A RAPID AND SIMPLE METHOD FOR DISTINGUISHING COLONIES OF PROTEUS FROM THOSE OF SALMONELLA AND SHIGELLA

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SUMMARY. A rapid and simple method is described by which colonies of Proteus can be distinguished from those of Salmonella and Shigella and other non-lactose-fermenting organisms growing on MacConkey's agar or desoxycholate citrate agar. The method is based on the ability of Proteus to produce urease constitutively. The enzyme was detected by the degradation of urea by the inoculum, thereby creating an alkaline reaction on pH-indicator paper.

INTRODUCTION

Examination of faeces for the presence of Salmonella and Shigella usually involves an initial screening of the non-lactose-fermenting colonies on media such as MacConkey's or desoxycholate citrate agar for the ability to degrade urea. Those that can do this are excluded from further examination. Proteus spp., which can degrade urea, are often found in faeces, and if few Salmonella or Shigella are present and not enough colonies are screened for the ability to degrade urea, the pathogens may go undetected despite the use of selective methods. The usual method for determining urease production involves inoculation of media containing urea, and observation for the development of alkalinity after incubation. Quicker methods have however been described, for example, that of Bergquist and Searcy (1963). The method described in this paper is much simpler and gives a result within minutes, thereby facilitating the screening of more colonies.

MATERIALS AND METHODS

Bacterial strains. All the strains used in this study were isolated from clinical specimens, were identified by appropriate biochemical and serological tests and were maintained as pure cultures on nutrient-agar slopes. Those used were: Proteus mirabilis (10 strains), P. vulgaris (10), P. morganii (10), P. rettgeri (10), Shigella sonnei (15), Sh. boydi (1), Sh. flexneri (1), Escherichia coli (1), Pseudomonas aeruginosa (5), Klebsiella pneumoniae (5), Salmonella typhi (1), S. typhimurium (15), S. anatum (1), S. infantis (1) and S. enteritidis (1).

Culture media. MacConkey Agar (Oxoid CM7) and Desoxycholate Citrate Agar (Oxoid CM35) were prepared as directed by the manufacturer and were inoculated with either the stock cultures or specimens of faeces received for routine bacteriological examination. The plates were incubated at 37°C overnight.

The test. Colonies were touched with a sterile straight wire and the inoculum transferred to a strip of Whatman-B.D.H. pH-indicator paper (range pH 6–8) lying on a piece of white blotting paper. No attention was given to any colour change of the indicator paper at this stage. A little aqueous solution of urea (2% w/v), just enough to moisten the indicator paper, was applied with a pasteur pipette over the inoculum and on to the indicator paper. When a proteus strain was tested, local alkalinity, about pH 9, appeared within 1 min as a diffusing sky-blue colour around the inoculum, and this was accepted as a positive reaction. The colour continued to develop and diffuse for a few minutes and began to fade after about 15 min. A negative reaction, such as was given by Salmonella and Shigella, did not produce the diffusing sky-blue colour. At the end of the test, the paper was burned or autoclaved.

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RESULTS AND DISCUSSION

In an attempt to hasten the recognition of Proteus from other non-lactose-fermenting colonies isolated from faeces, a method was sought that would be quick, simple to perform and cause a minimum change to the well established standard methods. Initially, attempts were made to identify proteus colonies in situ by incorporating urea and a pH indicator into culture media. Despite several attempts with different indicators, this approach proved unsatisfactory. Another approach was to add a loopful of the indicator to non-lactose-fermenting colonies. However, because of diffusion and uptake of dye by salmonella and shigella colonies growing close to proteus colonies, this method was also unsatisfactory.

Many different forms of urease are found in Proteus and although the enzymes are inducible, most strains form small amounts of urease constitutively (Senior, Bradford and Simpson, 1980). The urease test is usually performed by subculture in media containing urea in which growth and enzyme induction has to occur before a positive result can be recorded. This may take several hours, P. morgani in particular usually requiring overnight incubation.

The method described here eliminated the delay by detecting constitutively produced enzyme. In tests made on all the listed bacterial strains and 350 non-lactose-fermenting colonies isolated from faeces, only those belonging to the genus Proteus gave positive reactions when tested as described. No isolate subsequently identified as a species of Salmonella and Shigella gave a positive urease reaction by this test. Colonies of Pseudomonas spp. are rarely confused with Salmonella or Shigella and they usually gave a negative reaction although a few gave a very weak positive reaction (some species produce small amounts of urease). Other non-lactose-fermenting organisms did not produce apparent alkalinity.

A positive reaction arises through the development of alkalinity after breakdown of urea by constitutively formed urease. When urease inhibitors were added to the urea solution or the solution was replaced by water, the reaction did not occur.

Although it was difficult to standardise the size of the inoculum transferred to the indicator paper, the strength and development of the reaction seemed to be influenced by the species of Proteus under test. The strongest and most rapid positive reactions were given by P. morgani, then by P. mirabilis, P. vulgaris and finally P. rettgeri.

The method is simple to perform and requires no altered or additional media. If necessary, phenol can be incorporated into the urea solution to a final concentration of 1% (w/v) without apparently affecting the reaction. The greatest advantage of the test may be its speed, the results being obtained after 1 min, and the reaction is stable for long enough for the results to be recorded with ease. No discrepancies have been observed between this test and the traditional method.

REFERENCES
