Effect of removal capsular polysaccharides by rapid agitation on staphylococcus aureus pathogenesis

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Abstract

The capacity of capsular polysaccharides enable bacteria to resist phagocyte process and help it to dissemination in liver & spleen organs was tested in vivo by measurement of log number of bacteria isolated from these organs in addition to show organ section . Two mice groups was used the first one injected with encapsulated strain, the other one injected with capsule-removed strain

The results of this study confirmed the important role of capsule in improvement of the dissemination of encapsulated *S. aureus* bacteria into some organ of mice which observed by increase in log number of bacteria isolated from liver & spleen through an increase in the time of mice injection and reach maximum at 72h to (4.89) (5.32) respectively, but mice groups injected with capsule removed strain reach to (3.16) in liver and (3.78) in spleen at 24h , then decrease of low level in other injection time .

Liver section of mice injected with the same encapsulated *S. aureus* show congestion , hemorrhage combined by PMN cell infiltration in addition to fusion of white pulp in spleen . These pathological changes occur too ,but less severe in mice injected with capsule –removed strain .

الخلاصة

مقاومة لعملية البلعمة ومساعدتها في الانتشار إلى كبد وطحال الفئران *S.aureus*قابلية متعدد السكر يد ألمحفظي القادرة على جعل جرثومة المصابة أمكن ملاحظتها عن طريق حساب لوغارتم العدد الجرثومي الحي المسترد من أعضاء تلك الفئران بالإضافة إلى فحص الشرائح المكبسلة في حين حقنت الأخرى بنفس S.aureusالنسيجية لتلك الأعضاء ، حيث استخدمت مجموعتين من الفئران الأولى حقنت بجرثومة السلالة من الجرثومة ولكن بعد إزالة المحفظة منها بعملية الرج السريع .

وذلك من خلال الزيادة *S.aureus*أكدت نتائج هذه الدراسة أهمية الدور الذي يلعبه متعدد السكر يد ألمحفظي في أمراضية جرثومة الواضحة في لوغارتم العدد الجرثومي الحي المسترد من كبد وطحال الفئران المصابة بزيادة الوقت لتصل إعدادها عند 72 ساعة والبالغة (4.89) و (5.32) بالتعاقب . في حين المجموعة المصابة بالسلالة غير المكبسلة وصل العدد الجرثومي إلى (3.16) في الكبد و (3.78) في الطحال بعد مرور 24 ساعة من الحقن ، في حين انخفض العدد الحي المسترد بزيادة وقت الحضن .

المكبسلة احتقان دموي ونزف مصحوبا بارتشاح كمية كبيرة S.aureusبينت المقاطع النسيجية المأخوذة من كبد الفئران المصابة بجرثومة من خلايا الدم البيض مشكلة النواة بالإضافة إلى التوسع والاندماج الكبير في اللب الأبيض الطحالي لهذه الفئران ، هذه التغيرات النسيجية ظهرت أيضا ولكن اقل حدة في الفئران المحقونة بالسلالة الجرثومية غير المكبسلة .

INTRODUCTION

S.aureus produces a myriad of virulence factors that contribute to its ability to cause disease, allowing the organism to gain entry into tissues , evade the host immune system , attach to host cells (11). Some *S.aureus* bacteria form a protective structure called capsule that surrounds the cell wall and is especially important in protecting bacteria cells against phagocytosis by eukaryotic cell , these layers contribute to the ability of bacteria to attach or adhere to particular host cell or

tissue , this an important factor that determines the virulence of particular pathogens (4)

Having a capsule can be a major factor in determining the pathogenicity of *S.aureus* bacteria , because the non capsulated *S.aureus* strain are subject to phagocytosis by blood cells involved the immune response of the infect host organisms , on the other hand phagocytizing blood cells are unable or less able to adhere to , engulf , and digest those bacteria that have capsules (12) .

