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FEMS Immunology and Medical Microbiology 24 (1999) 105–114

FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Tissue culture adherence and haemagglutination characteristics of *Moraxella (Branhamella) catarrhalis*

Margaret Fitzgerald [a], Susan Murphy [b], Riona Mulcahy [b], Conor Keane [c], Davis Coakley [b], Thomas Scott [a,*]

[a] *Department of Biological Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland*
[b] *Mercer's Institute for Research on Ageing, St James's Hospital, Dublin 8, Ireland*
[c] *Department of Clinical Microbiology, St. James's Hospital, Dublin 8, Ireland*

Received 5 July 1998; received in revised form 2 February 1999; accepted 3 February 1999

Abstract

The haemagglutination and tissue culture adherence properties of 20 isolates of *Moraxella catarrhalis* obtained from the sputum of elderly patients with lower respiratory tract infections were compared with those of 20 isolates of *M. catarrhalis* obtained from the nasopharynx of elderly persons colonised by the organism. Eighty percent of isolates from the infected group as opposed to 5% of isolates from the colonised group haemagglutinated human erythrocytes ($P < 0.001$), indicating that the haemagglutinin might be a marker of pathogenicity for *M. catarrhalis*. There was a significant difference in the adherence to HEp-2 cells of isolates from the infected group in comparison to isolates from the colonised group ($P = 0.03$). Haemagglutination and tissue culture adherence properties were unrelated, indicating that separate adhesin systems are involved. The adherence of *M. catarrhalis* to HEp-2 cells was unaffected following pronase and trypsin treatment, however, sodium periodate pre-treatment of the bacteria significantly reduced the tissue culture adherence index, indicating that the adhesin by which the bacteria bind to HEp-2 cells may have a carbohydrate moiety. Transmission electron microscopy studies revealed that adherence of *M. catarrhalis* to HEp-2 cells was mediated by trypsin-resistant 'tack-/spicule-like' structures protruding from the surface of the bacteria.

Keywords: *Moraxella (Branhamella) catarrhalis*; Tissue culture adherence assay; HEp-2 cell line; Hemagglutination; Adhesin; Electron microscopy; Transmission electron microscopy; Spicule-like structure

1. Introduction

Moraxella (Branhamella) catarrhalis is now recognised as a human respiratory pathogen, although once dismissed as a commensal of the oropharyngeal tract [1,2]. *M. catarrhalis* is reported as the third most common bacterial pathogen after *Haemophilus influenzae* and *Streptocuccus pneumoniae* to be isolated from the sputum of patients with lower respiratory tract infections [3–6]. The organism is also a common cause of acute otitis media [7–9] and sinusitis [10,11] in children.

For bacteria to colonise and infect mucosal surfa-

* Corresponding author. Tel.: +353 (1) 4024747; Fax: +353 (1) 4024995; E-mail: tscott@dit.ie

0928-8244/99/$20.00
PII: S0928-8244(99)00015-2

ces, they usually attach to epithelial cells. One of the most important factors in determining this colonisation is bacterial adherence. Bacterial adherence is thought to be an important first step in the pathogenesis of many infectious diseases [12]. Bacterial adherence denotes the stable, essentially irreversible attachment of bacteria to surfaces. It is a specific event involving the union of bacterial surface structures called adhesins with complementary receptors on the host cell which has been compared to a lock-and-key interaction [12,13].

In an effort to elucidate the virulence factors involved in the pathogenesis of *M. catarrhalis* infection, a number of researchers have focused on studying the adherence properties of the organism. It has been found that haemagglutination is more commonly associated with *M. catarrhalis* strains isolated from the sputum of elderly individuals with lower respiratory tract infections due to *M. catarrhalis*, compared with isolates colonising the nasopharynx of healthy elderly individuals [14]. Recently, Kellens et al. reported that haemagglutination is associated with tracheal cell adherence [15]. However, adherence of *M. catarrhalis* to oropharyngeal epithelial cells has not been linked to haemagglutination [16,17].

