*Summary*

In this study, 159 milk samples were collected from Abu-Ghraib, Al-Mahmodia and Khanaqeen city from cows suffering from acute mastitis and apparently normal cows, cultured on blood agar and mannitol salt agar, after purification of cultured bacteria, biochemical tests and API Staph System, results showed that 56 out of 159 milk samples were positive for *S.aureus* ,the ability of these *S.aureus* isolates to produce biofilm were detected and the results showed that 46 out of 56 *S.aureus* isolates produced biofilm (82.14%) with different thickness ranged between (0.2-1.5)mm, while 10 isolates had not produce biofilm.

The results showed that protein concentration of biofilm was 9 mg/ml for one isolate and 18 mg/ml for another.

Optimal pH for biofilm production was studied, the result showed that optimal pH was (7) with mean thickness 0.834 mm, also optimal temperature for biofilm production was studied and ranged between (35-40) ºC with mean thickness (0.934 , 0.626)mm respectively.

The resistance of these *S.aureus* isolates against (8) of antibiotics (Ampicillin, Chloramphenicol, Tetracycline, Vancomycin, Gentamycin, Ciprofloxacin, Cloxacillin and Trimethoprim) were studied and results showed that all these isolates were susceptible 100% to Ciprofloxacin, in addition the results showed that the resistance of biofilm producer isolates and non-biofilm producer isolates against these antibiotics, all isolates of group of biofilm producer were more resistant than group of non-biofilm producer isolates against all (7) of antibiotics except Cloxacillin at which group of non producer biofilm was more resistant than group of producer biofilm.

Three types of bacterial antigens were prepared as follow ; Whole cell sonicated antigen (WCA) of biofilm producer *S.aureus* and Whole cell sonicated antigen of non-biofilm producer *S.aureus* and biofilm extract antigen (biA), then these antigens were injected in 60 healthy White BALB mice.

Results showed that survival time of animals in the immunized group with biofilm antigen of high protein concenteration (18 mg/ml) was longer (516 hrs) than animals immunized with whole sonicated antigen, heavy bacterial isolation was recorded in the internal organs of the immunized infected animals at (24-72) hours post infection, while moderate bacterial isolation at day 30 post infectin.

Histopathological examination showed large abscess which caused adhesion of liver with stomach and spleen with stomach and pancreas surround by dense cellular fibrous tissue, the result showed that all animals of control positive group,8 animals of group injected with (WCA)not produce biofilm,7 animals of group injected with (WCA) produce biofilm,7 animals of group injected with (biA) 4.5 mg/ml and 3 animals of group injected with (biA) 18 mg/ml were died during 24-72 hours post infection, acute suppurative reaction were seen in internal organs of animals died at 24-72 hours post infection, granulomatous lesions were seen in most internal organs of animals in group II , III and IV at day 30 post challenge, while mild inflammatory reaction was recorded in most internal organs of group IV , V at day 30 post challenge.