COMPARISON OF REVERSE PASSIVE LATEX AGGLUTINATION TEST AND IMMUNOBLOTTING FOR DETECTION OF STAPHYLOCOCCAL ENTEROTOXIN A AND B

A. DI PINTO1,3, V.T. FORTE1, G. CICCARESE2, M.C. CONVERSANO1 and G.M. TANTILLO1

1Dipartimento di Sanità e Benessere degli Animali
Università degli Studi, Bari
Provinciale per Casamassima, km 3, 70010 Valenzano
Bari, Italy

2Isituto Zooprofilattico
S.S. 7 ter, km 59, Zona Industriale, 73012 Campi Salentina
Lecce, Italy

Accepted for Publication April 27, 2004

ABSTRACT

Staphylococcal foodborne diseases resulting from consumption of food contaminated with staphylococcal enterotoxins (SEs) produced by certain strains of Staphylococcus aureus are the second most common foodborne illnesses in the world. Analytical methods are essential for routine monitoring purposes and safeguard public health. Different methods for SE detection have been proposed although their use in a complex matrix is often limited by the presence of substances that interfere with tests. In this article reverse passive latex agglutination (RPLA) and immunoblotting methods based on specific antibodies and currently available for SE detection have been compared. Culture filtrates from enterotoxin S. aureus strains isolated from cheese samples were identified by SET-RPLA. Then the culture filtrates identified as staphylococcal enterotoxin A and staphylococcal enterotoxin B by RPLA test were analyzed with immunoblotting. The results obtained suggest that either SET-RPLA or immunoblotting may be applied to culture filtrates for the detection of SEs with good correspondence of results. Although SET-RPLA represents a simple method for routine monitoring purposes, a positive result by a rapid method (RPLA) is only regarded as presumptive and must be confirmed by standard methods (Feng 1996), such as immunoblotting method.

3 Author for correspondence. TEL: +39 080 5443970; FAX: +39 080 5443855; EMAIL: a.dipinto@veterinaria.uniba.it

© Copyright 2004, Blackwell Publishing 231
INTRODUCTION

Foodborne diseases resulting from consumption of food contaminated with staphylococcal enterotoxins (SEs) produced by certain strains of *Staphylococcus aureus*, are the second most frequent foodborne illnesses in the world (Garthright et al. 1988; Levine et al. 1991; Bunning et al. 1997; Archer and Young 1998). Although the true incidence of staphylococcal food poisoning is unknown, in the U.S. about 1000 cases are reported each year (Rasooly and Balaban 2000). Staphylococcal food poisoning is characterized by a short incubation period (2–6 h) after ingestion of preformed toxins, followed by nausea, vomiting, abdominal pain and diarrhea. Death from staphylococcal food poisoning is very rare, although such cases have occurred among the elderly, infants and severely debilitated persons.

Foods frequently incriminated in staphylococcal food poisoning include meat and meat products, poultry, fish and shellfish, egg products and salads. Other contaminated food products included milk and dairy products.

Staphylococcal enterotoxins are basic, low molecular weight (MW 26,900–29,600) and highly heat-stable proteins. They are divided into seven serotypes: SEA, SEB, SEC 1–3, SED, SEE (Su and Wong 1997), although the synthesis of other enterotoxins (SEG, SEH, SEI, SEJ, SEK) has also been found in some *S. aureus* strains (Orwin et al. 2001). The 12 known SEs function as potent gastrointestinal toxins. After ingestion of contaminated food, the toxins are resorbed into the blood in the gastrointestinal tract, activate an emetic reflex and cause nausea, emesis, abdominal cramps and diarrhea (Tortora et al. 1995). SEs are resistant to inactivation by gastrointestinal proteases such as pepsin. Heat stability is one of the most important physical and chemical properties of SEs in terms of food safety (Denny et al. 1971; Humber et al. 1975; Lee et al. 1977; Hoover et al. 1983).