Mbaki et al. demonstrated that the adherence of *M. catarrhalis* to oropharyngeal cells correlates with respiratory tract infection [18]. A study by Carr et al. investigated the effect of the age of the host on the in vitro adherence of *M. catarrhalis* to the host's buccal epithelial cells [19]. In that study it was noted that there was a significantly higher level of adherence of *M. catarrhalis* to cells from both ill and healthy elderly subjects in comparison to cells from young subjects. It would appear from these studies that increased nasopharyngeal colonisation may be associated with infection. However, it would appear that separate adhesins may be involved in the infectious and colonisation processes as haemagglutination and nasopharyngeal cell adherence by *M. catarrhalis* do not correlate [16,17].

Tissue culture cell lines may be used as standardised epithelial cell models for studying bacterial adherence in vitro. In this present study the HEp-2 cell line was chosen as a means of evaluating the adherence of *M. catarrhalis* to epithelial cells in an effort to clarify the processes involved in the colonisation and infection of the upper and lower respiratory tracts, respectively. For completeness, the haemagglutination and tissue culture adherence characteristics of 40 isolates of *M. catarrhalis* isolates were compared and the nature of the adhesin involved in tissue culture adherence was investigated. A separate set of isolates from those used in the study by Murphy et al. were examined [14].

1.1. Aims of the study

The aims of this study were:

1. to compare the relative adherence characteristics of *M. catarrhalis* isolates obtained at a concentration of $>10^7$ cfu ml^{-1} from the sputum of 20 patients with respiratory tract infections with *M. catarrhalis* isolates from nasopharyngeal swabs from 20 elderly persons colonised by the organism;
2. to clarify, by chemical means and electron microscopy studies, if separate adhesin systems are involved in tissue culture adherence and haemagglutination by *M. catarrhalis*;
3. to further elucidate the processes involved in colonisation and infection by *M. catarrhalis*.

2. Materials and methods

2.1. Bacterial isolates and growth conditions

Forty isolates of *M. catarrhalis* were examined. Briefly, sputum cultures processed between 1990 and 1993 by the Clinical Microbiology Department at the Central Pathology Laboratory, St James's Hospital, Dublin were monitored. All *M. catarrhalis* isolates recovered in pure culture at a concentration of $>10^7$ cfu ml^{-1} from the sputum of patients >65 years and with clinically diagnosed lower respiratory tract infections were collected and stored at -70°C using the Protect Bead System (Technical Service Consultants, UK). For the purposes of this study, 20 of these isolates were randomly selected and were referred to as 'isolates from the infected group'. Healthy elderly subjects attending four day-care centres for the active elderly in the Dublin area

were screened at three monthly intervals over a 2-year period (1992 and 1993) for asymptomatic carriage of *M. catarrhalis* in the nasopharynx. Isolates collected were stored at −70°C as described above. Twenty of these isolates were randomly selected for the purposes of this study and were termed 'isolates from the colonised group'.

The identity of these 40 isolates was confirmed according to the criteria previously outlined [20].

2.2. Haemagglutination studies

The ability of the 40 isolates of *M. catarrhalis* to agglutinate human group O erythrocytes was determined by a microtitre method [21]. Briefly, bacteria from overnight cultures were resuspended in Dulbecco A PBS (pH 7.3) at a concentration of 10^9 cfu ml^{-1}. Serial two-fold dilutions of the bacterial suspensions (50 μl) were prepared in U-bottomed microtitre plates and 50 μl of a 2% (v/v) human group O erythrocyte suspension was added to each well. The plate was rocked at room temperature for 5 min. The titre was defined as the reciprocal of the highest dilution in which haemagglutination was clearly visible. The haemagglutination assay was performed at least three times on each of the isolates examined.

2.3. Tissue culture adherence assay

HEp-2 cells, a human epithelial cell line derived from carcinoma of the larynx, were maintained in RPMI 1640 (BioWittaker, Belgium), supplemented with 10% (v/v) foetal calf serum (FCS) (BioWittaker), 1% (v/v) 200 mM L-glutamine (BioWittaker), 50 IU penicillin ml^{-1} (BioWittaker) and 50 IU streptomycin ml^{-1} (BioWittaker). Cells were incubated at 37°C in the presence of 5% CO_2.

