Epidemiology of *Staphylococcus aureus* in a spray drying dairy plant

Von X.W.D. Chin[1)], C. Jacobs[2)] and P. Hammer[2)*)]

[1)] Christian-Albrechts-University of Kiel, Faculty of Agricultural and Nutritional Sciences

[2)] Institute for Hygiene and Food Safety, Federal Research Centre for Nutrition and Food – Location Kiel, P.O. Box 6069, D-24121 Kiel

1. Introduction

Foodborne pathogens have always been a threat to the food processing industry. In the case of a microbial contamination of final products, process lines and environment, the identification of strains among the isolates is a major step in determining the source of the outbreak or perpetuating contamination and in designing subsequent hygienic measures to fight it (1, 2).

In food manufacturing and processing plants, *Staphylococcus* (*S.) aureus* is often found in dust, in ventilation systems and cyclones, due to its resistance to drying. The bacterium may also colonize food-processing equipment that is difficult to clean and is left wet (3).

The 3 factories involved in this study are named A, B and C for the sake of anonymity. The samples wherefrom *S. aureus* was isolated were all collected by factory A. Factory A is a factory that produces skim milk powder and whey powder from respective concentrates. The concentrates are either from suppliers or produced internally. Factory B and C are suppliers for whey concentrates and skim milk concentrates. As factory A does not deal with unprocessed raw material, it does not have its own pasteurization facility and has to depend heavily on the quality of the concentrates. After delivery the concentrates are first further evaporated at 70-95 °C and then stored in two balance tanks. These balance tanks are cleaned alternately every time they are emptied to prevent microbial cross-contamination, which is on average once every 3 to 4 hours. The concentrate is then pumped using a progressing cavity pump to the spray drying tower whithout further heat treatment. The ambient air for spray drying is usually between 200 -220 °C. However, due to the high latent heat of water evaporation, the temperature at the center of the powder particle usually reaches a temperature of only 30-40 °C (4, 5).

During the outbreak in 2002, which lasted a total of 6 months, *S. aureus* was found in both the concentrates and the spray dried products. After a series of systematic investigations, it was found that the source of the lasting contamination was the progressing cavity pump. Although the balance tanks were cleaned regularly, the same pump was used without interruption and this pump was never completely emptied during operation. The temperature during these process steps is 40-45 °C and is very close to the optimal growth temperature required by *S. aureus*.

One possible source of contamination lies within the plant, a strain that has established itself somewhere along the production line (6, 7), as a so called “house strain”. A house strain is a bacterial species that has managed to colonize a factory and causes repeated contamination over a longer period of time. House strains usually are bacteria of one general DNA sub-type, ie. clones. The correct determination of the source of the contamination is either helpful to find an ongoing outbreak or to prevent similar events in the future. In order to find out if the *S. aureus* isolates from this outbreak belong to a clone or not, they were subjected to pulsed field gel electrophoresis (PFGE).

2. Material and methods

Bacterial strains

The reference strain used in this study was *S. aureus subsp. aureus* ATCC 29213.

A complete list of field isolates is presented in Table 1. Twenty six of the isolates were from factory A, and are labelled A. Four samples were isolated from products delivered from factory B, and 3 samples were isolated from supplies from factory C, labelled B and C respectively.The field isolates were collected between April and August 2002. They were frozen in BHI with an addition of 10 % glycerol and stored at -80 °C. All strains were regarded as *S. aureus* based on a positive test for hemolysis, coagulase and thermo-nuclease. Unfortunately 15 of these isolates showed an untypical stikky, clumping growth which did not change even after several passages and which therefore could not be typed by PFGE.

Culture for PFGE

In preparation for PFGE the bacterial strains were grown on blood agar (5% defibrinated sheep blood), scrutinized for strain purity and subsequently inoculated into BHI broth.

PFGE

The PFGE was performed according to a standard procedure published earlier (8). For the *S. aureus* isolates the restriction was performed with SmaI (Fermentas) 25 U overnight at 37 °C. The running parameters are as follows:

Initial Pulse:	2 s
Final Pulse:	20 s
Voltage:	6 V / cm
Angle:	120°
Time:	21 h
Temperature:	14 °C

The DNA restriction bands were analyzed using Fingerprinting II™ Software Version 3.0. The work of Tenover et al. (9) was used as an aid for the interpretation of the banding patterns.

