Hemagglutination properties of some intestinal bacterial pathogens isolated from clinical samples

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Abstract

Hemagglutinating properties of four intestinal bacterial pathogens*(Escherichia coli , Salmonella sp. , Klebsiella sp. , and Proteus sp.)*isolated from human clinical samples in Pediateric hospital at Kirkuk city were determined. Red blood cells of different origins as human blood group A,B,AB and O,Sheep, and Chicken were used in this study. Bacterial genera cultured in different solid media at 37c like Tryptose soy agar (TSA) and broth ,Brian heart infusion agar (BHI) and broth , and Luria Bertani agar (LBA) broth. The results showed that all bacterial isolates were able to express hemagglutinating activitywith human blood group A,B,and O and Sheep and Chickenerythrocyte, and this expression was better in isolates cultured on TSA agar than other exmined media. For the detection of Mannose – sensitive and mannose –resistant hemagglutination (HA), reactions were run with and without 1% (w/v) D-mannose, all bacterial genera grown on TSA produced two main kinds of hemagglutinins ; (a) mannose resistance hemagglutination (MRHA) of erythrocyte from human bloods group B and O,Sheep and Chicken ; (b) mannose sensitive hemagglutination(MSHA) of erythrocyte from human blood group A . Other sugars like D-mannitol, D- glucose ,D- maltose, D-galactose, D-fructose, D- xylose, and Dulcitol ,was tested in this study and we observed that all these sugars caused inhibition of hemagglutination of human blood group B erythrocyte by those organisms whereas they were not inhibitory to human group AandO, Sheep and Chicken erythrocytes.

Introduction

Studies on the virulence mechanisms of enteropathogenic bacteria have revealed an-other crucial factor, an organism must be able to attach to mucosal surfaces of the animal host if it is to cause gastrointestinal disease (Atkinson & Trust., 1980; Connell *et al* ., 1997).

The ability of variety of enteropathogenic bacteria to cause hemagglutination (HA) of erythrocyte are considered virulence factors , because HA activity was associated with bindig capacity of bacterial cells to epithelial cell surfaces (Hogan *et al* ., 1990). However many enteropathogenic bacteria have well characterized hemagglutinating properties which are indicative of an ability to adhere to intestinal mucosal surfaces , which are recognized as an-essential step in the pathogenesis of enteric infections . This interaction between microbs and hosts depends upon microbial surface adhesions which recognize specific receptors on the host cell surface (Jones &Richardson .,1981; Gilboa-Garber *et al*., 1997 ; Klemm&Schembri., 2000). These bacterial adhesions may also cause agglutination of erythrocytes for different species of animals, because the erythrocyte membranes possess different receptors , so the bacteria-erythrocytes interaction gives aclue as to the nature of the receptors for these pathogens in the intestinal mucosa (Qadri *et al* ., 1994 ; Alam *et al* ., 1997). Such HA reactions have been classified as Mannose sensitive or resistant, depending on whether d-mannose or its derivatives can inhibit the HA (VanDen-Bosch *et al* ., 1980 ; Duguid& Old., 1980; Goldhar.,1994; Simi *et al*., 2002). Correlation between HA ability, adhesion and bacterial pathogenicity for many intestinal bacterial pathogens has been shown(Aslanzaden&Paulissen ., 1992 ; Edwards *et al* ., 2000 ; Klemm *et al* ., 2000) .

The objective of this study was to determine the incidence of HA among some intestinal bacterial pathogens (*E.coli , Salmonella sp. , Klebsiella sp*. , and *Proteus*).

Material and methods

Bacterial genera:

Escherichia coli , Salmonella sp. , Klebsiella sp. , and *Proteus sp.* included in this study were obtained from the Pediateric hospital in Kirkuk city. Diagnosis of the isolates were based on biochemical and serological tests and then by API20E

Bacterial culture:

For the HA assay , bacteria were grown on various soild and liquid media at 37c to determine the conditions for optimal expression of hemagglutinating activity , those media included Tryptose soy agar (TSA) and broth , Brain heart infusion agar (BHIA) and broth , and Luria-Bertani agar (LBA) and broth . Bacteria were harvested and washed twice in phosphate-buffered saline (PBS; pH 7.2) and the cells were suspended in the same buffer to adencity of approximately 10 bacteria per ml in comperision with McFarland turbidity standards as described by (Atkinson & Trust., 1980) .