The tissue culture adherence assay involved the use of the 'static overlay technique' and the procedure of Scott et al. [22] was employed. Essentially, HEp-2 cell cultures were subcultured by trypsinising the monolayers with 0.25% (w/v) trypsin (Gibco BRL). The cells were resuspended in complete growth medium. Cells were counted using a Neubauer counting chamber and the cell concentration adjusted to 1.7×10^5 ml^{-1}. Using this suspension, 1-ml aliquots were placed in Trac tubes with number 3 coverslips already present (Sterilin). The preparations were incubated at 37°C in the presence of 5% CO_2 for 18 h.

M. catarrhalis isolates for tissue culture adherence studies were cultured on Columbia blood agar at 37°C for 18 h. Bacterial suspensions of 3×10^8 cfu ml^{-1} (equivalent to a MacFarland No. 1 turbidity standard) were prepared in Dulbecco A PBS (PBS-A). For each test, 1 ml of bacterial suspension was added to the Trac tube from which the growth medium had been removed. The preparations were incubated at 37°C for 1 h. The bacterial suspensions were removed and the monolayer washed five times in PBS-A in order to remove any non-adherent bacteria. The preparations were fixed in methanol for 90 s and then stained with 10% (v/v) Giemsa for 30 min. The preparations were washed twice in tap water, decolourised in acid-tap water [1 ml (25% (v/v) sulfuric acid in 1 litre tap water] for 20 s and washed twice more in tap water. The coverslip preparations were dehydrated in acetone and cleared in xylene. The coverslip preparations were attached to glass slides using DPX with the cell monolayers uppermost. These preparations were then mounted in DPX using number 1 coverslips (Chance Propper) and examined under the oil immersion lens for the presence of adherent bacteria.

In total 100 HEp-2 cells were counted per coverslip and the percentage of cells with greater than 10 bacteria adhering was determined. An average between two coverslip preparations was obtained for each isolate examined and expressed as an adherence index.

2.4. Investigation of the properties of the adhesins involved in tissue culture adherence

To investigate the properties of the adhesins involved in tissue culture adherence by *M. catarrhalis*, six tissue culture adherent isolates (21, 25, B4, K3, S540, S580) were treated with sodium periodate ($NaIO_4$) (10 mg ml^{-1}) and 0.4% (v/v) formalin in PBS-A at room temperature for 30 min prior to adhesion studies. Bacterial suspensions treated with PBS-A served as positive controls.

Bacterial suspensions were also pre-treated with pronase E, trypsin and wheat-germ lipase (2 mg ml^{-1}) and with PBS-A as a control at 37°C for 2 h.

Following treatment, bacteria were washed three times with PBS-A. and were resuspended to a concentration of 3×10^8 cfu ml^{-1} (equivalent to a MacFarland No. 1 turbidity standard) in PBS-A for the tissue culture adherence studies.

Bacterial suspensions 3×10^8 cfu ml^{-1} of the six tissue culture adherent isolates were prepared in PBS-A. Samples of these suspensions were heat treated at 60°C or 70°C for 1 h. Controls were run in parallel by incubating suspensions at room temperature for 1 h.

Following the various pre-treatment steps of the six isolates, tissue culture adherence studies were performed on the preparations, using the procedure as outlined above. All tests were repeated on three separate occasions.

2.5. Transmission electron microscopy (TEM) studies of bacteria

Bacterial suspensions of two tissue culture adherent isolates (21, K3) were examined by TEM. The suspensions consisting of 1.5×10^9 cfu ml^{-1} (equivalent to a MacFarland No. 5 turbidity standard) were prepared in 1-ml amounts in sterile distilled water and washed twice. The bacterial pellets were fixed using the procedure previously described [23].

Isolates 21 and K3 of *M. catarrhalis* were treated with trypsin (2 mg ml^{-1}) at 37°C for 2 h prior to TEM. Following washing, the pellets were prepared and fixed for TEM as described above.