Tab. 1: Isolates of *S. aureus* from factory A and its suppliers B and C

Strain No.	Date sample was collected	Description of sample	Typed by PFGE
A 2	April 10, 2002	Skim milk powder	
A 3	April 10, 2002	Whey powder	x
A 10	April 17, 2002	Skim milk concentrate produced 15.04.2002	x
A 11	April 17, 2002	Skim milk concentrate produced 16.04.2002	x
A 12	April 17, 2002	Evaporator 1	
A 13	April 17, 2002	Progressing cavity pump	
A 14	April 17, 2002	Fluid bed – inlet	
A 15	April 17, 2002	Fluid bed – outlet	x
A 17	April 24, 2002	Whey powder	x
A 18	April 24, 2002	Skim milk powder	x
A 22	April 24, 2002	Skim milk concentrate produced 17.04.2002	x
A 23	April 24, 2002	Skim milk concentrate produced 17.04.2002	x
A 27	April 25, 2002	Progressing cavity pump	
A 28	April 25, 2002	Fluid bed – inlet	
A 29	April 25, 2002	Fluid bed - outlet	
A 30	April 30, 2002	Concentrate balance tank 1	x
A 31	April 30, 2002	Concentrate balance tank 2	x
A 34	April 30, 2002	Whey powder	
A 36	April 30, 2002	Skim milk powder	
A 43	May 08, 2002	Skim milk concetrate produced 07.05.2002	x
A 44	May 07, 2002	Evaporator 1	
A 45	May 07, 2002	Progressing cavity pump	
A 46	May 07, 2002	Fluid bed – inlet	
A 47	May 08, 2002	Fluid bed - Sector 3	
A 54	May 22, 2002	Whey powder	
A 55	May 22, 2002	Skim milk powder	
B 82	July 25, 2002	Skim milk concentrate produced 22.07.2002	x
B 83	July 25, 2002	Skim milk concentrate produced 22.07.2002	x
B 89	Aug. 08, 2002	Whey concentrate produced 06.08.2002	x
B 90	Aug. 14, 2002	Whey concentrate produced 08.08.2002	x
C 72	July 17, 2002	Whey concentrate produced 15.07.2002	x
C 73	July 17, 2002	Whey concentrate produced 16.07.2002	x
C 94	Aug. 28, 2002	Whey, not concentrated produced 27.08.2002	x

3. Results

After PFGE was completed, the DNA restriction band patterns were analyzed and a dendrogram was produced as presented in Fig. 1. According to Tenover et al. (9), there can be 4 different categories of genetic and epidemiologic relatedness depending on the number of fragments differing from the outbreak strain. The criteria used to interprete the PFGE patterns in this study are summarized in Table 2.

Tab. 2: Criteria for interpreting PFGE patterns (Tenover et al., 1995)

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiologic interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2 – 3	Isolate is probably part of the outbreak
Possibly related	2	4 – 6	Isolate is possibly part of the outbreak
Different	≥ 3	≥ 7	Isolate is not part of the outbreak

PFGE patterns can be altered as a result of random genetic events like point mutation resulting in creation or loss of a restriction site and insertions or deletions of DNA (9).

Unfortunately, 15 of the 33 isolates could not be typed as the sediment was abnormally sticky and the cells could not be subjected to proper lysis. Most of these isolates were from areas or products that were already exposed to high heat and it is speculated that that could be the reason for this anomaly. When the chromosomal DNA from the 18 isolates in this study were digested with *SmaI* and subjected to PFGE, 8 to 12 fragments were generated for each strain. The fragments ranged in sizes from 20 to 500 kilobase pairs (kbp).

Among the isolates that could be typed, 10 distinct chromosomal digestion patterns were identified (see Fig.1). All the isolates from deliveries from B showed a similarity of 100 %. While two of the isolates of supplies from C (C 72 and C 73) were 86 % homologous, the third isolate from supplier C (C 94) was unrelated to the other two.