Capsular polysaccharides have been shown to be produced from more than 90% of *S.aureus* isolates , the production of capsule in strain of *S.aureus* has been correlated with its virulence properties in both in vitro phagocytic assay and in vivo mouse lethality assay (14). The capsule increases virulence of *S.aureus* bacteria in laboratory animals through the rabid dissemination of bacteria into several mice organs (21) and the capsule prevents the interaction of the opsonins with its receptors on bacterial surface , that cause protected bacterial cells from phagocytosis (2).

Several studies examined the ability of capsulated and un capsulated *S.aureus* bacteria to spread through the host body and proliferation in mice liver and spleen by measurement of colony forming unit (CFU)of cell isolation from these organ and found the high CFU of capsular *S .aureus* bacteria isolated from mice organs compared to the CFU isolated from mice infected with *S.aureus* bacteria after removal there capsule by different methods (19, 10) . found the removal of capsular polysaccharides from *S.aureus* bacteria reduce the persistence of *S.aureus* in its hosts , based on microscopic examination of organ section take from mice infected that showed difference in the organ lesions caused by the capsulated and un capsulated *S.aureus* cell .(16)

The aim of this study was to investigation the important role of capsular polysaccharide in dissemination the *S.aureus* to many organ in the host body and to inhibit the phagocytic engulfment for bacterial cell .

Material and Methods

1- Bacterial strain and growth conditions

The *S.aureus* strain used in this study was isolated from the wound swab of patients admitted to the Mussaib hospital , the diagnosis of this bacteria was acarried out according to the (6) and investigate for the presence of the capsule by using negative stain and slide clumping factors methods according to (3) .

The bacteria were grown in (100ml)of brain heart infusion broth (BHI) at 37°C with genetal shaking for 18h , then culture was centrifuged at (2000 rpm) and the pellet was resuspended in 10ml of medium to give a cell density of (10^4 cell \ ml) (4) .

2- preparation capsule-removal strain suspension.

The removal of capsular polysaccharide from bacterial cell was done by used rabid agitation, as shown in (9,8).

3-Quantitative organ culture.

Tow group of Balb female mice (20 mice in each one) , 8 to 12 week old were used . the first group were injected intraprotinally with (10^4 cell \ ml) of capsular strain *S.aureus* while the other injected with 10^4 cell \ml of capsule – removal strain *S.aureus* suspension.

At each time period (18-24-48-72h) following bacteria inoculation five mice from each group were sacrificed , the liver & spleen were removed &portions from these organs were homogenized separately in phosphate buffer stain containing 0.05 % Triton × 100, with a tissue grinder . after homogenization aliquots of these organ suspension were serially diluted in PBS

plated on trypticase soy agar contain 5% sheep blood . colonies were counted after 24h of incubation at 37C° and bacteria per gram were enumerated (7)

4- Preparation of Histological sections

Over a period at 3 days the liver & spleen mice were fixed in 10% Para formaldehyde and processed by routine methods to provide paraffin wax section which were stained with hematoxylin &eosin stain to detect bacteria & other pathological change(1)

Results & Discussion

The study achieved to investigate the role of the capsular polysaccharide in pathogenicity and disseminated process of *S.aureus* bacteria in liver and spleen of mice by recovered this bacteria from these organs & performed its sections.

The *S.aureus* bacteria survival in these organs was monitored at each time point (18, 24,48,72h) by isolation the number of viable bacteria in liver & spleen of mice infected intrapertonally with 10^4 cell\ ml of capsular strain & capsule-remove strain .

As shown in (Fig 1) the log number of capsule-remove strain *S.aureus* recovered from liver of mice increased slightly to reach (3.16) at 24h post inoculated and decreased at low level (1.99) at 72h and reach to (2.34) at 18h & 48h . whereas capsular *S.aureus* bacteria , increase with increase time injection (from 18-72h) to reach maximum at 72h (4.89) .

The (Fig2) show the slightly increased of log number of capsular *S.aureus* recovered from spleen mice which reach to (5.13) at 48 h & (5.32) at 72h , but the capsule –remove *S.aureus* bacteria isolated in high log number (3.78) at 24h and then decrease clearly at 48h (2.82) &72h (2.53).