2.6. TEM of tissue culture adherence

The nature of the interactions occurring when *M. catarrhalis* adheres to HEp-2 cells was examined by TEM. Isolates 21, B4 and K3 were examined. Monolayers of HEp-2 cells were prepared by incubating 15 ml of 1.7×10^5 cells ml^{-1} in a 75-cm^2 tissue culture flask at 37°C in 5% CO_2 for 18 h. The culture medium was removed and the monolayers were washed three times in PBS-A. A 15-ml bacterial suspension containing 3×10^8 cfu ml^{-1} prepared in PBS-A was added to the monolayer and incubated at 37°C for 1 h. The monolayer was washed five times in PBS-A. The cell sheets were then removed using a sterile cell scraper and transferred into an Eppendorf tube. The cells were pelleted at $200\times g$ for 5 min and the deposit fixed as previously described [23].

Two tissue culture adherent isolates (21 and B4) were treated with trypsin (2 mg ml^{-1}) at 37°C for 2 h. Following washing, the bacterial suspensions were adjusted to 3×10^8 cfu ml^{-1} with PBS-A. These bacterial suspensions were used in the tissue culture adherence assay for TEM, as described above.

2.7. Statistical analysis

The haemagglutination results were analysed by the χ^2 test, using Yates' correction, while the tissue culture adherence data were analysed using the Mann-Whitney two-tailed test.

3. Results

3.1. Haemagglutination studies

Seventeen of the 40 isolates of *M. catarrhalis* examined agglutinated human erythrocytes. A marked difference in the number of haemagglutinating isolates obtained from the infected group as opposed to the colonised group was noted: 80% (16/20) of isolates from the infected group as opposed to 5% (1/20) of isolates from the colonised group agglutinated human erythrocytes ($P<0.001$) (Table 1).

3.2. Tissue culture adherence properties

The ability of the 40 isolates of *M. catarrhalis* to adhere to HEp-2 cells was determined by the static overlay technique. Adherence levels were expressed as an index.

Eighteen of 20 (90%) isolates from the infected group and 12 of 20 (60%) isolates from the colonised group had an adherence index of greater than 10% (Table 1). The average adherence index for isolates from the infected group was 76% as opposed to 50% for isolates from the colonised group. The difference in adherence between the isolates from the infected group and isolates from the colonised group was found to be statistically significant ($P=0.03$).

Tissue culture adherence of isolates did not correlate with haemagglutinating activity: 65% (15/23) of

Table 1
Tissue culture adherence and haemagglutination characteristics of *M. catarrhalis*

Infected group			Colonised group		
Isolate	TCA index[a], % (S.E.M.)	HA[b]	Isolate	TCA index[a], % (S.E.M.)	HA[b]
21	97.5 (2.5)	0	K1	1 (1)	0
25	98 (1)	0	K2	84 (4)	0
B4	100 (0)	64	K3	100 (0)	0
K16	81 (1)	0	K4	91 (7)	0
K29	98 (1)	16	K5	25.5 (4.5)	0
K32	91 (5)	32	K6	3 (3)	0
K36	95.5 (1.5)	8	K8	0.5 (0.5)	0
K38	98.5 (1.5)	4	K9	4 (4)	0
K43	99 (1)	32	K10	3 (3)	0
K44	96 (2)	4	K11	3.5 (3.5)	0
K46	84.5 (4.5)	2	K12	4.5 (2.5)	0
K47	29.5 (1.5)	V!	S7	98.5 (1.5)	0
K48	30.5 (8.5)	V!	S17	93.5 (6.5)	0
K51	77.5 (3.5)	64	S22	96 (3)	0
S15	1.5 (0.5)	0	S23	15.5 (3.5)	0
S109	99 (1)	16	S64	73.5 (1.5)	16
S274	13 (1.9)	32	S68	99.5 (0.5)	0
S407	39 (2.4)	64	S75	24 (2)	0
S540	99 (1)	64	S76	97 (2)	0
S580	100 (0)	64	S89	82.5 (4.5)	0

[a]TCA index, % of 100 HEp-2 cells counted per coverslip with >10 bacteria adhering per cell. Results based on the mean of two separate experiments and the standard error of the mean (S.E.M.) was calculated.
[b]Haemagglutination titres.
V!, the haemagglutination titres for isolates K47 and K48 varied between 0 and 4 with human erythrocytes.

non-haemagglutinating isolates had an adherence index greater than 20%.

