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# Protective ability of locally extracted Protein A against pathogenic organisms

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**Summary**

This study was conducted to find out the ability of Protein A obtained from a local isolate of *Staphylococcus aureus* to protect rats against infection by pathogenic organisms. A good amount (5000µg) of Protein A was extracted from a small quantity (approximately 40g) of *Staphylococcus aureus* culture using lysostaphin technique. This extract was found to have protective property against pathogenic *Escherichia coli* and *Pseudomonas aeruginosa* in rats even at a low concentration of 50µg. The crude Protein A extract also compared favourably with imported standard Protein A in the study.

**Keywords:** *Staphylococcus aureus, Protein A extract, pathogenic organisms.*

**Résumé**

Cette étude était conduite pour rechercher l'habilité de la protéine A obtenue d'un isolat local de *Staphylocoque aureus* á protéger les rats contre l'infection des microorganismes pathogéniques. Une quantité importante (5000µg) de Protéine A était extraite de 40g de culture de *Staphylocoque aureus utiisant la technique de* lysostaphine. Cet extrait démontrait une propriété protectrice contre *Escherichia coli* et *Pseudomonas aeruginose pathogénique chez les rats a la concentration faible de* 50µg. L'extrait pure de protéine A était favorablement comparable a la protéine A standard importée

## Introduction

*Staphylococcus aureus* is an important human pathogen incriminated in several clinical disease conditions that are often fatal with high mortality rate. These include: staphylococcal meningitis, septicaemia, bronchial pneumonia, urinary tract infection, conjunctivitis in children, post-operative infections, otitis media, wound infections etc, which have been frequently reported in hospitals and clinics. Several dental infections have also been caused by *S. aureus*. *Staphylococcus aureus* is aerobic, grows at 35-37°C. Some strains produce heat-stable enterotoxin which, if ingested, gives rise to acute food poisoning. The hospital-acquired strains (nosocomical) are usually more resistant to antibiotics than strains encountered in open communities [1-5].

*Staphylococcus aureus*, like other living microorganisms, constantly synthesize materials primarily for growth and adaptation to environment. These biosynthetic activities of microorganisms often bring a huge advantage to mankind [6]. Protein A from a few strains of *Staphylococcus aureus* have been well documented to have a lot of beneficial uses [7-9]. The major feature of Protein A, as far as Immunologists are concerned, is its extraordinary affinity for immunoglobulin, notably IgG [10]. This affinity for IgG was first detected by an apparent high incidence of "natural antibodies" to *Staphylococcus aureus* in normal human serum, a reaction subsequently shown to be "pseudo-immune" [11]. This interesting and very useful property has been widely exploited as immunological tool. It has been of great interest to develop efficient methods for the production of staphylococcal Protein A [12]. In earlier methods of investigations, Protein A has been produced from *Staphylococcus aureus* strain COWAN I type by treatment of the whole bacteria with lysostaphin [13-14]. Dialysis and dialysis culture techniques were also used to optimize staphylococcal Protein A production [15-16].

The properties of Protein A from *Staphylococcus aureus* has been extensively explored in developed countries, and its immunomodulatory properties against cancer, tumors, carcinogens, bacteria, and bacteria toxins have been known [17-29]. Protein A is attached to the peptidoglycan layer of the cell wall [30]. This study was therefore conducted with the aim of extracting protein A from a local strain of *Staphylococcus aureus*, determine its protective ability against infections by *E. coli* and *Ps. aeruginosa* in rats and also compare its activities with a standard protein A.

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## Materials and methods

*Chemicals*

Standard Protein A, lysostaphin, Neomycin were purchased from SIGMA, USA. Other materials and reagents, culture media were procured locally.

*Microorganisms*

Bacteria strains were isolated in the department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan, Baptist Medical Centre, Ogbomoso and Shalom Medical Centre, Ogbomoso. Control bacteria were obtained from American Type Culture Collection (ATCC) in lyophilized form.

*Experimental animals*

Eight weeks old albino rats were purchased from the central animal house, College of Medicine, University of Ibadan.

