Vet. Med. J. Giza 38, No. 1, 45-51 (1990).

TYPING OF STREPTOCOCCI BY MEANS OF SPECIFIC ANTIBODY ADSORBED TO PROTEIN-A CONTAINING STAPHYLOCOCCI

BY

M. ISMAIL

Department of Microbiology, Faculty of Veterinary Medicine, Cairo University.

(Received: 16.11. 1989).

INTRODUCTION

Staphylococcus protein-A (SPA) is a cell wall constituent of *Staphylococcus aureus* which has an extra - ordinary affinity for binding to the FC region of immunoglobulin G (IgG) from various mammalian species. Cell bound SPA is found to be useful as an adsorbent for detection of antigen-antibody complex (Goding, 1978, Kessler, 1981 and Lindmark et al., 1983).

The precipitation test is widely used for typing of streptococci by means of their carbohydrate substance or antigen "C" in their cell walls (Crusickshank et al., 1975). This test however, more laborious and time consuming as a routine test. A slide agglutination method is here described, that renders streptococcal typing quick and easy to be performed. When specific anti-streptococcal antibodies, were added to a stabilized suspension of staphylococci, they became bound to the protein A on the cell wall via the FC structure of IgG (Kronvall and Williams,1969 and Kronvall and Frommel, 1970), therefore, orienting the Fab-located antibody-combining sites outwards (Lind et al., 1972). When streptococci of the corresponding group are added, coagglutination occurs with the antibody coated staphylococci. Thus, this work was done to determine the more economic, rapid and easy method for typing of the examined streptococci.

MATERIALS AND METHODS

1. MATERIAL

a) Bacterial strains:

- **Cowan 1 strain:** of Staphylococcus aureus (NCTC Code No. 8530) capable of producing large amounts of protein A, was used throughout the present investigation for the preparation of typing reagent. This strain was obtained in lyophilized ampoule from Namro 3, Egypt.

- **Streptococcal strains:** A total of 45 streptococcal strains were used which were identified as 15. *Str. pyogenes* (group A), 15 *Str. agalactiae* (gorup B) and 15 *Str. equi* (gorup C). Streptococcal strains were obtained from routine isolations in Department of Microbiology, Fac. of Vet. Med., Cairo Univ.

b) Diagnostic streptoccoccal antisera:

Bacto-streptococcal rabbit antisera for serological typing of streptococcus groups (groups A, B and C) were obtained from Difco laboratories, Detroit , Mitchigan, U.S.A.

2. METHODS

- Characterization of the used streptococcal strains:

Pure colonies of streptococci were characterized morphologically, culturally and biochemically according to Sneath et al. (1986) and serologically according to El-Kholy et al. (1974) and Finegold and Martin (1982).

- Preparation of stabilized staphylococci:

This was performed according to Grangeot-Keros et al. (1982) as follows:

Staphylococcus aureus strain (Cowan 1) was grown onto trypticase soya agar plates. Cells from a fourty hours old culture at 37°C were harvested and washed twice with phosphate buffered saline (PBS), pH 7.4. The bacteria were then suspended in 0.5% formaldehyde in PBS and kept at room temperature for 3 hours. The formaldehyde-treated suspension was then washed four times in PBS and finally adjusted to a concentration of 10% (V/V) and stored at -20°C.

- **Preparation of typing reagent** (Kronvall, 1973) :
To 1.0 ml of a 10% (V/V) suspension of formaldehyde treated staphylococci, 0.1 ml of streptococcal serum was added. After mixing, the treated staphylococci was washed and resuspended in PBS, containing 0.1% sodium azide to a final concentration of 1% and then stored at 4°C for use.

