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Activity of some Mucolytics Against Bacterial Adherence to Mammalian Cells

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Abstract In this article, some mucolytic agents were tested for their activity to prevent bacterial adherence to mammalian cells. Preliminary screening for antiadherent activity showed that ambroxol, bromhexine, ammonium chloride, and ammonium acetate but neither guaiphenesin nor carbocysteine significantly reduced the adherence of the tested clinical isolates to cultured mammalian cells. The antiadherent effect of such agents was observed when mammalian cells were treated with these agents either before or after bacterial adherence, and this effect was exhibited in a dose-dependent manner. The minimum concentrations of ambroxol, bromhexine, ammonium chloride, and ammonium acetate required for mammalian cells treatment to prevent bacterial adherence were 2.5, 5, 50, and 20 ng/ml, respectively, whereas significantly higher mucolytic concentrations were required to eradicate bacteria that adhered to mammalian cells. Upon treatment of mammalian cells with mucolytics, the maximum reduction in adherence of the tested isolates attained by ambroxol, bromhexine, ammonium chloride, ammonium acetate were 99%, 98%, 75%, and 54% to that of control, respectively. Insignificant difference existed between the antiadherent activities of ambroxol and bromhexine, while both agents had significantly higher effect than ammonium chloride and ammonium acetate. Pretreatment of the immobilized mucin with ambroxol, bromhexine, ammonium chloride, or ammonium acetate reduced the adherence of *Pseudomonas aeruginosa*, *Escherichia coli*, and staphylococcal isolates to this receptor analogue. A strong correlation was observed for the antiadherent activity of the tested mucolytics in case of mammalian cells and immobilized mucin. Moreover, pretreatment of the immobilized receptor analogues chondroitin sulfate-B and heparin with the abovementioned agents significantly reduced the adherence of *Staphylococcus aureus*, *P. aeruginosa*, and *E. coli* isolates to such immobilized glycoproteins.

Keywords Bacterial adherence · Mammalian cells · Mucolytics

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Introduction

The noted dramatic increase in the incidence of severe, life-threatening infections [1] in addition to the recent spread of new multidrug resistant variants in the hospital and community [2] created a need for more effective antimicrobial therapy. Attempts were made to find novel agents and alternative approaches for prevention and treatment of microbial infections. It became widely accepted that bacterial adhesion to tissues was prerequisite step in the infectious process [3–6]; hence, numerous trials have been made to prevent microbial diseases by reducing the binding of the pathogen to epithelial surfaces [7]. Although the antiadherent effect of some antibiotics at sub-MICs was extensively reported [8, 9], they did not find similar acceptance between clinicians. Clinicians are hoping for new drugs, which are capable of avoiding and therefore preventing the adherence of bacteria without adverse effects. Antibiotics are not always efficient and cannot be administered for long period as microbial adherence preventive drugs due to their side effects. At the end of the twentieth century, it was shown that *N*-acetyl cysteine (NAC) reduced adhesion of *Streptococcus pneumoniae* and *Haemophilus influenzae* to oropharyngeal epithelial cells in vitro [10]. Similarly, NAC reduced attachment of *Moraxella catarrhalis* to pharyngeal epithelial cells [11]. The effect of NAC on bacterial adherence is still relatively unknown [12], and a better understanding of bacterial responses to NAC may facilitate efficient use of similar compound for therapeutic purposes. The question is whether this antiadherent activity of NAC is related to its mucolytic action or it is mediated through another mechanism. In other words, it is important to clarify whether the reported antiadherent activity is extended to other mucolytics or it is limited to NAC. Accordingly, the present study aimed to investigate the effect of some mucolytic agents on adherence and hence infectivity of the *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, as well as *Escherichia coli* isolates to mammalian cells. Moreover, it is concerned with clarifying the mechanism by which such agents exert their possible microbial antiadherent effect.

