Adherence of local and standard strains of *Salmonella* to human Uro-epithelial cells

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Abstract

This study include determination of the ability to adhere to human uroepithelial cells in three types of local isolates of *Salmonella (S.typhi , S.typhimurium , S. montevideo*) and compared this ability with standard strains *(S.typhi 5535 , S.paratyphi B 5542 , S. paratyphi C a-55108*). The results showed that all isolates were able to adhere to Human uroepithelial cells, and found that the standard strains adhered more efficiently than local isolates, but the statistical analysis revealed that there was no significant difference in adhesion rates between local and standard isolates. The results also showed that among the local isolates, the bacteria *S. montevideo* adhered in higher numbers than did other species, whereas the highest adherence among standard strains was by *S. typhi 5535*.

Introduction

Salmonella species are enteric pathogens that infects humans of all ages (Lindquist et al., 1987). At least four major groups of infection associated with Salmonellosis are recognized, these groups include gastroenteritis, septicemia with or with out focalized infection, and two syndromes (enteric fever and carrier state) traditionally associated with *Salmonella typhi* infection (Janda & Abbott, 1998). In addition to these four syndromes, anumber of less–well-described extraintestinal illnesses due to *Salmonella* have been reported, among these illnesses are Urinary tract infections (UTIs). (Cohen et al., 1987; Mathai et al., 1995 and Abbott et al., 1999). But its extremely rare are occurs in individuals with structural or functional abnormalities of the urinary tract, or immunosuppressive status. and may occur in conjunction with gastroenteritis .(Wilson & Feldman., 1982; Kappor et al., 1992; Ramos et al.,1996; Embil & Nicolle., 1997; Abbott et al.,1999; Richard.,2003; Mourani et al.,2005 and Gagnon et al.,2007). OGrady &Cattell (1966) reported that *Salmonella* enter the urinary tract either hematogenously or by direct invasion of the bladder via the urethra.

In order to initiate all those infections mensioned above, Salmonella species must be able to attach to its host mucosal surfaces in the intestine or urinary tract and subsequent invasion of epithelial cells (ECs) (Baumler et

al .,1997) hence microbial attachment to mucosal surfaces is a first step in mucosal infection. Specific interaction between microbial surface ligands and host receptors influence the distribution of microbes in their sites of infection. Adhesion has often been regarded as a sufficient end point, explaining tissue tropism and bacterial persistence at mucosal sites. Adherence, however, is also a virulence factor through which microbes gain access to host tissues, upset the integrity of the mucosal barrier, and cause disease. (Deman et al., 1990; Connell et al., 1997). In gram negative bacteria adhesion is often mediated by fimbriae, which are thin filamentous organelles expressed on the surface of the bacterial cells (Clegg et al., 1987). The aim of the present study was to determine the adherence ability to human uro-epithelial cells by local and standard strain of *Salmonella* and hence comparing between them.

Material and Method

Bacteria:-

Local isolates used in this study include :- *(S.typhi, S.typhimurium , and S.montevideo)* which belonged to groups D, B, and C respectively . These organisms were isolated from patients with typhoid and enteric fever, attending Azadi general hospital in kirkuk city. Diagnosis of the isolates were based on biochemical and serological tests (Andrews &Hammack., 2003) and then by API20E.

Standard strains include: - (*S. typhi 5535, S. paratyphi B 5542, S. paratyphi C a-55108)* .These strains were obtained from University of Mosul-college of science– Dep of Biology.

Preparation of bacterial suspension:-

Cultures were grown at 37c٥ in Luria Bertani broth medium for 24h., after incubation period the broth cultures was centrifuged at 5000gx for 10 min. and the pellet was washed twice in phosphate buffered saline (PBS) and resuspended in the same buffer at a final concentration of (10^9) cells /ml by Mcfarland standard solution (Guzman et al.,1989)

Epithelial cells (ECs):-

Human uro-epithelial cells were collected by centrifugation of fresh morning urine from healthy woman. ECs were washed three times in phosphate buffered saline (PBS pH =7.2) and resuspend the pellet in the same buffer, the suspension passed through filter paper (Whattman No.1),and the epithelial cells retained on the filter placed on microscope slides (Guzman et al.,1989).

Adherence assay: -

The slides that contain epithelial cells placed in a plate, and 0.5 ml of the bacterial suspension was added then the plate was incubated for 1hr. At 37c° with shaking, after incubation the slides renced twice with PBS to eliminate unattached bacteria .then the cells on the slides were fixed with ethanol, and stained using Giemsa stain. (Van-DenBosch et al., 1980; Guzman et al., 1989 and Bories et al., 1989). The binding results were expressed as the number of bacteria adhered to each of 100 epithelial cells.

Statistical analysis:-

Adherence was expressed as the mean number of bound bacteria /epithelial cell ± the standard deviation. Comparisons were analyzed by student t-test.