Of all the isolates from A, 5 clusters can be identified. Isolates A 11, A 22, and A 43 were indistinguishable. Isolates A 10 and A 15 were also indistinguishable and were closely related to isolate A 23 (94 %). The third cluster included isolates A 17 and A 3 which were identical and isolate A 18 which was 93 % related to the other two. The remaining two isolates A 30 and A 31 showed distinct restriction patterns and were unrelated to the other strains.

None of the isolates from A were related to those from B and C.

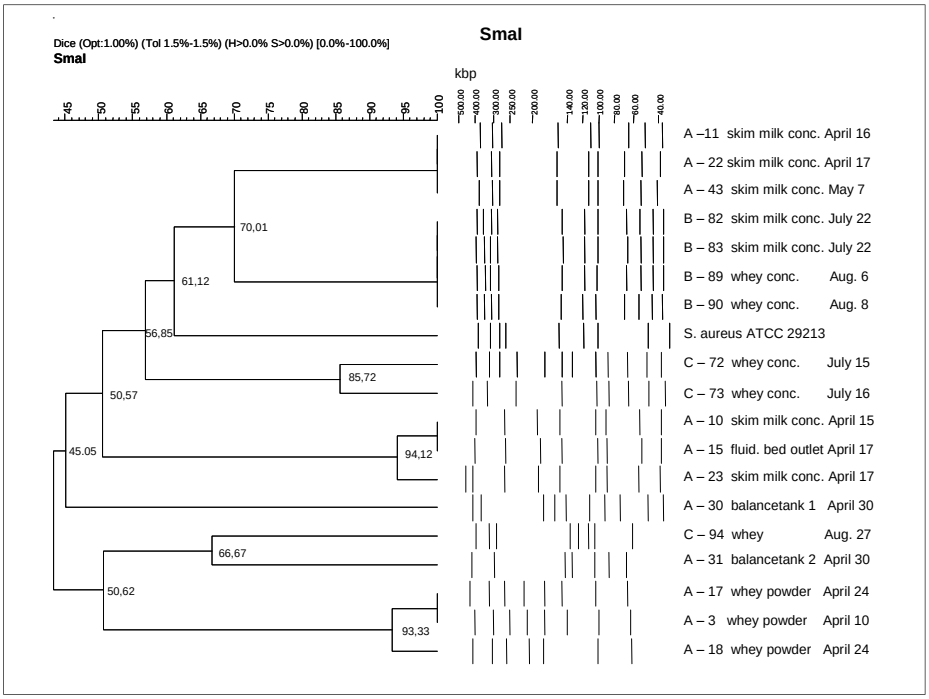

Fig. 1: Dendrogram of the percent similarity between *Staphylococcus aureus* isolates from a manufacturer of spray dry milk products after restriction with SmaI.

4. Discussion

Several conclusions can be drawn based on the results of this study.

First, all the isolates from B were indistinguishable and can be considered to represent the same clone although the sampling occurred over a period of more than 2 weeks and in two different products. From this, the conclusion can be drawn that factory B had common source of contamination, at least during the sampling period. Following the results of the microbiological investigation in 2002 this source must have been eliminated as in the concentrates delivered afterwards *S. aureus* was not detectable. Details about the source and the means for elimination are not known, however. Isolates from C sampled on the same day were identical (C 72 and C 73) but another isolate taken 5 weeks later (C 94) was unrelated to the first two. This was evidenced for at least two contamination events in factory C. A thorough investigation to see if the present process parameters sufficiently eliminate targeted microorganisms was carried out, resulting in concentrates not containing detectable *S. aureus* as in factory B. Details on the source of contamination and the measures for elimination are also not known.

For factory A, the first cluster of isolates A 11, A 22, and A 43 were all from skim milk concentrate. Samples were taken over a period of 3 weeks. This showed that factory A was having a problem with contaminated primary supplies during the outbreak.

The second cluster involving isolates A 10 and A 15 showed, as already mentioned earlier, that the drying process was insufficient in deactivating microorganisms, in this case *S. aureus*. This is evident because isolate A 10 was from a sample of skim milk

concentrate, a supply, whereas A15 was isolated from a sample taken from the outlet of the fluid bed, which is almost at the end of the process line. This means that the successful elimination of *S. aureus* in the supplies has to be carried out prior to the drying step.