Several studies reported that capsule play an important role in staphylococci infection and dissemination within the host organs by inhibit phagocytic engulfment (11) .

Many workers provided evidence that peptidoglycan is the key cell wall component promoting opsonization of *S.aureus* when removal capsule by many automatic procedure such as agitation and washing(5).

The infected tissue with *S.aureus* exhibited large amount of leukocytic cells particularly PMN , which lead to lysis of microorganism by phagocytosis process and the capsule has been described as virulence factors with the capacity to interference with the innate host defense system by preventing Nutrophil cell to phagocytic bacterial cell (10) .

Histological examination was performed on liver &spleen sections taken from mice at 72h post inoculation with capsular and capsule – remove *S.aureus* bacteria. The histopathological study of liver revealed some pathological changes as a result of infected with capsular *S.aureus* showing multiple foci of inflammation with high average of PMN migration , combined with congestion , hemorrhage and necrosis (Fig 3) .
In the other hand the examination of spleen show severe hemorrhage , congestion in red pulp with marked necrosis, oedema combined with hyperplasia of white pulp and increase number of megakaryotic cell (Fig 4).

It has been shown only minimal to mild lesions in liver usually represented by high infiltration of inflammations cell to this organ, that combined by widest of white pulp (Fig 5) ,and increased in number megakaryocytic in spleen of mice infected with un capsulated *S.aureus* (Fig 6) (×100) , by 20h following incubation , most (80 to 90 %) of the capsular *S.aureus* inoculated were trapped in liver and spleen with majority in liver & clear difference was observed between capsulated and un capsulated *S.aureus* strain(17) . 8% of mice injected with capsular *S.aureus* bacteria died within 8 days after injection , while only 44% mice injected with un capsulated strain died within the same period , this results show that removal capsule attenuates virulence of bacteria (13,15).

Several studies show high accumulation of neutrophils in the liver after injection with capsular &un capsular *S.aureus* bacteria , that responsible in elimination of bacteria from this organ and these data support the conclusion that capsule blocks the removal of M.O from liver therefore the number of bacteria increase and combined with sever pathological effects (20) .

Infected tissue with *S.aureus* exhibited PMN in liver and spleen , this may be discus that reason for the presence of large infiltration foci in these organs section (18) .