Erythrocytes :

erythrocytes were obtaind from various species including human blood group A,B,AB and O, Sheep and Chicken , before use, blood cells were washed two time in PBS (pH 7.2) and a 1% (vol /vol) erythrocyte suspension was prepared .(Qadri*et al* ., 1994 ; Jane *et al.,2004)*

Microtiter HA assay :

Samples for HA were titrated in a 96-well U-bottom microtiter plate , serial doubling dilutions of the bacterial suspension (starting concentration , 10^9 CFU/ml) in PBS were made in 50 ul volumes, after which an equal volume of 1% suspension of erythrocytes in PBS was added to each well . the plate was incubated at 4c for 2hrs , and the result was read. The HA unit was defind as the reciprocal of the highest dilution of the bacterial suspension causing visible agglutination of the erythrocytes, reaction was compared with negative control (50 ul of PBS and 50 ul of blood cell) .(Qadri*et et al* ., 1994 ; Jane *et al* ., 2004) .

Carbohydrate inhibition of HA :

The inhibitory effect of sugars on hemagglutinating activity was determined as described above. Fifty ul of 1% erythrocyte suspension containing 1% (wt/vol) sugar was added to each well of plate containing 50ul of serially diluted bacterial suspension (Jane *et al* .,2004) ,

the plates were incubated and the HA recoded as described above . the tested sugars were D -mannose , D - mannitol , D- maltose , D - glucose D, -galactose , D - fructose , D - xylose and Dulcit .

Results and Discussion

The adhesive ability of an enteropathogen is usually assessed by determining the hemagglutinating ability , because the erythrocyte membrane is believed to possess the homologus of the mucosal substances involved in bacterial adherence to epithelial cells (Yamamoto *et al* ., 1988 ; Uchimura& Yamamoto ., 1992 ; Nagayama *et al* ., 1994 ; Jane *et al* ., 2004) .

Figure (1) and Table (1) demonstrate that bacterial genera tested in this study were able to expressehemagglutination activity when cultured on different solid media, this activity were seen with erythrocyte from human group A,B,O, Sheep, and Chicken and the results was as follow:- Bcterial grown on TSA agar showes HA activity for human blood group A,B,O and Chicken erythrocytes except *Klebsiella sp.* isolate which couldn't agglutinate Chicken erythrocyte, and agglutination of Sheep erythrocyte was caused only by *Salmonella sp.* cultured on this medium (table-1).

*E.col*i and *Klebsiellasp*. from BHI agar agglutinate Sheep and Chicken erythrocyte but did not.agglutinate human groups, on the contrary , *Proteus sp.* agglutinate human blood group erythrocyte but Sheep and Chicken did not . Where as *Salmonella sp*. caused hemagglutination of Chicken erythrocyte only .(table-1).

Bacteria grown on LB agar showed HA of Sheep and Chicken erythrocytes. Human blood group A and B was agglutinated only by *Proteus sp.*, while group O agglutinated by *E.coli.*

Varing degrees of hemagglutination for red blood cells of different origin as human blood group, Sheep ,Rat, Horse ,and Chicken have been described previously in *Escherichia coli, Salmonlla sp., Proteus mirabilis, Klebsiella pneumoniae* . *(*Cosar., 1990; Yano *et al*., 1996; Sekowska & Gospodarek., 2008).The results showed that the optimum culture medium for the expression of hemagglutinating activity was TSA agar ,hemagglutinins were produced on BHI and LB agar , but they were not strong (table 1). These results agree with previous studies on the expression of hemagglutinins which concluded that growth on solid media like colonization factor antigen (CFA) agar, Trytose soy agar ,promote the expression of hemagglutinins (Atkinson & Trust ., 1980; Jiwa & Mansson ., 1983 ; Cosar ., 1990; Woodward *et al* ., 2000; Jane *et al* ., 2004) . No reaction was observed when the bacterial genera were grown in liquid media (data not shown). The differences in hemagglutinin production between broth and agar –grown cultures suggested that surface contact was important in regulating of the expression of hemagglutinin .(Jane *et al*., 2004). other researchers have reported that the expression of *Salmonella sp.*, is enhanced by growth on agar surface indicating that surface contact is an environmental signal for fimbrial expression (throns*et et al* .,1992; Walker *et a* ., 1999) . Goldhar (1994) shwod that growth on solid media promotes the expression of mannose - resistant hemagglutinin (MR-HA) but diminishes the expression of mannose – sensitive hemagglutinin (MS-HA), whereas cultures in broth produce MS-HA, but Jane and here workers (2004) showed that the best production of MS-HA was obtained when the strains of *S. enteritidis*cultivated on solid media . thus the correct choice of media and other culture condition like tempreture and pH (which not done in the present study) for bacterial growth is important when evaluating the capacity for hemagglutination .