The last cluster within factory A occurred in end products over a period of two weeks, and despite the time apart, the isolates belonged to the same clone. This confirmed that somewhere along the process line, one of the *S. aureus* strains was able to establish itself and was contaminating the end products. During the outbreak, in order to pin point the source of the contamination, systematic investigations were carried out. Systematic investigations allow the step by step elimination of possible points in the process line where *S. aureus* contamination could occur. This led to the discovery that the source of the contamination was indeed the progressing cavity pump. The pump was then completely disassembled and cleaned and the contamination was effectively eliminated.

In addition to the three clusters of strains two single strains were isolated at the same day from samples taken from both balance tanks, one isolate from each tank. These were unrelated and did not match with any of the clusters either.

Because many isolates of *S. aureus* were found in the factory during the outbreak, thorough cleaning and disinfecting measures were carried out for the entire production area. A more rigorous cleaning plan with more regular cleaning intervals was implemented until monitoring showed that the occurrence of *S. aureus* was reduced significantly. More stringent control of the supplies as well as the personnel and transport machinery was also carried out to reduce the possibility of a new entry of *S. aureus*.

As the human carrier is the most important source of enterotoxin-producing *S. aureus*, the lack of proper personal hygiene and wrong handling of food are often causes of a *S. aureus* contamination (3). Although proper personal hygiene is indeed of utmost importance when it comes to preventing the contamination of food with *S. aureus*, supplies rarely come into contact with the personnel in a fully automated plant that processes fluids like factory A. In cases like these, it is important to also pay attention to factors other than personal hygiene. For example, if possible, the inside of the machinery should be checked constantly to make sure that the surface is not damaged. Cracks on surfaces often provide optimal niches that allow the establishment of microorganisms. Equipment with proper hygienic design is also very important. Poorly designed equipment with crevices and dead areas that cannot be cleaned easily can be very dangerous (10). This was the case with factory A. If the progressing cavity pump were designed such that it is completely emptied inbetween batches, the chances of a cross-contamination between batches would have been lower.

Another problem that factory A might have had is product deposit in the machinery. The fat, sugar, and protein content of dairy products gives hygroscopic and sticky characteristics at temperature and humidity conditions present in the plant (11). Hence, the possibility of product deposit formation is very high. Product deposit provides the ideal base for microbial growth because there is unlimited food supply. Minimizing product deposit not only reduces the chance of microbial growth within the machinery, it also allows longer plant operation.

New standards for hygiene and sanitation in food manufacturing like good manufacturing practices (GMP) and HACCP introduced to satisfy the increasing standard of food quality demanded by the modern consumer places a great emphasis on the prevention of eventual contamination (12). In accordance with the concept of HACCP, environment- and product monitoring plays a significant role in the quality management of food. Environment and product monitoring by taking routine microbial samples is a useful tool because it gives insight where weak points in a production system could lie and also on

the pathways of microbial distribution within a plant (13). Knowledge of this kind will help prevent eventual outbreaks of foodborne diseases. Monitoring also gives feedback on the effectiveness of the current hygienic measures.

Although factory A was having problems with contaminated supplies, the strains found in the final products and also elsewhere in factory A were not related to those from suppliers B and C and this shows that there was no microbial cross-contamination between factory A, B and C. The mere presence of *S. aureus* in the supplies however means that there is always the danger of a cross-contamination and a constant monitoring will be essential to allow swift discovery in the event of a contamination.

Acknowledgements

For excellent technical assistance we would like to thank Mrs. Beate Bonniksen for performing the PFGE and Mrs. Susanne Cochoy for isolation and phenotypical typing of the bacterial strains.