RESULTS AND DISCUSSION

Typing of streptococcal strains with antibody coated staphylococci:

Staphylococci coated with specific antibody in this manner were used in typing of 45 strains of streptococci. One or more colonies of the strain to be tested were emulsified in two drops of typing reagent on a slide. The mixture was observed for 2 min., and the presence or absence of agglutination was recorded as illustrated in **(Fig. 1 & 2)**. In most instance, a positive reaction occurred within seconds. All streptococcal strains tested could be easily and quickly typed by the slide-agglutination method and the results obtained were in complete agreement with the results of typing by means of precipitation reaction but not needs the extraction of the carbohydrate antigens from the surface of the typed strains. This findings agree with the work of Kronvall (1973) who typed 89 strains of pneumococci using staphylococci coated with specific antibodies and the result goes

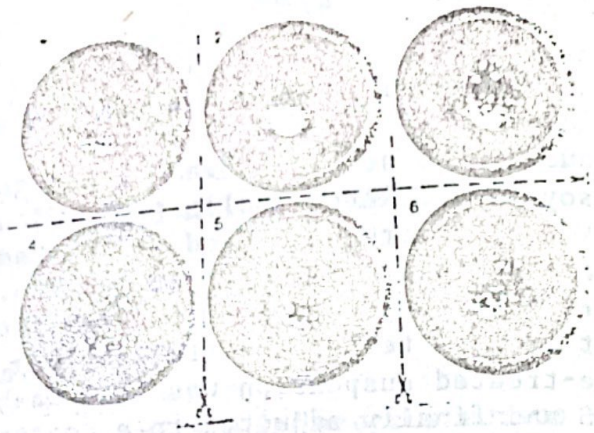
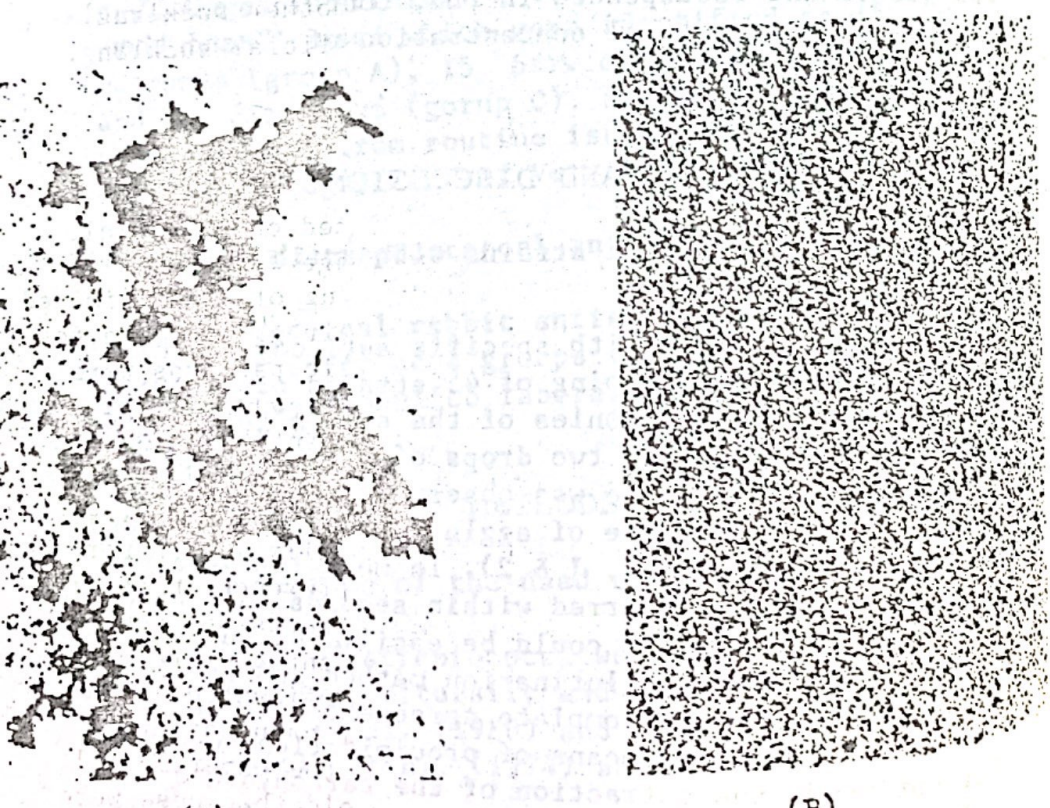


Fig. 1: Slide agglutination test using type specific streptococcal antiserum bounded with SPA. 1, 3, 4, 5, & 6 showing macroscopical agglutination patterns obtained with specific streptococcal antiserum bounded with SPA and the corresponsing streptococcus strains. No. 2 revealed negative results.