*Staphylococcus aureus* strains were isolated from blood, pus, aspirates and urine of patients in the hospital: Two milliliters (2mls) of blood obtained by venepuncture were inoculated into 20mls Brain Heart Infusion (BHI) broth. These were incubated at 37°C aerobically, and subcultured after 72 hours on Blood Agar (BA) and McConkey (MCC). These were incubated at 37°C for 24 hours. Pus, aspirates and swabs were plated on BA, MCC and Mannitol salt agar (MSA).

Pure isolates characteristic of staphylococcus species obtained were tested for coagulase, using slide and tube methods. BHI, BA, MCC and MSA were prepared in our laboratory. Neomycin-resistant strain of *Staphylococcus aureus* are known to possess good amount of Protein A in their cell walls [31]. Therefore 30µg Neomycin disc were prepared as described by Monica [1], and resistance to neomycin was determined as follows: one hundred different isolates of *Staphylococcus aureus* were screened for neomycin sensitivity by disc diffusion. Colonies of *Staphylococcus aureus* were inoculated into sterile peptone water. Inoculum was standardized, and a loopful was applied to the centre of Muller Hinton sensitivity agar. It was spread evenly across the centre third of the plate.

Similarly, control *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were inoculated across the upper and lower thirds of the plate. The prepared Neomycin discs were placed on the test and the controls, incubated at 37°C for 24 hours. The zones of inhibition were measured for the test and the controls, and were compared with WHO standard sensitivity table [32].

*Extraction of Staphylococcal Protein A*

Protein A was extracted from the Neomycin-resistant strains of *Staphylococcus aureus* using modified Janet method [33]: 0.5g wet weight of screened *S. aureus* was mixed with 25µg/ml lysostaphin powder in Phosphate Buffered Saline (PBS) pH 7.2. The mixture was allowed to stand at 37°C for 3 hours, centrifuged at 10,000 revolutions per minute for 2 hours. The supernatant containing the Protein A was retained while the deposit was discarded.

*Lysostaphin efficiency test*

Smears were made from bacteria suspension before and after lysostaphin action. Smears were Gram stained and examined microscopically. The lysed portion was cultured on BA, MSA, MCC and incubated for 24 hours at 37°C aerobically.

*Quantitation of Protein A extract*

Twenty millilitres (20mls) of extract were mixed with 20mls ethanol and kept at 40°C for 24 hours. Precipitate was concentrated by centrifugation. Supernatant was carefully decanted, and the deposit dried at 37°C for 24 hours. The quantity of Protein A was determined by weighing.

*Investigating the effects of crude Protein A extract on prevention of bacteria infection*

Ability of seven days prophylactic effect of Protein A extract to protect against *E coli* and *Ps aeruginosa* infection in rats were investigated using Bill method [34]: Eight weeks old albino rats were fed on pellets and sterile distilled water. An aliquot 50µg Protein A extract in sterile distilled water was administered intraperitoneally into each animal. 0.2mls of ($4.0 \times 10^7$/ml) bacteria suspension was administered intraperitoneally into each animal 7 days post Protein A administration. Infection was followed for 10 days. Survival was recorded daily. Surviving animals were sacrificed by cervical dislocation after 10 days, livers excised aseptically, and weighed. Whole liver was homogenized in 20ml sterile Nutrient broth. The homogenate was seeded on Nutrient agar, and incubated at 37°C aerobically for 24 hours for bacteria load count, using Miles and Mizra method [1].

*Comparison of crude Protein A extract with Standard Protein A:*
Fifty microgram standard Protein A was administered intraperitoneally into another set of animals, and followed by infection with bacteria after 7 days, and treated as above.The control groups consisted of unprotected animals infected with *E coli* and *Ps. aeruginosa.*

## Results

*Isolation and identification of Staphylococcus aureus*
A total of 250 staphylococcus spp were isolated. 150 were coagulase-negative and were discarded. 100 coagulase-positive (*Staphylococcus aureus*) strains were identified and screened for Protein A.

*Qualitative screening for Protein A*
70 (70%) showed partial resistance to 30µg Neomycin with zones of inhibition ranging between 2-6mm. 29 (29%) were sensitive to 30µg Neomycin with zone of inhibition ranging between 18-22mm. 1 (1%) was absolutely resistant (zone of inhibition 0) (Fig. I). This absolutely resistant strain was multiplied and used for Protein A extraction. The efficacy of the prepared Neomycin disc was evident on the Neomycin-sensitive control *S. aureus* ATCC 25923, *E. coli* ATCC 25922 strains (Fig. 1).