5. References

(1) Vanderlinde P. B., Fegan N., Mills L., Desmarchelier P.M. Use of pulsed field gel electrophoresis for the epidemiological characterisation of coagulase positive *Staphylococcus* isolated from meat workers and beef carcasses. International Journal of Food Microbiology **48** 8- 85 (1999)

(2) Wei H.L., Chiou C.S. Molecular subtyping of *Staphylococcus aureus* from an outbreak associated with a food handler. Epidemiology of Infections **128** 15-20 (2002)

(3) International Commission on Microbiological Specifications for Foods (ICMSF): Microorganisms in Foods 5 Microbiological Specifications of Food Pathogens. Blackie Academic & Professional, 1st Edition (1996)

(4) Becker, H., Terplan, G. Salmonellen in Milch und Milchprodukten. Journal of Veterinary Medicine B **33** 1-25 (1986)

(5) LiCari, J.J., Potter, N.N. Salmonella survival during spray drying and subsequent handling of skim-milk powder. I. Salmonella enumeration. Journal of Dairy Science **53** 865-870 (1970)

(6) Luppertz, K. Mikrobiologie von Milchtrockenprodukten unter Berücksichtigung der Herstellung – eine Literaturübersicht. Dissertation med. vet. München (1990)

(7) Terplan, G., Becker, H. Salmonellen, E.coli, Staphylokokken und Listerien in Trockenmilch-produkten. Molkereitechnik **82** 71-75 (1988)

(8) Hammer P., Knappstein K., Teufel P. Umfeld versus Endproduktmonitoring – Praktische Erfahrungen bei der Kontrolle von Salmonella-Kontaminationen in Umfeld einer Milch-trocknungsanlage. Kieler Milchwirtschaftliche Forschungsberichte **58** (1) 59-80 (2006)

(9) Tenover F.C., Arbeit R.D., Goering R.V., Mickelson P.A., Murray B.E., Persing D.H., Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. Journal of Clinical Microbiology **33** 2233-2239 (1995)

(10) European Hygienic Equipment Design Group (EHEDG): Hygienic equipment design criteria. Trends in Food Science and Technology **4** 225-229 (1993)

(11) Cari (12) M.: Concentrated and Dried Dairy Products. VCH Publishers, 1st Edition (1994)

(13) Sinell H.J.: Einführung in die Lebensmittelhygiene. Parey Verlag, 4th Edition (2004)

(14) Hanslik N., Kitzmüller C., Woidich A. Hygiene-Management. Behr's Verlag, 1st Edition (2000)

6. Summary

Chin X.W.D., Jacobs, C., Hammer, P.: **Epidemiology of *Staphylococcus aureus* in a spray drying dairy plant.** Kieler Milchwirtschaftliche Forschungsberichte **59** (3) 181-189 (2007)

06 Veterinary medicine and hygiene (Staphylococcus aureus, milk powder, epidemiology, Pulsed Field Gel Electrophoresis)

Foodborne pathogens as *Staphylococcus* (*S.*) *aureus* have always been a threat to the food processing industry. In the case of a microbial contamination of final products, process lines and environment, the identification of bacteria strains among the isolates is a major step in determining the source of the outbreak or of the perpetuating contamination and in designing subsequent hygienic measures to fight it.

In this study, isolates of *S. aureus* from a factory that produces spray dried milk products were analysed. The outbreak in this factory involved contamination of concentrates and of the end product. The correct determination of the source of contamination is helpful to fight an ongoing outbreak and to prevent similar events in the future. In order to investigate the epidemiology of *S. aureus* in the factory during the reported outbreak the isolates were subjected to pulsed field gel electrophoresis (PFGE).

Within a group of 18 isolates several clusters of strains were identified, and could be allocated to supplies of concentrates, technical equipment and end products. Because many different isolates of *S. aureus* were found in the factory during the outbreak, thorough cleaning and disinfecting measures were carried out for the entire production area. A rigorous cleaning plan with shorter cleaning intervals was implemented until the microbiological monitoring showed that the occurrence of *S. aureus* was reduced significantly. More stringent control of the supplies as well as of the personnel and transport machinery contributed to reduce the possibility of a new entry of *S. aureus* in the factory.