Results and Discussion

The ability of bacteria to adhere to epithelial cells (ECs) has been shown to be an essential step in the pathogenesis of bacterial infections (Guzman et al., 1989). Furthermore, it has been shown that the propensity of certain bacteria to infect specific tissues is often related to the ability of these bacteria to adhere to the respective target cell in vitro (Beachey, 1981; Klemm & Schembri., 2000 and Mikcha et al., 2004).

In this report, adhereing by all *Salmonella* (local and standard strains) were clearly seen on the surface of uro-epithelial cells (Fig 1). The results summarized in table (1) shows few differences in the dgree of attachment between local and standard strains, in fact standard strains shows higher rates of adherence than local isolates did, but no significant differences were observed in the adherence rates between them. These results are in agreement with the results of experiments reported by Shareef et al. (2009) on the adherence of the same *Salmonella* species to epithelial cells of rat intestine. There is an evidence that *Salmonella spp*. possesses some virulence factors on their cell surface such as type 1 fimbriae that mediates binding of bacterial cells to specific receptors on the surface of cells in the intestine or the urinary tract.(Stocker & Makela.,1986; Vanderveldn et al.,1998; Althouse et al.,2003;White et al., 2003; Duncan et al.,2005 and Shareef et al.,2009).

From the results that shown in table (1) it was observed that the highest ability of adherence among local isolates was by *S. montevideo* while among the standard strains was by *S.typhi 5535*, this may be due to the number of fimbriae present in these two species than in the other species . Similar observation were reported by Shareef et al., (2009). Also Weinstein et al (1998) refers in their study that *S. typhi* have greater ability to attach to ECs in human and animal intestine compared to *S.typhimurium*

In addition more recent studies on urinary tract infection caused by *Salmonella spp.* indicated that the most frequently serotypes isolated from patients urine were *S. montevideo* and *S.typhi* then *S. typhimurium.* (Wilson & Feldman., 1982; Allerberger et al., 1992; Paterson et al., 1997 and Abbott et al., 1999).

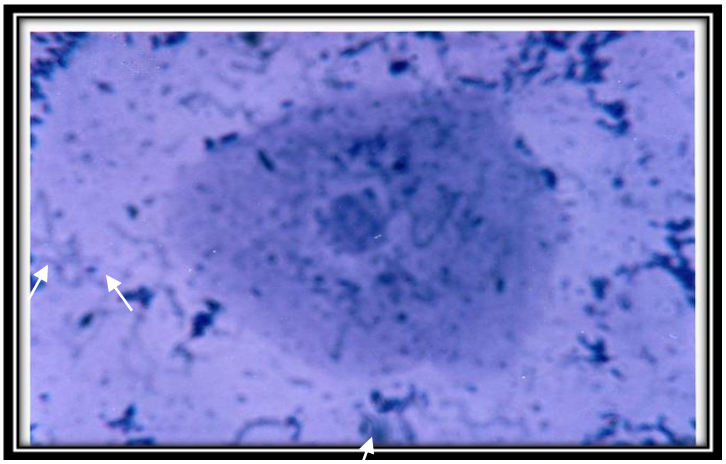

Fig (1):- Salmonella spp. in association with human uro-epithelial cell.

Table (1):- Adherence rates of Salmonella species to human Uro-epithelial cells.

Species	**Number of bacteria bound/ uro-epithelial cell** **Mean ± standard deviation**
S. typhi *S.typhi 5535* *t-value*	25.40 ± 10.o9 30.95 ± 13.37 *t= o.34 NS p>o.o5*
S. typhimurium *S. paratyphi B 5542* *t-value*	20.2 ± 10.31 24.3 ± 10.36 *t= o.67 NS p>o.o5*
S. Montevideo *S. paratyphi C a-55108* *t-value*	26.2 ± 9.48 27.05 ± 11.91 t=o.906 NS P>o.o5

NS= No significant difference

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التصاق جراثيم سالمونيلا المحلية والقياسية بالخلايا الظهاريه البولية للإنسان

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الخلاصة

تضمنت هذا البحث تحديد قابلية الالتصاق بالخلايا الظهاريه البولية للإنسان لثلاثة أنواع من جراثيم سالمونيلا معزولة محليا(*S.typhi , S.typhimurium , S.montevideo*) وتم مقارنتها مع السلالات القياسية (*S.typhi 5535 , S.paratyphi B 5542 , S. paratyphi C a-55108*) على التوالي . أظهرت النتائج قدرة جميع العزلات على الالتصاق ، وكانت معدلات الالتصاق للسلالات القياسية أعلى مقارنة بالعزلات المحلية ، إلا إن التحليل الإحصائي بين عدم وجود فرق معنوي بين معدلات الالتصاق لجراثيم السالمونيلا القياسية والمحلية . كما وبينت النتائج إن أعلى معدل للالتصاق ضمن العزلات المحلية قد أظهرتها النوع*S.Montevideo* ، *في* حين ضمن السلالات القياسية كانت أعلى معدل للالتصاق من قبل *S.typhi 5535*