Proteoytic Activity and Swarming Growth of Proteus spp. Isolates.

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Abstract

Many clinical isolates of proteus spp. (30 isolates of *P. mirabilis* and 30 isolates of P. *vulgaris*) from patients with urinary tract infections (UTIs) were examined for their ability to produce proteolytic enzymes and their ability to form swarming growth. Most (90%) of *P. mirabilis* and 60% of *P. vulgaris* isolates secreteal proteolytic engymes. A strong correlation was found between the ability of a strain to secreted proteases and it's ability to form swarming growth. Non- swarming isolates invariably appeared to be non- proteolytic. However, some isolates (12 isolates of *P. valgaris*) were non- proteolytic even when they formed swarming growth.

Introduction

All species of proteus are widespread in nature and cause many infections urinary tract infections (UTIs), Blood and Wound infections and other condition in man)(1)*P. mirabilis* is the most frequently species cause of UTI (70- 90%), *P. valgaris* and *P. penneri* cause similar types of infection their because habitats and virulence factors are similar, but they are isolated less frequently and they many by less virulent.(2, 3). Strains of proteus spp. form seveal virulence factors.

These include: Urease production, cell invasiveness, cleavage of IgG and IgA by aproteolytic enzymes, outer- memberane proteins, motilty by flagella and resistance to nomal serum.(4, 5, 6, 7).

The proteolytic enzymes play a role in generating products such as glutamine, which is important in inducing swarm cell

formation. In- vivo experiment in mice have shown that proteinase-negative mutants can infect the bladder, but have reduced ability to infect the kidney and form abscesses.(8). In view of the above and the well established proteolytic capabilities of proteus strains, this study aimed to investigate the proteolytic enzymes produced by protease spp. And to determine the relationship between the ability of protease spp. To form swarming growth with their ability to produce proteolytic enzymes.

Materials and Methods

Bacteria isolates: A total of 60 isolates of proteus spp. (*P. mirabilis* (30 isolates)) and (30 isolates) of *P. vulgaris* were studied. All were identified API- 20E system (Bio merieux). All were clinical isolates form paitents with (UTIs) and were maintained in pure culture on nutrient ager slants at 4C°.

-Detection of proteoytic activity of isolates. The proteolytiz activity at all isolates was examined on casein medium (contained cystime 3.6% W/v) in 0.05M Tris- HCL buffer (pH: 8.0), supplemented with casein 1% w/v, this medium was sterilized by autoclaving at 121C° for 15 min. the isolates were inoculated by loop onto plates of casein medium form nutrient broth cultures (18h. at 37C°), the plates incubated in 5% co2 at 37 C° and examined for proteoltic activity after (18- 24)h, this was revealed as azone of clearing around the growth of the bacteria. (Addition of INHCL to the medium, enhanced the definition of the zone of proteolysis if this was not clear).(9).

-Examination of swarming growth. All isolates of proteus spp. (that appeared tobe non- proteolytic or proteolytic on casie medium) were examined to form swarming culture. Their nutrient broth culturer (of each isolates from casien medium) were palted on 2 plates of blood agsr, and incubated at 37C° for 18h. them examined for swarming growth.

Results and Discussion

-Proteolytic activity of proteus isolates. The proteolytic activity of 60 proteus isolates (30 isolates of *P. mirabilis* and 30 isolates of *P. vulgaris*) was determined on casein medium. 90% (27). Of *P.*

mirabilis siolates and 60% (18) of *P. vulgaris* isolates were found to be proteolytic on casein medium. Most of these isolates showed wide zones (4- 6 mm) of proteolysis after incubation for 18h. at 37 C° but the diameter zones of *P. mirabilis* isolates were bigger than their formed by *P. vulgaris*. Our results showed, most isolates of *P. mirabilis* were found to produce proteolytic enzymes, and the lowest proportion of proteolytic isolates was found among *P. vulgaris* isolates. Therefor it was thougth important to investigate the secreted proteases formed by members of the genus to determine the proteolytic enzymes that might act as virulence factors in proteus infections of man, because in nature, strains of proteus spp. produce many proteolytic enzymes and the differentiation in ability of proteus spp. To produce proteolytic enzymes may be due to the diversity of this bacteria isolates in genomospecies.(10).

-Proteolytic activity and swarming growth. The 38 isolates of proteus spp. (23 *P. mirabilis* and 15 *P. vulgaris*) were able to form swarming growth on blood agar and degrade casein on casein medium, whereas 15 isolates (3 *P. mirabilis* and 12 *P. vulgaris*) formed swarming growth, but not degraded casein. A clear association was found between the ability of isolates to swarm and their ability to form aproteolytic enzymes.(9), was explained, many strains of proteus spp. that were unable to form swarming growth did not secrete proteolytic enzymes and appeared to be non- proteolytic, they remained like this until such time as this stage proteolytic activity became detectable. While (6) supports this association between swarm cell formation and protease production. However, it s hould be noted that swarming growth is not always associated with protease production because some swarming strain of proteus spp. developed after reprated sub culture of their non- swarming parents, do not proudce proteolytic enzyme.

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الفعالية الحالة للبروتين والنمو المحتشد لعزلات أنواع من بكتيريا Proteus

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الخلاصة

تـــم فحـــص قابلية العديد من العزلات المرضية لبكتيريا *Proteus spp.* (30 عـــزلة من بكتيريا *Proteus mirabilis* و 30 عزلة من بكتيريا *Proteus vulgaris* المعـــزولة مـــن مرضى مصابين بالتهابات القناة البولية لانتاج الإنزيمات الحالة للبروتين وقابليـــتها علـــى تكـــوين الـــنمو المحتشد. لوحظ ان معظم (90%) عزلات بكتيريا *P. mirabilis* و 60% من عزلات *P. vulgaris* كانت منتجة للإنزيمات الحالة للبروتين. كمـــا لـــوحظ وجـــود علاقة قوية ما بين قابلية هذه العزلات على إنتاج الإنزيمات الحالة للبـــروتين وقابليتها للنمو بشكل محتشد. ووجد انه ليس بالضرورة ان تكون العزلات غير المكونة للنمو بشكل محتشد غير منتجة للأنزيمات الحالة للبروتين وبالعكس، حيث ظهرت بعض العزلات لبكتيريا *P. vulgaris* (12 عزلة) مكونة للنمو بشكل محتشد على الرغم من عدم إنتاجها للأنزيمات الحالة للبروتين.