Regarding inhibition of hemagglutinatig activity by sugars ,the activity was observed in the absence and presence of D- mannose. As shown in (table-2), all bacterial genera grown on TSA produced MR-HA of erythrocytes from Human group B and O,,Sheep, and Chicken ,and at the same time produced MS-HA of erythrocytes from Human group A. Our results are in agreement with those of limited studies with strains of *Enteroaggregative*and *Toxigenic Esherichia coli*and also with *Klebsiella pneumoniae*, *Proteus mirabilis* and *Salmonella sp.* isolated from various geographical locations like India , South American , United Kingdum , Turky , Mexico and Bangladesh (Scotland *et al* ., 1991 ; knnttone*t et al*., 1992 ; Qadri *et al*., 1994),who detected in their studies more than one type of HA in the presence of D- mannose. This finding indicates that most strains of those organism produce two different types of fimbriae that mediated adhesion to host cells; type 1 fimbriae cause MS-HA and play an important role in urinary tract infection, and Type 3 fimbriae mediate MR-HA (mobley*et et al*., 1988; Podschun *et al*., 1993; Podschun*et et al*., 2000; Schembri&klemm., 2001; Boddicker *et al*., 2002 ; Ong*et et al*., 2008 ; struve*et et al* ., 2008). Other sugars as D-mannitol, D-glucose ,D-maltose , D-galactose, D-fructose, D-xylose, and Dulcitol were tested in this study and the result revealed that all bacteria produce hemagglutinins sensitive to those sugars with Human group A erythrocyte, while those sugars were not inhibitory for the hemagglutinating activity with Human group B and O,Sheep,and Chicken by all bacteria genera , these observations confirmed in some of the previous findings reported for those organisms (Old., 1972 ; Feutrier *et al*., 1986; Yamamoto *et al.,* 1991; Qadri *et al*.,1994) . Presumably , the sugars that inhibited hemagglutination resemble or are identical to residues available for binding to adhesions on mammalian cell membranes.

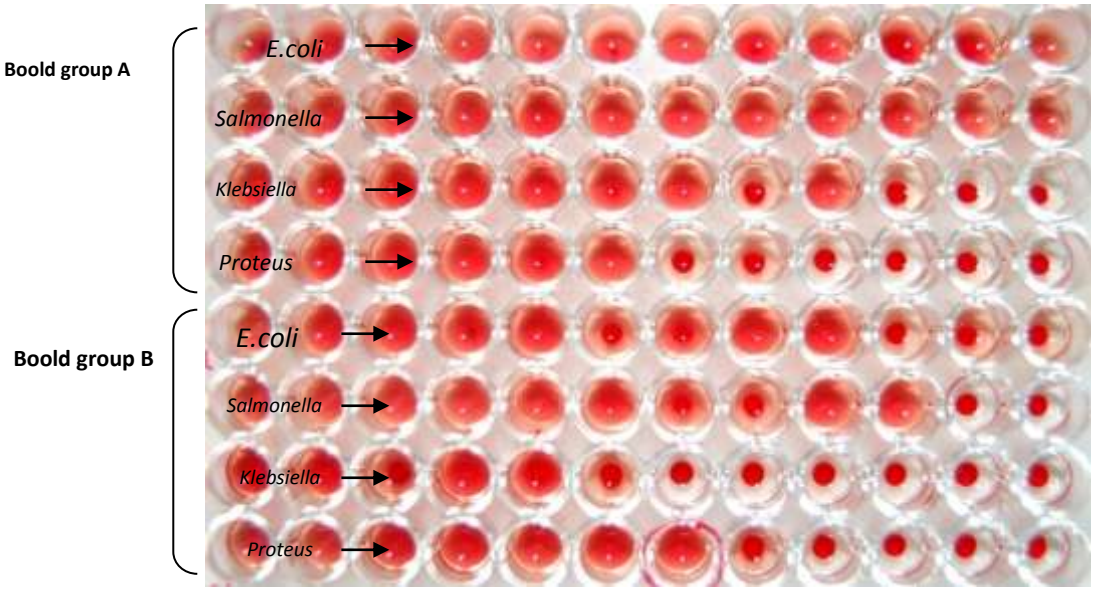

Figure (1) : Hemagglutination assay performed in amicrotiter plate using human blood groug A and B.when hemagglutination is not occur erythrocytes settle to the bottom of the well as aspherical red button

Table (1) :- Hemagglutinations of human and animal erythrocytes by some intestinal bacterial pathogens cultured on different solid media.