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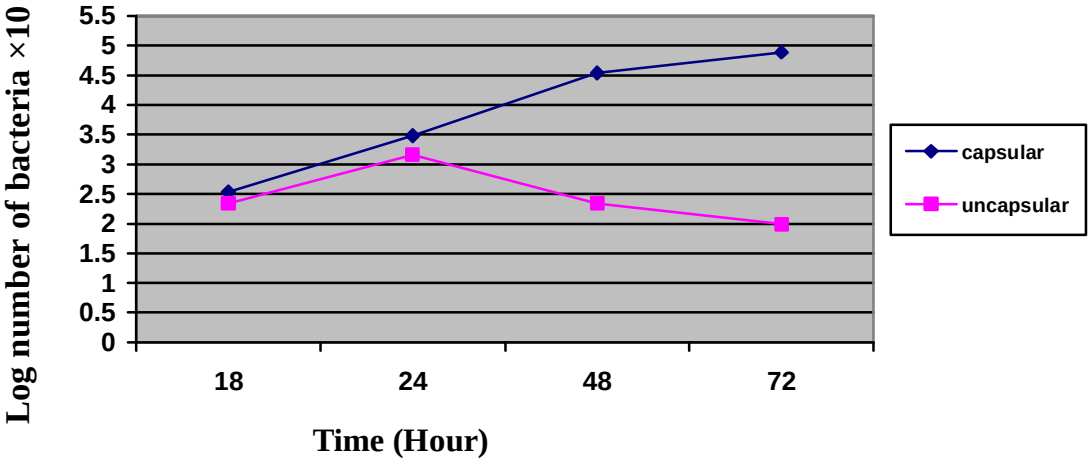
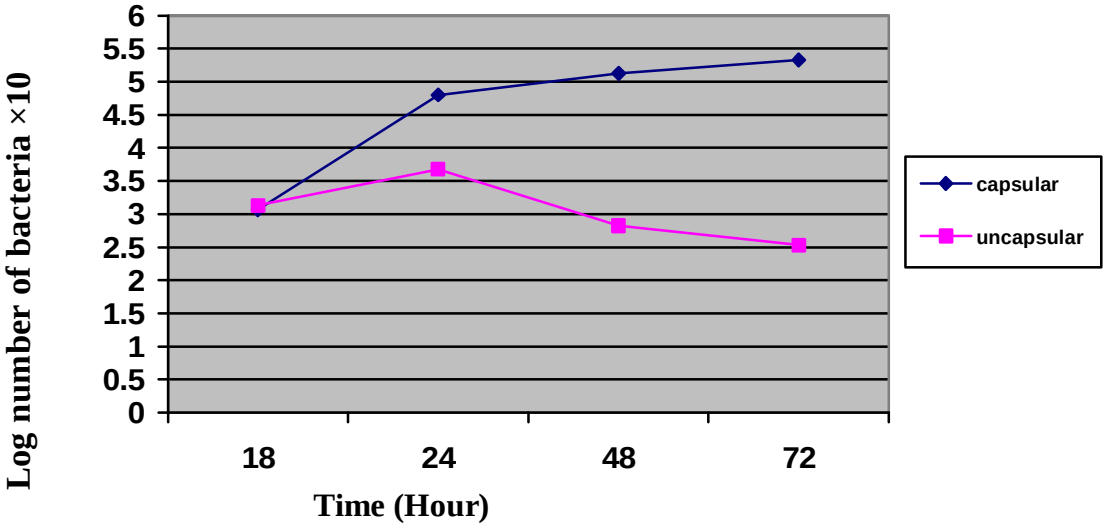
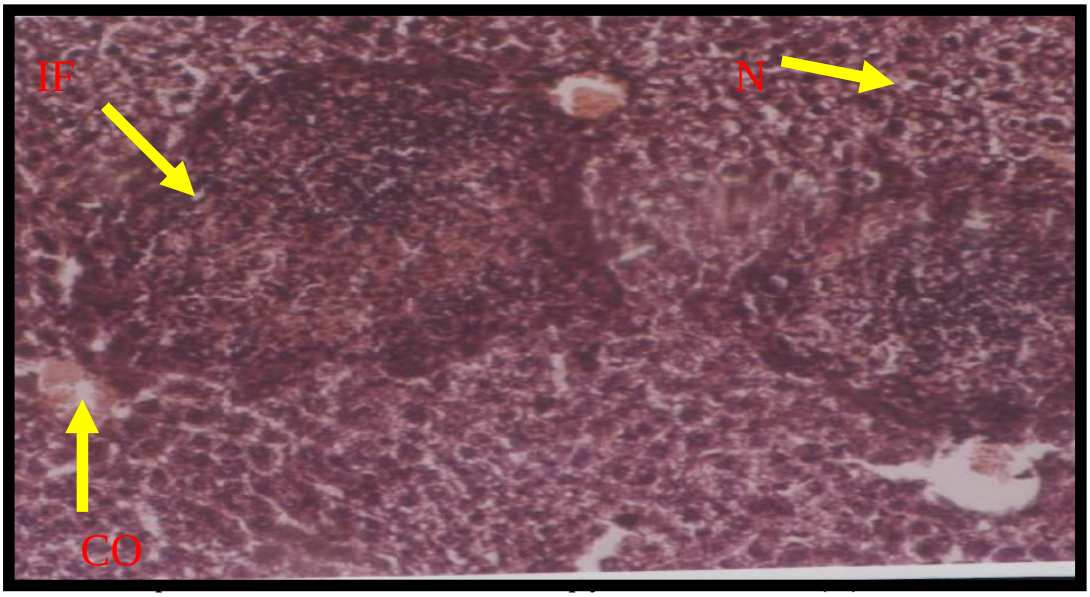


