**Table 2: Recovery of *S. aureus* from peritoneal fluid obtained from mice challegened with live *S. aureus***

| Bacterial challenge (CFU) | $10^6$ | $10^7$ | | $10^8$ | |
|---|---|---|---|---|---|
| Time | 36 hrs post infection | 18 hrs post infection | 36 hrs post infection | 18 hrs post infection | 36 hrs post infection |
| Control group (non-immunized) | 0 | $1.08 \pm 0.14 \times 10^6$ | $8.98 \pm 1.94 \times 10^6$ | $1.11 \pm 0.20 \times 10^8$ | [2]N/A |
| PBS | 0 | $4.92 \pm 0.94 \times 10^5$ | $1.54 \pm 0.94 \times 10^6$ | $3.16 \pm 1.94 \times 10^6$ | [2]N/A |
| IFA | 0 | $2.35 \pm 1.12 \times 10^5$ | $1.23 \pm 1.14 \times 10^6$ | $1.80 \pm 0.22 \times 10^6$ | $2.16 \pm 1.10 \times 10^6$ |
| PHIS-01 | 0 | $3.64 \pm 1.44 \times 10^5$ | $1.13 \pm 2.31 \times 10^6$ | $8.90 \pm 3.20 \times 10^5$ | $5.60 \pm 0.94 \times 10^5$ |
| PHYTOL | 0 | $4.0 \pm 0.25 \times 10^3$ | 0 | $8.90 \pm 1.12 \times 10^4$ | 0 |

[1] Peritoneal fluid samples from all groups of mice were diluted in LB medium (1:200), and 10 μl of diluted samples were streaked on agar plates using calibrated loops. CFUs were scored 18 and 36 hours following bacterial challenge and recorded as CFU/ml. The results represent the average of two experiments (n = 4 for each group) ± SEM. [2] N/A: Not assessable since all mice were dead within 18 hrs.

such as phytol or phytol-derived compounds, as adjuvants. In our ongoing study [12], we have tested the adjuvant activity of phytol, and its reduced derivative, PHIS-01, and observed efficient stimulation of antibody response against hapten antigens conjugated to a protein carrier. We have observed that the response due to these adjuvants appeared superior to what has been observed with conventional adjuvants, such as CFA/IFA, TiterMax, and Alum. PHIS-01, in particular, also efficiently evokes cellular immunity, including tumor-specific cytotoxic and helper T cell responses [12]. In this report, we have assessed the usefulness of the adjuvant potentials of phytol and PHIS-01 in augmenting efficacy of vaccines against the common infectious agents *S. aureus* and *E. coli*.

Our vaccine formulations contain heat-inactivated bacteria emulsified with standard IFA, or either of the two experimental phytol-based adjuvants. The latter, unlike IFA, have been used without any emulsifying or surface-active agents. In spite of these differences, these new adjuvants are effective not only in augmenting anti-bacterial humoral responses against both *E. coli* and *S. aureus*, but also in preventing bacteremia and death caused by these infections. Phytol and its derivative seem to be excellent adjuvants for their ability to enhance and sustain quality antibody responses (preventing bacteremia) over a longer period of time. Thus, phytol-based novel adjuvants significantly improve vaccine efficacy by modulating immunogenicity and toxicity of the heat-killed bacterial inocula, responsible for gram-negative bacteremia [16-18]. However, phytol and PHIS-01 adjuvants differ in their effectiveness against gram-positive *S. aureus*. Phytol is better at increasing specific antibody responses and preventing bacteremia and death due to *S. aureus*.

It has been well known that IgG2a is the most desirable antibody isotype for therapeutic applications involving normal immune responses [19]. This isotype is more effective in activating complement, promoting antibody-dependent cellular cytotoxicity, and conferring protection against tumors or parasite invasion than any other isotype. In mice immunized with *E. coli*, phytol and in particular, PHIS-01 exert their effects in raising mouse serum levels of all major IgG subclasses, specifically IgG2a antibody. In contrast, mice vaccinated with *S. aureus* lysates emulsified with phytol register higher levels of IgG1-type antibody, and are better protected, whereas IFA and PHIS-01 do not exert much effect on this isotype switch. Both IFA and PHIS-01 promote induction primarily anti-staph IgM response, which is not associated with the immunological memory. This induction of IgG1 antibody against gram-positive *S. aureus* observed with phytol implicates Th2-type cellular responses and the establishment of immunological memory.

Adjuvants facilitate the persistence of antigens at injection sites, the so-called depot effect. The qualitative differences in adjuvant efficacy can also be gleaned from the analyses of antigens involved in immune responses. The *E. coli* antigens recognized by immune sera due to phytol and PHIS-01 are clearly discernible on western blots as compared to immune sera obtained from IFA-immunized mice. A 45 KDa antigen was recognized by antibodies from mice immunized with phytol and PHIS-01 only. Similarly, IgG antibodies only from the phytol group recognized four unique *S. aureus* antigens (approximately 45, 74, 90 and 95 KDa). Our findings suggest that phytol and PHIS-01 differ from the conventional adjuvant IFA in their ability to augment the immunogenicity of bacterial antigens. This may explain why phytol and its derivative provide better protection against re-exposure to the pathogens. The biochemical nature of these antigens remains to be elucidated.

The efficacy of phytol and PHIS-01 as adjuvants is also evident in the quality of protection that the vaccines pro-