(A) (B)

Fig. 2: Microscopical agglutination pattern:
a) in positive strain with the corresponding antiserum adsorbed to SPA.
b) Negative reaction on using strain with non specific antiserum adsorbed to SPA i.e. no agglutination.

parallel with those of typing by Neufeld's capsule swelling methods. Also, these, results concide with those obtained by Kronvall and Frommel, (1970) , Kessler, (1981) and Lindmark (1983) who reported that, cell bound staphylococcus protein-A act as immuno-sorbent for detection of antigen-antibody complex. Generally, positive results (agglutination) were obtained on usign SPA coated antisera with the corresponding streptococcal strains only.

SUMMARY

- A slide agglutination method was used for serological typing of unknown strains of streptococcus species.

- Type-specific streptococcal antibodies were bound to stabilized staphylococcus protein-A through the FC region.

- This method is more economic, easily applied and not time consuming as regards to the precipitation reactions.

REFERENCES

1. Crusickshank, R.; Duguid, J.; Marmion, B. and Swain, R. (1975): Medical microbiology, 12th Ed. Vol. I & II. Charchill Living Stone, Edinburgh London and New York.

2. El-Kholy, A.; Wannamaker, L.W. and Krause, R.M. (1974): Simplified extraction procedure for serological grouping of Beta-haemolytic streptococci. App. Microbiol., 28 (5), 836-839.

3. Finegold, S.M. and Martin, W.J. (1982): Bailey and Scott's Diagnostic Microbiology, 6th Edt. The C.V. Mosby Co., St. Louis, Toronto, London.

4. Goding, J.W. (1978): Use of staphylococcal protein-A as an immunological reagent. J. Immunol. Meth., 20, 241-253.

5. Grangeot-Kerose, L.; Lebrum, L.; Briantais, M. and Pillot, J. (1982): A critical study of the use of staphylococci containing protein A for separation of IgG and IgM antibodies. J. Immunol. Meth., 51, 183-195.

6. Kessler, S.W. (1981): Use of protein-A bearing staphylococci for the immunoprecipitation and isolation of antigens from cells. Meth. Enzymol. 73, 442-259.

7. Kronvall, G. and Frommel, D. (1970): Definition of staphylococcal protein-A reactivity for human immunoglobulin G fragments. Immunochem., 7, 124-126.

8. Kronvall, G. and Williams, R. (1969): Differences in anti-protein A activity among IgG subgroups. J. Immun., 103, 828-830.

9. Kronvall, G. (1973): A rapid slide-agglutination method for typign pneumococci by means of specific antibody adsorbed to protein A-containing staphylococci. J. med. Microbiol., 6, 187-190.

10. Lind, I.; Reyn, A. and Birch-Andersen, A. (1972): Electron microscopy of staphylococcal protein-A reactivity and specific antigen-antibody reactions. Acta. Path. Microbiol. Scand., 80, 281-286.

11. Lindmark, R.; Thoren, K. and Sjoquist, J. (1983): Binding of immunoglobulins to protein A and Immunoglobulin level in mammalian sera. J. Immunol. Meth., 62, 1-13.

12. Sneath, P.A.; Mair, N.S.; Sharpe, M.E. and Holt, J.G. (1986): Bergey's Manual of systematic bacteriology. Vol. 2. 1st Ed. Williams and Wilkins, Baltimore, London, Los Angeles, Sydney.

Fig. 1: Slide agglutination test using type specific streptococcal antiserum bounded with SPA. 1, 3, 4, 5, & 6 showing macroscopical agglutination patterns obtained with specific streptococcal antiserum bounded with SPA and the corresponsing streptococcus strains. No. 2 revealed negative results.

Fig. 2: Microscopical agglutination pattern:
a) in positive strain with the corresponding antiserum adsorbed to SPA.

b) Negative reaction on using strain with non specific antiserum adsorbed to SPA i.e. no agglutination.