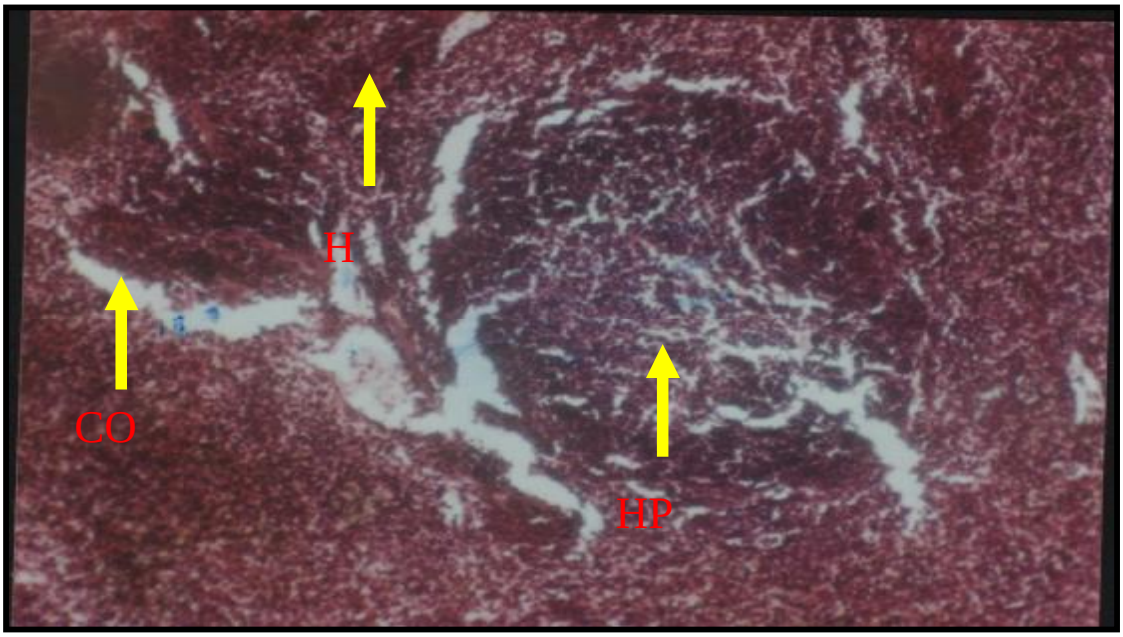
Fig(1)Numbers of capsulated and capsule – removal *S.aureus* bacteria isolated from mice liver following intraperitonally injection .



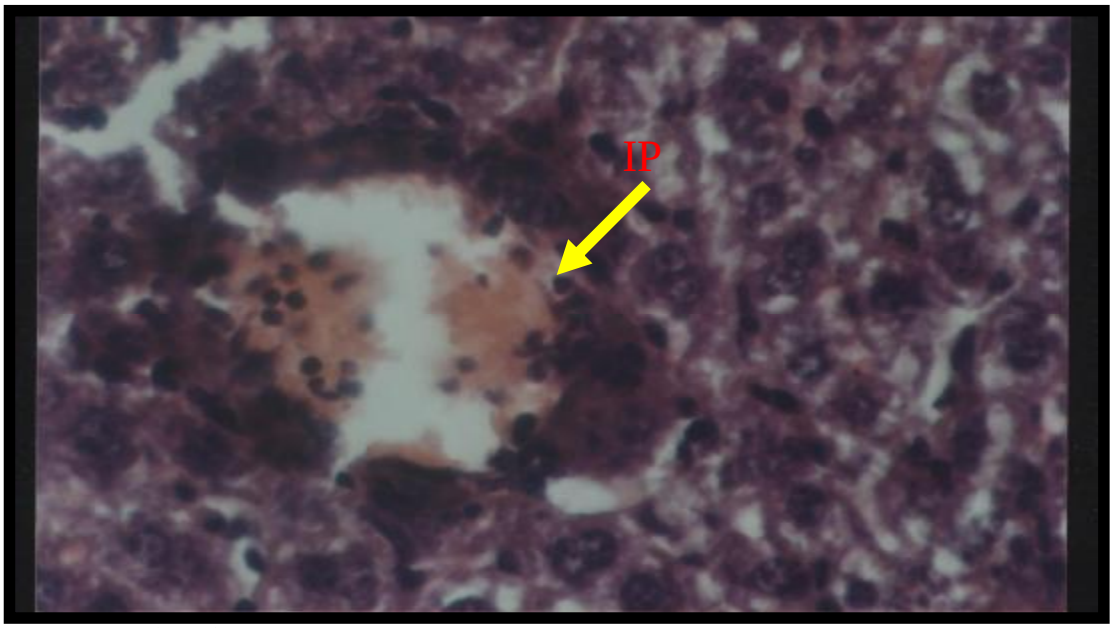
Fig(2)Numbers of capsulated and capsule – removal *S.aureus* bacteria isolated from mice spleen following intraperitonally injection.