3.3. Tissue culture adhesin

To determine the nature of the adhesin involved in the adherence of *M. catarrhalis* to HEp-2 cells, six tissue culture adherent isolates (21, 25, B4, K3, S540 and S580) were pre-treated with a range of enzymes, chemicals and heat prior to the adhesion studies.

Pronase and trypsin treatment did not dramatically reduce the level of tissue culture adherence, with the exception of isolate K3 (Table 2). Similarly, wheat-germ lipase pre-treatment of bacteria did not have an effect on tissue culture adherence, with the exception of isolate 21 for which the tissue culture

Table 2
Effect of enzyme pre-treatments of *M. catarrhalis* isolates on tissue culture adhesion

Isolate	% HEp-2 cells with >10 bacteria attaching[a]			
	Untreated	Pronase	Trypsin	WGL[b]
21	98.7 (0.3)	94 (2.9)	95 (4)	52 (3.6)
25	99 (0.6)	50.7 (1.8)	88 (6.1)	89.7 (2.8)
K3	100	0.7 (0.3)	21.7 (2.7)	90.7 (3.8)
S540	99.7 (0.3)	89.3 (2.7)	99.7 (0.3)	98.7 (1.3)
S580	100	100	99.3 (0.7)	100
B4	100	100	100	100

[a]Mean of three experiments, standard error of the mean (S.E.M.) in parentheses.
[b]WGL, wheat-germ lipase.

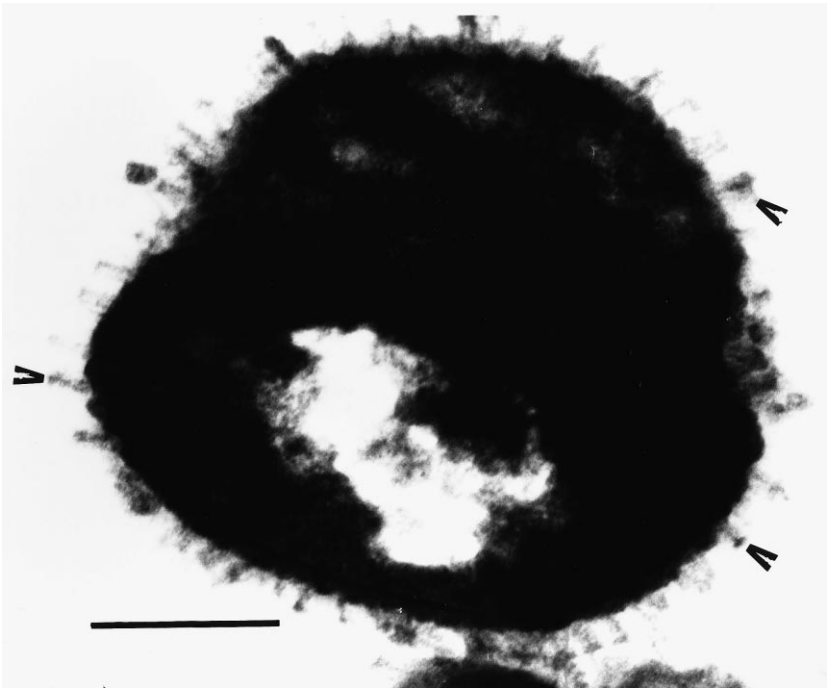

Fig. 1. TEM of a tissue culture-adherent but non-haemagglutinating isolate of *M. catarrhalis* (isolate 21). Note the absence of the fibrillar type coat, but the presence of 'tack-/spicule-like' structures on the bacterial cell surface (indicated by arrows). These structures were unaffected following trypsin treatment. Bar, 100 nm.

adherence index was reduced from 98.7% to 52% (Table 2).

Treatment of bacteria with sodium periodate ($NaIO_4$) and heat at 70°C significantly reduced the ability of the six tissue culture adherent isolates examined, to attach to HEp-2 cells. However, isolate B4 was an exception, as its adherence index was not reduced following heat treatment at 70°C (Table 3).