Blood samples/culture media																		
	A			B		AB			O		Sheep			Chicken				
Isolates	TSA	BHI	LBA	TSA	BHI	LBA	TSA	BHI	LBA	TSA	BHI	LBA	TSA	BHI	LBA	TSA	BHI	LBA
E.coli	++	-	-	++	-	-	-	-	-	++	-	++	-	+	+	+	+	++
Salmonella sp.	++	-	-	++	-	-	-	-	-	++	-	-	++	-	+	+	++	++
Klebsiella sp.	++	-	-	++	-	-	-	-	-	++	-	-	-	++	+	-	++	++
Proteus sp.	++	+	+	++	++	++	-	-	-	++	-	-	-	-	+	+	-	++

Hemagglutination :are the reciprocals of the highest dilutions at which hemagglutination was detectable .
++ : strong hemagglutination
+ : weak hemagglutination
- : no hemagglutination
TSA :-Trytose soy agar ; BHI :- Brian heart infusion agar ; LBA :- Luria Bertani agar

Table (2) :- Inhibitory effect of d- mannose on hemagglutination by bacterial genera after growth onTrytose soy agar .

	MSHA		MRHA									
	A		B		O		Sheep		Chicken			
Isolates	With out Mannose	with Mannose	With out Mannose	with Mannose	With out Mannose	with Mannose	With out Mannose	with Mannose	With out Mannose	with Mannose		
E.coli	++	-	++	+ +	++	++	-	-	+	+		
Salmonella sp.	++	-	++	++	++	++	++	++	+	+		
Klebsiella sp.	++	-	++	++	++	++	-	-	-	-		
Proteus sp.	++	-	++	++	++	++	-	-	+	+		

MSHA :- Mannose – sensitive hemagglutination
MRHA :- Mannose – resistant hemagglutination .

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خواص التلازن الدموي لبعض الممرضات البكتيريه المعويه المعزولة من نماذج سريرية

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الملخص

تم تحديد خاصية التلازن الدموي لاربعة اجناس بكتيريه من الممرضات المعويه وهي (*Escherichia coli, Salmonella sp.* , *Klebsiella sp.* , *Proteus sp.)* والتي عزلت من نماذج سريريه من مستشفى الاطفال في مدينة كركوك ، واستخدمت في هذه الدراسة اصناف مختلفه من الدم مثل مجاميع الدم ABO للانسانودم الاغنام والدجاج. ونميت الاجناس البكتيريه على اوساط صلبة مختلفه مثل (Luria Bertani agar (LBA) ,Brian heart infusion agar (BHI), (Tryptose soy agar (TSA) على درجة حراره ٣٧م . اظهرت النتائج ان جميع الاجناس المدروسه كانت ايجابية التلازن لكريات الدم الحمراء لمجاميع Aو B و Oللانسان وكريات الدم الحمراء للاغنام والدجاج ، وبينت النتائج ان افضل وسط صلب لاظهار الملزنات البكتيريه هي وسط Tryptose soy agar (TSA)مقارنة بالاوساط الاخرى .

وللكشف عن وجود ملزنات حساسه ومقاومه للمانوز تم اجراء تفاعلات التلازن بوجود وعدم وجود سكر المانوز وبتركيز ١% (وزن/حجم) ،١ واظهرت النتائج ان جميع الاجناس البكتيريه الناميه على وسط TSA انتجت نمطين من الملزنات الدموية وهي : أ- ملزنات مقاومه للمانوز مع كيات الدم الحمراء لمجاميع B و O للانسان وكذلك مع كريات الدم الحمراء للاغنام والدجاج . ب- ملزنات حساسه للمانوز مع كريات الدم الحمراء لمجموعة دم A للانسان .

كما وتم في الدراسه الحاليه اختبار التأثير التثبيطي لسكريات اخرى مثل (D-mannitol , D- glucose ,D- maltose , D- galactose , D-fructose , D- xylose , Dulcitol) ، على التلازن الدموي الذي احدثه الاجناس البكتيريه قيد الدراسه ، واظهرت النتائج ان جميع هذه السكريات تسببت في تثبيط التلازن الدموي مع كريات الدم الحمراء لمجموعة B للانسان ، في حين لم يكن مثبطا للتلازن مع مجموعة دم A و O وكريت الدم الحمراء للاغنام والدجاج .