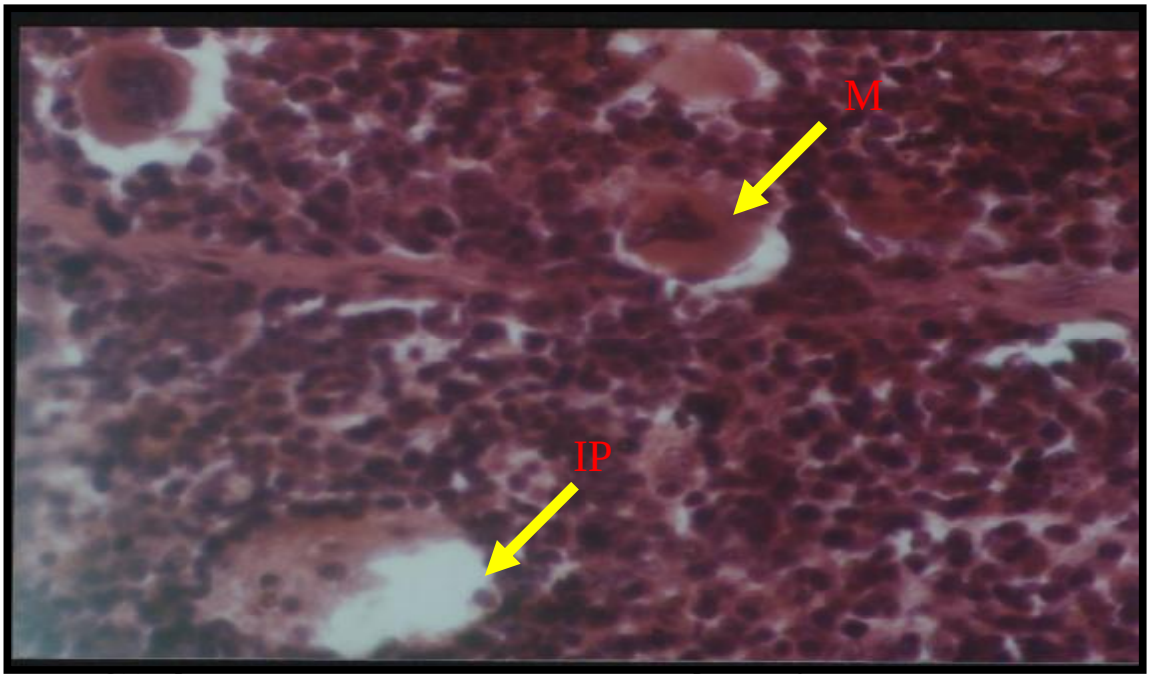
Congestion (CO),necrosis (N) (×100) .



Fig(4): Spleen section taken from mice inculcated intraperitonally with capsulated *S.aureus* bacteria showed hemorrhage(H),Odema(O) and hyperplasia(HP)(×100) .



(400X) .



capsule – remove *S.aureus* bacteria showed increase number of megakaryocytic(M)& infiltration PMN(IP) (400X) .