Detection of the presence of toxins involves the analysis of incriminated foods for enterotoxigenic staphylococci. In cases where the food may have been treated to kill the staphylococci, as in pasteurization or heating, direct microscopic observation of the food may be an aid in the diagnosis. Different methods for SE detection have been developed; in particular the rapid methods based on specific antibodies, such as enzyme-linked immunosorbent assays and reverse passive latex agglutination (RPLA) are commonly resorted to. In staphylococcal intoxication, the use of Polymerase chain reaction assays and DNA probes is limited in detecting only the presence of *S. aureus* genes, but not in detecting the presence of preformed toxins.

In this article RPLA and immunoblotting methods for SE detection have been compared. Culture filtrates from enterotoxin *S. aureus* strains isolated from cheese samples were identified by SET-RPLA. Then the culture filtrates
identified as staphylococcal enterotoxin A (SEA) and staphylococcal enterotoxin B (SEB) with the RPLA technique were subjected to immunoblotting to confirm the RPLA results and consequently evaluate the RPLA test reliability in routine monitoring purposes.

MATERIALS AND METHODS

Samples

The culture filtrates from enterotoxin *S. aureus* strains, obtained by Normanno *et al.* (2001), were used for the enterotoxin detection with the RPLA technique and SDS-PAGE followed by Western blotting.

Reverse Passive Latex Agglutination

The detection of staphylococcal enterotoxins in the culture filtrates was carried out with the SET-RPLA Staphylococcal enterotoxin test kit (Oxoid, Basingstoke, Hampshire, UK) following the manufacturer’s instructions.

Protein Assay

The protein content of the culture filtrates was determined spectrophotometrically at 595 nm by Biorad Protein Assay (Biorad Laboratories Gmbh, Munchen, Germany). The method is based on the observation of the maximum absorbance for an acidic solution of Comassie Brilliant blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs, according to Bradford (1976).

SDS-PAGE and Immunoblotting

Aliquots (750 ng) of proteic extracts were added with an equal volume of Sample Buffer Laemmli (Sigma), heated at 95C for 3 min, and loaded onto 12.5% SDS-PAGE homogeneous gel (Amersham Biosciences, Germany). Protein molecular weight standard (Rainbow Coloured protein molecular weight marker, Amersham Biosciences, Germany) was also loaded onto the gel. A Multiphor II Electrophoresis System (Amersham Biosciences, Germany) was used. Gels were run at 150 V for 3 h, then electrobotted with a semidry transfer unit (Multiphor II NovaBlot electrophoretic transfer unit) to a nitrocellulose membrane (Amersham Biosciences, Germany) at 90 mA for 3 h. The membrane was blocked with blocking solution (Washing solution and dried milk 1%) overnight and incubated with polyclonal rabbit anti-SEA and anti-SEB (Sigma) raised against native SEs,
diluted 1 : 20000 (v/v) in blocking solution, for 60 min. The membrane was then washed with washing solution (Sodium phosphate 20 mM pH 7, NaCl 150 mM and Tween 20 0.1%), and incubated in goat anti-rabbit alkaline phosphatase conjugate (Biorad), diluted 1 : 3000 (v/v) in blocking solution. After washing, an alkaline phosphatase conjugate substrate solution (Biorad) was used as a color reagent to detect bound antibodies. Purified SEA (Sigma, St. Louis, MO) and SEB (Sigma, St. Louis, MO) were used as the positive controls. A filtrate enterotoxin-free was used as negative control in SDS-PAGE.

RESULTS

The culture filtrates from enterotoxin S. aureus strains isolated from cheese samples and detected as SEA and SEB by SET-RPLA were confirmed by immunoblotting analysis. The SET-RPLA test and immunoblotting gave same results.

The immunoblotting analysis of culture filtrates (Fig. 1) shows the specific bands of SEA and SEB as expected. The presence of nonspecific bands was probably a result of cross-reactions related to the use of polyclonal antibodies either for SEA or for SEB.