3.4. TEM of bacteria

When the tissue culture adherent isolates (21, K3) were examined by TEM, very distinctive structures were evident on the bacterial surface of these isolates (Fig. 1). These structures were not evident on tissue culture negative isolates. These structures resembled 'tack-like' structures previously described on *Yersinia enterocolitica* [24] or perhaps the 'spicule' structures demonstrated on *M. catarrhalis* [25]. These structures were resistant to treatment by trypsin.

3.5. TEM of tissue culture adherence

TEM studies of *M. catarrhalis* adhering to HEp-2 cells revealed that the 'tack-/spicule-like' structures described above appeared to mediate attachment of *M. catarrhalis* (21, B4 and K3) to the tissue culture cell line (Fig. 2A). Following trypsin treatment, *M. catarrhalis* was still capable of adhering to the HEp-2 cells, and furthermore, these 'tack-/spicule-like' structures still appeared to be involved in the tissue culture adherence process (Fig. 2B).

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Fig. 2. TEM of a haemagglutinating and tissue culture-adherent-positive isolate of *M. catarrhalis* isolate B4 (untreated) adhering to HEp-2 cells. Note the involvement of the tack-like structures on *M. catarrhalis* (indicated by arrows), in attachment to HEp-2 cells (A). Trypsin treatment of isolate B4 abolished neither tissue culture adherence nor the involvement of the tack-like structures in the adherence process (B). Bars, 100 nm.