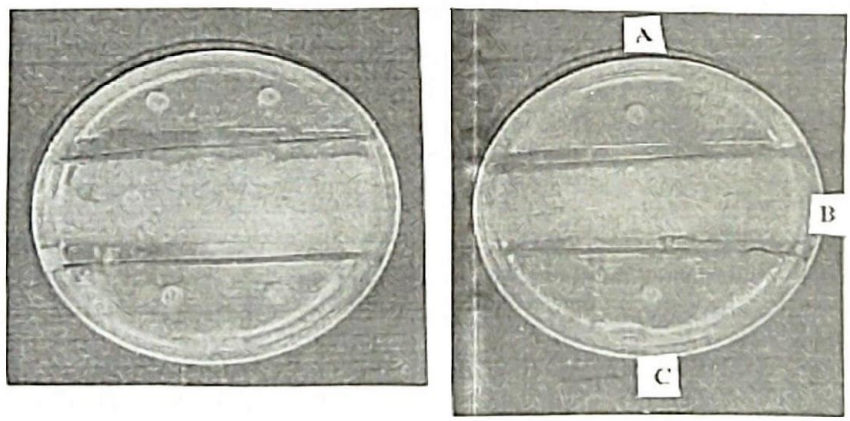
*Quantitation of Protein A extract*
40g wet weight of *Staphylococcus aureus* used yielded 5000µg.

*Effect of crude Protein A extract on bacteria*
Fifty microgram protein A extract administered 7 days before bacteria infection protected the rats. Similarly, 50µg standard protein A protected the rats. Survival rate of the rats was 100% with the protein A extract and standard protein A. In the control groups infected with *E coli* and *Ps aeruginosa* without protection with protein A, all the rats died – survival rate was zero (0) (Table 1).

Table 2 shows the result of bacteria load count: there was no organism found in the livers of rats protected with protein A extract and standard protein A. *E coli* and *Ps aeruginosa* were found in the livers of the control groups without protection with protein A. The results found with crude protein A extract was identical to that of standard protein A. (Tables 1 and 2).

## Discussion

Several studies have been conducted on the immunological properties, and extraction of staphylococcal protein A. The researchers used

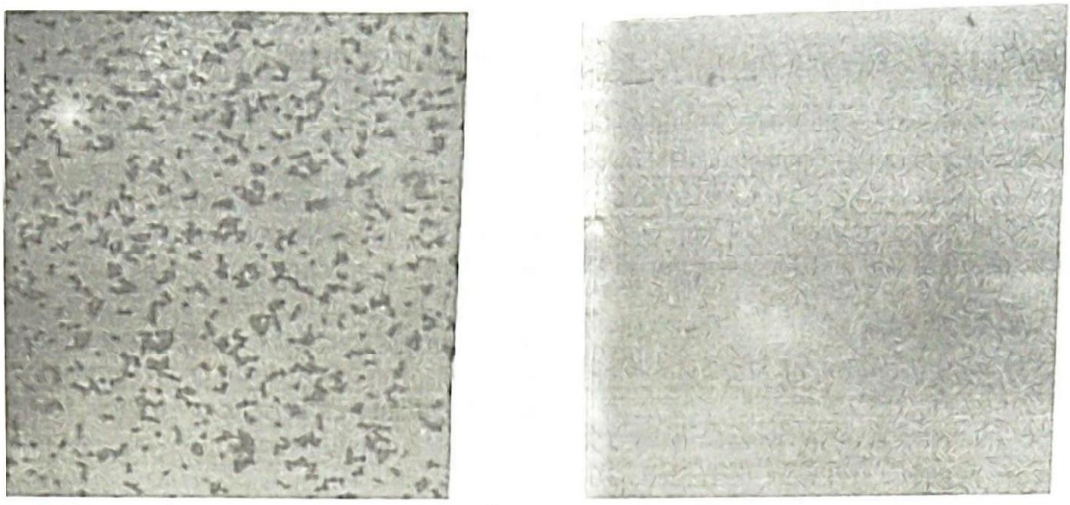



A- *Control 1: Staphylococcus aureus ATCC 25923. (Zone of Inhibition = 28mm)*
B- *Local strain Staphylococcus aureus resistant to Neomycin (Zone of inhibition = 0)*
C- *Control 2: E coli ATCC 25922 (Zone of inhibition - 26mm).*

**Fig. 1:** Photograph showing the sensitivity pattern of standard control bacteria and locally isolated strain of *staphylococcus aureus* to Neomycin.