Moreover, purified SEA and SEB used as positive controls and subjected to RPLA and immunoblotting gave the same results. Assessment of the specificity of the latex reagents against standard enterotoxins yielded the expected results. The toxin-free filtrate used as negative controls subjected to RPLA and immunoblotting tested negative. These results refer to 10 experiments and did not show a variability.

DISCUSSION

Staphylococcus aureus enterotoxin producing strains are the common agents of food poisoning, the most important of which belong to the family of heat-stable SEs. A great deal of epidemiological evidence points to the importance of having specific methods for the routine investigations of dairy products contaminated with SEs. The current rapid methods for detecting SEs include immunoassays. The highly specific binding of antibodies to antigens plus the simplicity and versatility of this reaction has facilitated the design of a variety of antibody assays and formats, and they comprise the largest group of rapid methods currently used in food testing (Feng 1996). Among different basic formats of antibody assays, the simplest is latex agglutination (LA), in
FIG. 1. IDENTIFICATION OF STAPHYLOCOCCUS AUREUS ENTEROTOXINS A AND B BY IMMUNOBLOTTING

(A) Immunoblotting of SEA. Lane 1: MW marker; lane 2: positive control (purified SEA); lane 3: negative control; lane 4: sample 1; lane 5: sample 2; lane 6: sample 3; lane 7: sample 4; lane 8: sample 5; lane 9: sample 6; lane 10: sample 7

(B) Immunoblotting of SEB. Lane 1: MW marker; lane 2: positive control (purified SEB); lane 3: negative control; lane 4: sample 1; lane 5: sample 2; lane 6: sample 3; lane 7: sample 4; lane 8: sample 5; lane 9: sample 6; lane 10: sample 7.
which antibody-coated latex beads or colloidal gold particles are used for quick serological identification or typing of pure culture isolates of bacteria from foods. A modification of LA, known as RPLA, tests for soluble antigens and is used mostly to test for toxins in food extracts or toxin production by pure cultures (Feng 1996).

In this study the RPLA test has been applied to detect SEs, thanks to its rapid processing time. The obtained results showed that commercially available SET-RPLA test is a sensitive, quick and versatile method for the detection of SEs in culture filtrates. The high sensitivity is probably related to removal of inhibitory substances, which are important for test efficiency. Thus, the SET-RPLA test kit, a semiquantitative assay, may be used to detect SEs in a wide variety of foods. Moreover, it is ideal for use in quality control programs to quickly screen large numbers of food samples for the presence of small amount of enterotoxin necessary to cause intoxication. In particular a toxin dose of less than $1.0 \mu g$ in contaminated food will produce symptoms of staphylococcal intoxication (Evenson et al. 1988). This toxin level is reached when $S.\ aureus$ populations exceed $100,000\ \text{per gram}.$

The immunoblotting analysis applied to identify the culture filtrates does not indicate discrepancies between the RPLA and the immunoblotting methods for detection of SEA and SEB. However, some samples identified as SED with SET-RPLA were detected as SEA when subjected to immunoblotting procedure (data not shown), data probably a result of RPLA nonspecific reactions which are in agreement with previous observations (Rose et al. 1989). Moreover, the nonspecific reactions are because of the use of polyclonal antibodies used by the two methods.

Thus, the results obtained suggest that either SET-RPLA or immunoblotting may be applied to culture filtrates for the detection of SEs with good correspondence of results. Although SET-RPLA represents a simple method for routine monitoring purposes, a positive result by a rapid method is regarded as presumptive and must be confirmed by standard methods (Feng 1996), such as immunoblotting method. As suggested by Orden et al. (1992), immunoblotting may overcome some problems, because it reveals the molecular weight of the cross-reacting substances and it could be used to identify both native and heat-treated SEs at the same level of sensitivity (Balaban and Rasooly 2000). Finally, considering the epidemiological links between contamination of food and staphylococcal food poisoning in humans, the RPLA test and immunoblotting appear to be suitable tools for routinely monitoring food samples for the presence of SEs.

Further studies are required to develop specific and sensitive methods for the detection of SEs in food to build a new and efficient sanitary control approach.
REFERENCES


