurrence, characterization and antimicrobial resistance of enterotoxigenic ***Staphylococcus aureus*** isolated from meat and dairy products. International Journal of Food Microbiology. 115 :290–296.

Quinn, P.J.; Markey, B.K.; Carter, M.E. and Donnelly, W.J.C.; Leonard, F.C. and Maguire, D. (2002): Veterinary Microbiology and Microbial diseses.1 published Blackwell science 1st d.

Rania,M.K.;Mohamed,A.B. and Salah,F.A.(2013):MRSA detection in raw milk,some dairy products and hands of dairy workers in Egypt, amini-survey.Food Control. 33: (1): 49-53.

Sambrook, J.and Russell, D.W. (2001): Molecular Cloning: A Laboratory Manual. 3rd edition.Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Sajith, K. A.K.; Preetha, J. S.; Lakshi, S. Y.; Anandi, C. and Ramesh, R. (2012): Detection of *mecA* genes of Methicillin-Resistant ***Staphylococcus aureus*** by Polymerase Chain Reaction. International Journal of Health and Rehabilitation Sciences . 1 (2):64-68.

Sergelidis, D. ; Abrahim, A.; Papadopoulos, T.; Soultos, N.; Martziou, E.; Koulourida, V.; Govaris, A.; Pexara, A.; Zdragas, A. and Papa, A. (2014): Isolation of methicillin-resistant *Staphylococcus* spp. from ready-to-eat fish products.LettApplMicrobiol. 59(5):500-506.

Shena, S.S. and Sanjeev, S. (2007): Prevalence of enterotoxigenic *Staphylococcus*

aureus in fishery products and fish processing factory workers, Food Control, 18:1565-1568.

Sichewo, P.R.; Gono, R.K.; Muzvondiwa, J.V.; and Sizanobuhle, N. (2013): Isolation and Identification of Pathogenic Bacteria in Edible Fish: A Case Study of Fletcher Dam in Gweru, Zimbabwe. International Journal of Science and Research (IJSR). **2:**269-273.

Van Eys, G.M.; Gravekamp, C.; Gerritsen, M.; Quint, W.; Cornlissen, S.J. and Terpstra, W. (1989): Detection of leptospira in urine by PCR.j.clinc.microbiol.27:2258-2262.

Walther, B.; Friedrich, A. W.; Brunnberg, L.; Wieler, L. H. and Lubke-Becker, A. (2006): Methicillin-resistant ***Staphylococcus aureus*** (MRSA) in veterinary medicine: a "new emerging pathogen"?.Berl. Munch TierarztlWochenschr. 119(5-6):222-232.

التصنيف الجزيئي لعترات المكور العنقودى الذهبى مارسا المعزولة من المنتجات المصنعة في محافظة بورسعيد

محمد السيد عناني[1]- عبدالعظيم محمد الجمال[1]- إيهاب محمود احمد هلال[2] - محمد عطية [1]

[1]قسم البكتيريولوجي و المناعة و الفطريات-كلية الطب البيطري- جامعة قناة السويس

[2]قسم البكتيريولوجي-معهد بحوث صحة الحيوان-معمل بورسعيد

فى هذه الدراسة تم إلقاء الضوء على تواجد المكور العنقودى الذهبى في عدد 200عينة عشوائية من الاسماك المصنعة (100عينة برجر أسماك و 100 عينة فيليه أسماك).

وقد أظهرت نتائج الفحص البكتريولوجي والتعريف البيوكيميائي للعينات المستخدمه ايجابية 23 عينه (11,5%) من عينات الأسماك المصنعه المستخدمه فى هذه الدراسه (200 عينه). و كانت نتيجة اختبار التجلط ايجابية 18 معزولة من المكور العنقودي الذهبي بنسبة (9%) بينما كانت 5 معزولات سالبة لاختبار التجلط.

وقد وجد ان العينات المعزوله الموجبه لاختبار التجلط من برجر الأسماك 14 معزولة بنسبة (14%) بينما وجد ان عددالعينات المعزوله السالبة لاختبار التجلط من فيليه الأسماك يمثل 4معزولات فقط بنسبة (4%). وكانت المعزولات الموجبه لاختبار التجلط من برجر الأسماك يمثل 4 معزولات بنسبة (4%) أما السالبه لاختبار التجلط فقد كانت معزولة واحدة فقط بنسبة (1%).

وبدراسة حساسية جميع عترات المكورالعنقودى الذهبى المعزولة وعددها الاجمالى23 عزلة من عينات الاسماك المصنعة للميثسلين واظهرت نتائج الدراسه التالى:

ثلاثة معزولات أظهرت مقاومه للميثسلين من اجمالي 23 معزولة موجبة للميكروب العنقودي الذهبي من عدد 200 عينه من الأسماك المصنعه. و كانت الثلاث معزولات المقاومة للمثيسيللين جميعها من عدد الاربعة عشر عينة برجر الأسماك موجبة التجلط. و للتأكيد على وجود (mec A gene) في الثلاث معزولات المقاومة للمثيسيللين, تم اجراء تفاعل انزيم البلمرة المتسلسل للثلاث معزولات, و تم اثبات تواجد (mec A gene) بهم.