*Lysostaphin efficiency test*
The lysed portion cultured on BA, MCC and MSA yielded no growth. Microscopic examination of stained smears showed complete lysing (Fig. 2).

*Staphylococcus aureus* Cowan I strain. Its antiviral [29], anti-tumour [23,25,28], antitoxic [24,37], anti-carcinogenic [28], and anti-bacteria [37] properties have been documented. Many methods have been employed for the extraction of staphylococcal protein



*Before Lysing* *After Lysing*

**Fig. 2:** Photomicrograph showing Staphylococcus aureus before and after lysostaphin lysing (x 2500)

**Table 1:** Survival rate of 10 rats post protein A extract administration against *E coli* and *Ps. aeruginosa*

| Rat groups | No of death | No of survival | Percentage (%) survival |
|---|---|---|---|
| Post protein A extract administration against *E coli* | 0 | 10 | 100 |
| Post standard protein A administration against *E coli* | 0 | 10 | 100 |
| Unprotected rats infected with *E coli* | 10 | 0 | 0 |
| Post protein A extract administration against *Ps aeruginosa* | 0 | 10 | 100 |
| Post standard protein A administration against *Ps aeruginosa* | 0 | 10 | 100 |
| Unprotected rats infected with *Ps aeruginosa* | 10 | 0 | 0 |

**Table 2:** Bacteria load count of 10 rats post protein A extract administration against *E coli* and *Ps aeruginosa*

| Rat groups | Bacteria load per gram liver weight of each rat | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Post protein A extract administration against *E coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Post standard protein A administration against *E coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unprotected rats infected with *E coli* | 800 | 900 | 1200 | 1200 | 1250 | 1400 | 1400 | 1500 | 1600 | 1600 |
| Post protein A extract administration against *Ps aeruginosa* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Post standard protein A administration against *Ps aeruginosa* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unprotected rats infected with *Ps aeruginosa* | 780 | 850 | 1000 | 1250 | 1400 | 1500 | 1600 | 1700 | 1800 | 1800 |

A, some of which are sophisticated and expensive, while some are simple and inexpensive [13,16,20].

This present study concentrated on a *Staphylococcus aureus* strain isolated in our locality. The immunological activity against Gram-negative bacteria was investigated in rats. It protected the rats against infection by the pathogenic bacteria and this result correlates with the reports of other workers in other countries, Australia, India, America [12,18,37].

The treatment of whole bacteria with lysostaphin was employed in this study. Complete lysis was achieved (Fig. 2). Approximately 40g wet weight

of *Staphylococcus aureus* yielded 5000μg protein A. This quantity is significantly low compared with the report of other workers where 40g wet weight of *Staphylococcus aureus* Cowan I (NCTC 8530), yielded 67,000μg protein A [13].

Neomycin-resistance method of screening *Staphylococcus aureus* for protein A was effective in this study, as was also reported by another worker in Denmark [31].

Other workers reported that staphylococcal protein A is not associated with pathogenicity [10,20,35], unlike in *E coli* where it has been proved to be associated with neonatal meningitis [36]. In this study too, protein A extract was found not to be pathogenic to the rats.

## Conclusion

Protein A extract obtained from a local isolate of *Staphylococcus aureus* has immunological property in rats even at low concentration. It compared favourably with imported standard protein A obtained from Cowan I strain of *Staphylococcus aureus*. The treatment of whole bacteria with lysostaphin remains effective and useful technique for extraction of staphylococcal protein A. The protein A extract was not associated with pathogenicity. Therefore production of staphylococcal protein A locally is recommended to promote our local microbial biotechnology, and conserve foreign exchange. Further research should also be intensified towards appropriating the benefits of staphylococcal protein A in our environment.

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**Abbreviation:** NCTC: National Collection of Typed Cultured

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