The specific detection of *E.coli* using commercial polyclonal serum for the development of a biosensor

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INTRODUCTION

Escherichia coli, is a rod shaped gram negative bacterium. It is always found in the presence of faeces and is therefore a direct indicator of faecal contamination. *E. coli* is easy to culture and although most strains are not pathogenic, its presence may indicate the possibility of pathogenic organisms in water. The means to identify *E. coli* specifically and sensitively is still only available to highly specialized laboratories and methods still remain too cumbersome and costly to be employed for routine drinking water analysis.

Most rural homes in South Africa collect their water in polyethylene or galvanized steel containers[1]. This water is collected directly from boreholes or rivers and can be stored for up to 48 hours, during which time the water is used for direct consumption, cooking and cleaning. It was shown that both forms of these containers supported the growth and survival of coliforms such as *E. coli*[1]. It is reported that 30% of the South African population do not have access to an adequate supply of potable water and have to rely on raw surface or ground water for drinking[2]. A routine test that can detect *E. coli* sensitively and specifically is greatly needed in South Africa.

The aim of this study was to determine whether the specificity required for a biosensor can be achieved using polyclonal antibodies.

METHODS

Enzyme linked immunosorbent assay was used to determine antibody binding efficiency to whole cells as well as LPS extracts and protein digested whole cells.

Whole cell protein digest by trypsin was carried out by supplementing 10^8 cells/ml suspended in phosphate buffer with 0.2% trypsin (w/v) and allowing overnight incubation with gentle shaking at 37˚C.

Hot phenol extraction of lipopolysaccharide (LPS) on whole bacterial cells was performed using the procedure initially published by Westphal & Jann[3].

RESULTS & DISCUSSION

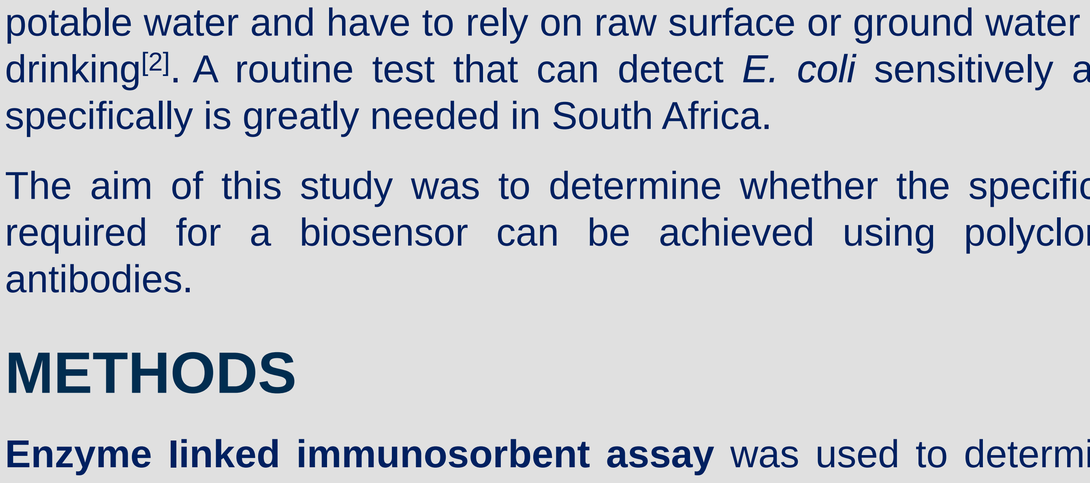

Fig 1: Serum sample at 1:100 dilution against titrated concentrations of *E. coli* and *A. baumannii* . Error bars represent standard deviation (n=3)

Figure 1 illustrates cross reactivity of the polyclonal serum to *Acinetobacter baumannii*, a water borne bacterium also isolated from waste water. Further cross reactivity to other bacteria from the family of Enterobacteriaceae is evident in figure 2. This highlights the lack of specificity of the polyclonal serum to *E. coli*.