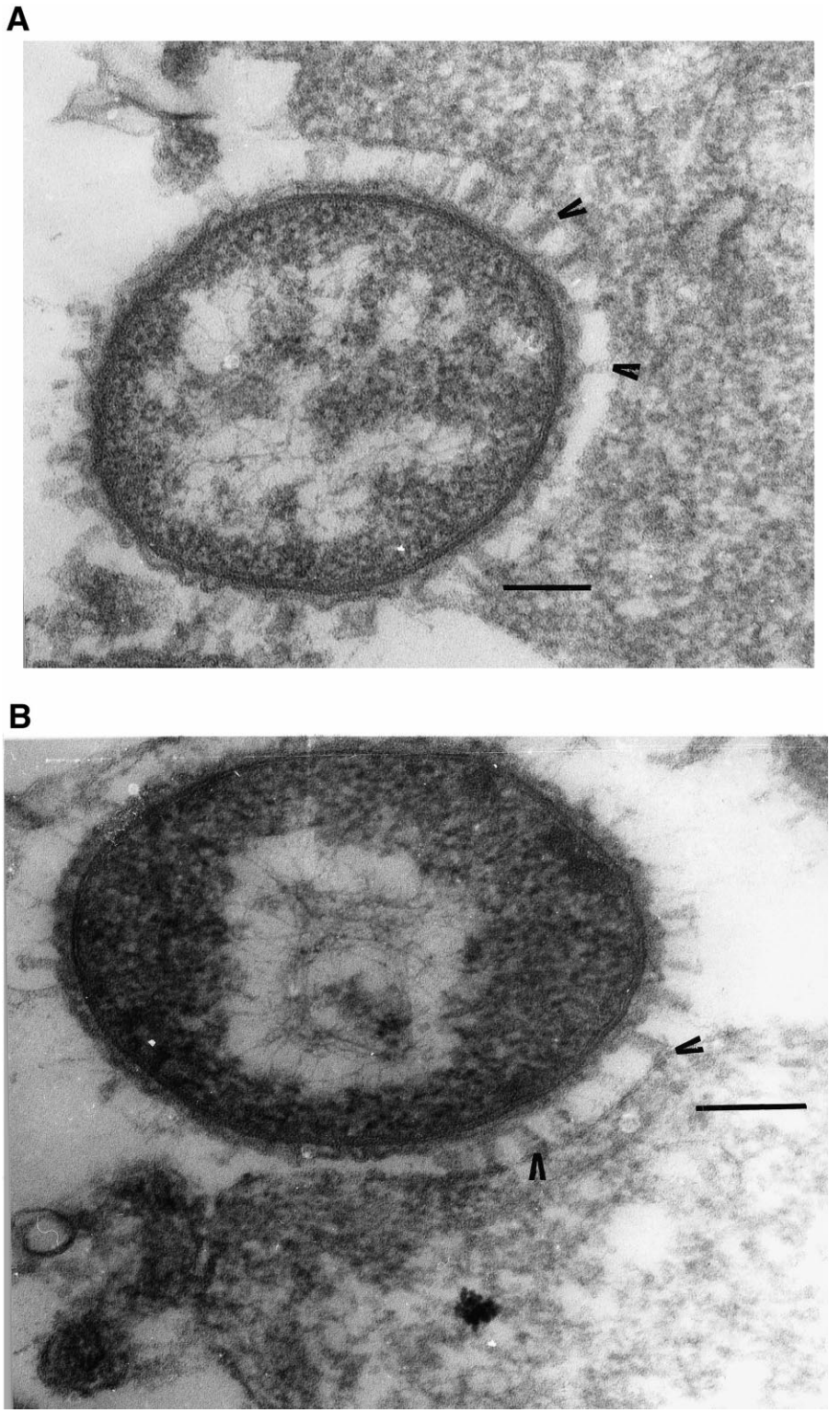
A
B

Table 3
Effect of chemical and heat treatments of *M. catarrhalis* isolates on tissue culture adhesion

Isolate	% HEp-2 cells with >10 bacteria attaching[a]				
	Untreated	Formalin	$NaIO_4$[b]	60°C	70°C
21	95.3 (1.9)	86.7 (2.8)	6.7 (1.8)	67 (4.2)	2 (0.6)
25	98.7 (0.7)	93 (5.1)	2.7 (1.2)	93.7 (2)	3.3 (1.2)
K3	100	93.3 (2)	1.3 (0.9)	56.3 (2.7)	0
S540	99.7 (0.3)	75.3 (1.8)	1 (0.6)	5.7 (0.9)	0.3 (0.3)
S580	100	94.7 (3.9)	0.7 (0.7)	2.7 (0.9)	1 (1)
B4	100	96.3 (3.7)	4.3 (1.9)	99.3 (0.7)	95 (2.2)

[a]Mean of three experiments, standard error of the mean (S.E.M.) in parentheses.
[b]$NaIO_4$=sodium periodate.

4. Discussion

Microbial pathogenicity is a complex phenomenon encompassing various mechanisms. The emergence of *M. catarrhalis* as an important respiratory pathogen has prompted researchers to investigate the possible virulence mechanisms contributing to the organism's pathogenicity. Studies have indicated that the attachment of bacteria to mucosal surfaces is the initial event in the pathogenesis of many infectious diseases. Adherence studies have found that the attachment of *M. catarrhalis* to oropharyngeal cells correlates with the occurrence of disease, indicating that the adherence of the organism to the mucosal surface may play an important role in at least the initial colonisation and infectious process [18].

To investigate the adherence properties of *M. catarrhalis*, the haemagglutination status and tissue culture adherence properties of 40 isolates were examined. The failure to establish a clear link between haemagglutination and tissue culture adherence of *M. catarrhalis* in this study suggests that at least two separate adhesins may be involved in these adherence processes. In two previous studies, no correlation was found when the organism's ability to haemagglutinate human erythrocytes and to adhere to human nasopharyngeal cells was compared [16,17]. The presence of more than one type of adhesin is not uncommon in bacteria [26,27]. Haemagglutinating activity does not always correlate with epithelial cell adherence, as has been demonstrated in the case of *Salmonella typhimurium* [28], *Gardnerella vaginalis* [29] and *H. influenzae* [30,31].

The properties of the *M. catarrhalis* adhesin involved in haemagglutination have been previously described as a trypsin-sensitive protein [15,21] and haemagglutination has been found to be associated with the presence of a 200-kDa protein [32]. To further elucidate the adherence characteristics of *M. catarrhalis*, the properties of the *M. catarrhalis* HEp-2 cell adhesin which have not been previously characterised, were investigated in this present study.