Zusammenfassung

Chin X.W.D., Jacobs, C., Hammer, P.: **Betriebsepidemiologie von *Staphylococcus aureus* in einem Trocknungswerk für Milchpulver.** Kieler Milchwirtschaftliche Forschungsberichte **59** (3) 181-189 (2007)

06 Veterinärmedizin und Hygiene (Staphylococcus aureus, Milchpulver, Epidemiologie, Pulsed Field Gel Electrophoresis)

Erreger von durch Lebensmittel übertragenen Erkrankungen, wie *Staphylococcus* (*S.) aureus* stellen immer eine Herausforderung für die Lebensmittelindustrie dar. Bei einer mikrobiellen Kontamination von Endprodukten, Prozesslinien und dem Produktionsumfeld ist es wichtig, die Identität der beteiligten Bakterienstämme zu kennen. Dies trägt dazu bei, die Ursache für das Kontaminationsereignis oder für kontinuierliche Kontaminationen zu finden und entsprechende Bekämpfungsmaßnahmen einzuleiten.

In dieser Untersuchung wurden Isolate von *S. aureus* aus einem Betrieb zur Sprühtrocknung von Milchpulver analysiert. Das Kontaminationsereignis betraf Konzentrate vor der Trocknung und Endprodukte. Eine genaue Identifizierung der Kontaminations-

quelle trägt einerseits zur Bekämpfung der Kontamination bei, andererseits zur Prävention, um derartige Ereignisse zukünftig verhindern zu können. Für die Aufdeckung der betriebsepidemiologischen Zusammenhänge wurden in diesem Fall die Isolate von *S. aureus* einer Feintypisierung mit der Pulsfeld-Gelelektrophorese (PFGE) unterzogen.

Innerhalb einer Gruppe von 18 Isolaten konnten mehrere Stamm-Cluster identifiziert werden, die sich jeweils Konzentratanlieferungen, technischer Einrichtung oder Endprodukten zuordnen ließen. Da es somit viele verschiedene Isolate gab, wurden generell verstärkt Reinigungs- und Desinfektionsmaßnahmen im gesamten Produktionsbereich durchgeführt. Im Reinigungsplan wurden solange engere Intervalle gesetzt bis das mikrobiologische Monitoring ein signifikantes Absinken der Kontamination ergab. Zusätzlich wurde die Kontrolle der angelieferten Konzentrate sowie des Personals und der Produktionsmittel verstärkt, um ein erneutes Eindringen von *S. aureus* in den Betrieb zu unterbinden.

Résumé

Chin X.W.D., Jacobs, C., Hammer, P.: **Épidémiologie industrielle de *staphylococcus aureus* dans une entreprise de séchage pour du lait en poudre.** Kieler Milchwirtschaftliche Forschungsberichte **59** (3) 181-189 (2007)

06 Médicine vétérinaire et hygiène (*staphylococcus aureus*, lait en poudre, épidémiologie, **E**lectrophorèse en **C**hamps **P**ulsé (ECP) (Pulsed Field Gel Electrophoresis)

Des agents pathogènes comme *staphylococcus* (S.*) aureus* transférés par des aliments représentent toujours un défi pour l'industrie alimentaire. Lors d'une contamination microbienne des produits finis, des installations de traitement et de l'environnement de production, il est important de connaître l'identité des souches de bactéries associées. Cela contribue à trouver la cause de la contamination ou des contaminations continues et à introduire des mesures de lutte correspondantes.

Dans cette étude, des isolats de *S. aureus* d'une entreprise de séchage pour du lait en poudre ont été analysés. La contamination s'était produite dans des concentrés avant le séchage et dans des produits finis. Une identification précise de la source de contamination contribue d'une part à la lutte contre la contamination, d'autre part à la prévention de contaminations futures. Pour détecter les causes inhérentes à l'entreprise, les isolats ont été soumis à une typisation détaillée au moyen de l'électrophorèse en champ pulsé.

Dans un groupe de 18 isolats, plusieurs clusters de souches pouvaient être identifiés permettant l'affectation à des livraisons de concentrés, des équipements techniques ou à des produits finis. Puisqu'il y avait tant d'isolats différents, des mesures de désinfection et de nettoyage ont été mises en oeuvre généralement de manière renforcée dans le secteur de la production complète. Des intervalles de plus en plus étroits ont été fixés dans le plan de nettoyage jusqu'à ce que le contrôle microbiologique ait montré une diminution significative de la contamination. En plus, le contrôle des concentrés fournis ainsi que du personnel et des moyens de production a été renforcé pour empêcher une nouvelle entrée de *S. aureus* dans l'entreprise.