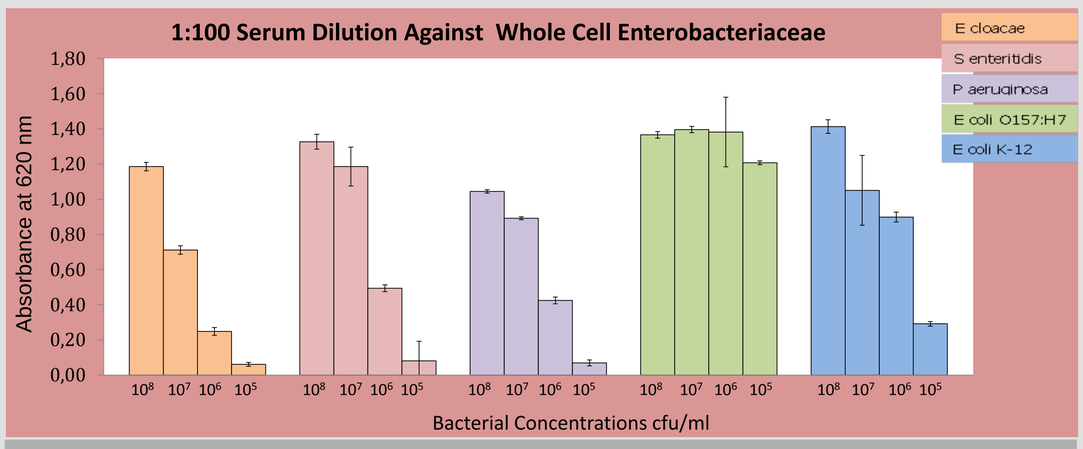

Fig 2: Serum sample at 1:100 dilution against titrated concentrations of *E. cloacae, S. enteritidis, P. aeruginosa, E. coli O157:H7 and K-12* . Error bars represent standard deviation (n=3)

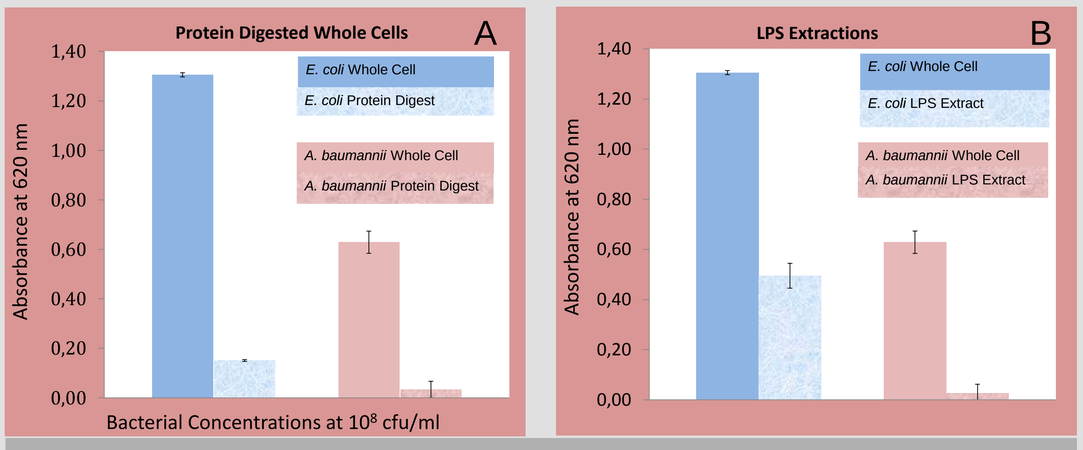

Fig 3A: Serum sample at 1:100 dilution against protein digested whole cells of *E. coli* and *A. baumannii* showing reduction in non specific binding. B) LPS extractions from whole cell *E. coli* and *A. baumannii* at 4.1g/ul and 4.2g/ul respectively. Error bars represent standard deviation (n=3)

Figure 3A shows an ELISA with polyclonal serum to protein digested whole cells of *E. coli* and *A. baumannii* showing a complete reduction in cross reactive signal of *A. baumannii*. Figure 3B shows an ELISA with LPS extracted from whole cell again showing complete reduction of cross reactive signal to *A. baumannii*.

CONCLUSION

Initial studies show a high degree of cross reactivity with polyclonal serum to whole cells. Experiments were repeated using a second polyclonal serum from a different manufacturer yielding similar results. Results indicate cross reactivity of the polyclonal serum is proteinaceous in nature and that specificity may be attained from the LPS antigen. Cross reactivity of polyclonal serum makes this a bad choice of bioprobe and therefore cannot be used effectively in the development of a biosensor. However, the specific binding of the polyclonal antibody to LPS antigen can be exploited for use in a biosensor to yield specificity that is comparable to current gold standards.

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