The tissue culture adhesin appeared to have a carbohydrate moiety present since sodium periodate pre-treatment of the bacteria dramatically reduced their ability to adhere to HEp-2 cells compared to the untreated controls. Furthermore, the fact that the tissue culture adhesin was resistant to trypsin and pronase treatment suggests that this structure may not be protein in nature, which contrasts with the proteinaceous nature of the haemagglutinin [15,21]. These observations provide additional evidence that separate adhesin systems are involved in haemagglutination and tissue culture adherence.

TEM examination of tissue culture adherent isolates revealed the presence of trypsin-resistant 'tack-/spicule-like' structures protruding from the cell surface. These structures closely resembled the structures described by Ahmed et al. as peritrichously arranged fimbriae [33,34] or the structures described by Marrs and Weir as short, thick fimbriae with knobby ends, that were seen extending outward peritrichously from the bacteria [35]. For the purposes of this article, these structures will be described as 'tack-/spicule-like' structures, as the authors remain unconvinced of the fimbrial nature of these appendages.

The 'tack-/spicule-like' structures on *M. catarrhalis* appeared to mediate attachment to the HEp-2 cells and additional TEM observations demonstrated

that, following trypsin treatment, these structures were still involved in the *M. catarrhalis*-HEp-2 cell interactions. These observations would indicate that these 'tack-/spicule-like' structures are involved in the adherence of *M. catarrhalis* to HEp-2 cells. Furthermore, the fact that these 'tack-/spicule-like' structures were trypsin-resistant and that the level of tissue culture adherence was unaffected following trypsin treatment indicates that these distinctive structures are involved in tissue culture adherence by *M. catarrhalis*. In a previous study it was established, following TEM examination, that haemagglutination by *M. catarrhalis* was mediated by a trypsin-sensitive outer fibrillar coat [36], thus providing further evidence that separate adhesins are involved in haemagglutination and tissue culture adherence.

The fact that 80% of isolates of *M. catarrhalis* from the infected group were haemagglutinating, in comparison to 5% of isolates from the colonised group, indicates that the haemagglutinin is in some way involved in the infectious process in the elderly population and could be used as a marker of pathogenicity of strains infecting this age group. These findings corroborate the findings of Murphy et al. [14]. It was also found that significantly more isolates from the infected group compared with isolates from the colonised group were tissue culture adherent (90% and 60%, respectively). Furthermore, isolates from the infected group showed a higher level of HEp-2 cell adherence than the isolates from the colonised group, these observations would support previous findings, whereby increased levels of nasopharyngeal adherence by *M. catarrhalis* correlated with increased risk of respiratory infection [18,19]. Thus, it is possible that the HEp-2 cell adherence model may reflect adherence processes during *M. catarrhalis* colonisation of the upper respiratory tract.

To examine if a relationship exists between HEp-2 cell adherence and nasopharyngeal colonisation, future work should focus on *M. catarrhalis* adherence to exfoliated nasopharyngeal cells to establish if these cells share a similar mechanism of adherence with the HEp-2 cell model to *M. catarrhalis*. Should such a link exist between nasopharyngeal cell and HEp-2 cell adherence, the HEp-2 cell model would be most useful in studying the adherence process involved in colonisation by *M. catarrhalis*, as this system is far easier to standardise and far less prone to variation when compared to the exfoliated epithelial cell adherence model.

4.1. Conclusions

To obtain an improved understanding of the adherence characteristics of *M. catarrhalis*, the adherence of the organism to epithelial cells using the HEP-2 cell line as an adherence model was evaluated and compared with haemagglutination. A separate adhesin from the haemagglutinin mediated adherence to the HEp-2 cells. This adhesin was carbohydrate in nature and resistant to trypsin treatment. TEM studies revealed that adherence of *M. catarrhalis* to HEp-2 cells was mediated by trypsin-resistant 'tack-/spicule-like' structures. Increased adherence was more likely to occur with the isolates from the infected group. Future work should focus on assessing whether the HEp-2 cell line may be used as an adherence model for studying nasopharyngeal cell adherence and thus elucidating interactions involved when *M. catarrhalis* colonises the upper respiratory tract.

Acknowledgments

Special thanks are due to the Electron Microscopy unit, St James's Hospital, Dublin, especially Ms Ann Mynes for her help with the electron microscopy work. This work was supported by a Health Research Board grant to M.F.

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