Materials and Methods

Bacterial Strains

A total of 45 bacterial pathogens including ten *S. aureus*, ten *S. epidermidis*, five *Staphylococcus saprophyticus*, ten *P. aeruginosa*, and ten *E. coli* clinical isolates were used in this study. The *S. aureus* and *S. epidermidis* isolates were recovered from swabs taken from wound infections and aspirated pus obtained from abscesses, while *S. saprophyticus*, *P. aeruginosa*, and *E. coli* isolates were recovered from urine specimens obtained from patients suffering from urinary tract infection. These bacterial pathogens have good adherence to both Vero and Hep-2 cells [13 and our unpublished data].

Cell Lines and Growth Conditions

Human epithelial cells derived from an epidermoid carcinoma of larynx (Hep-2 cells; ATCC No. CCL-23) and African green monkey kidney epithelial cells (Vero Cells; ATCC No. CCL-81) were maintained and manipulated as described by Hafez et al. [13].

Studying the Effect of Mucolytics on Adherence of the Tested Isolates to Mammalian Cells

Selected Drugs and their Concentrations

A total of six mucolytics (namely, ambroxol, bromhexine HCL, carbocysteine, guaiphenesin, ammonium chloride, and ammonium acetate) were tested for their antiadherent activity. Each mucolytic was dissolved in Eagle's minimum essential medium (MEM) to the required concentration. The agents were primarily screened at three concentration levels (0.1×, 1×, and 10× of therapeutic plasma concentration of each agent), and those that showed antiadherent activity were then tested at a complete concentration spectrum.

Preparation of Bacterial Inoculum

An 18-h nutrient broth culture of the tested microorganism was centrifuged, washed twice with phosphate-buffered saline (PBS, pH 7), and then standardized to 3×10^8 CFU/ml using Eagle's MEM. The count was adjusted using McFarland standard No. 1 and verified by viable count.

Preparation of Mammalian cell monolayer

Mammalian cells maintained in tissue culture flasks were trypsinized and suspended to a count of 10^4–10^5 living cells/ml in Eagle's MEM supplemented with 5% and 10% fetal bovine serum (Gibco) in case of Vero and Hep-2 cells, respectively. The count of living cells was carried out using hemocytometer (Shanghai, China) after staining with trypan blue solution (0.4% in PBS). Aliquots of 100 μl of mammalian cell suspension were transferred to wells of tissue culture plate. The plate was then incubated at 37°C in presence of 5% CO_2 and humid atmosphere for 48 h to form a confluent monolayer.

Adherence Assay

Adherence assay was carried out principally as described by Plotkowski et al. [14]. Quantitative determination of the adherent viable bacteria of the tested organism was carried out depending on the difference between the total number of adherent and uptaken bacteria by mammalian cells and the number of uptaken bacterial cells as described by Hafez et al. [13].

Testing the Ability of the Agents to Prevent Bacterial Adherence to Mammalian Cells

This was carried out by using two models (namely M1 and M2). In case of M1 model, aliquots of 100 μl of the mucolytic solution (in Eagle's MEM) were first incubated with the mammalian cells for 30 min at 37°C. The mucolytic was then discarded, and the monolayer was washed twice with PBS. The adherence of different isolates to the treated monolayer was assessed as mentioned before. In case of M2 model, the bacteria were preincubated with the mucolytic for 30 min at 37°C. After incubation, the suspension was centrifuged, and the cells were washed twice with PBS and resuspended in fresh Eagle's MEM to the count of 3×10^8 CFU/ml. This bacterial suspension was used in adherence assay described before. In both models, the control was similarly treated except that the tested agent was omitted [15].

Testing the Ability of the Agents to Eradicate Bacteria Preadhered to Mammalian Cells

The experiment (test model 3, M3) was carried out as described by Yassien and Khardori [16]. Briefly, the bacterial suspension was incubated with mammalian cell monolayer for 2 h at 37°C to allow adherence. The monolayer was then washed three times with PBS to remove nonadherent bacteria, then aliquots of 100 μl of the mucolytic solution (in Eagle's MEM) were added to the monolayer and incubated at 37°C for further 2 h. After incubation, the supernatants were discarded, and the monolayer was washed twice with PBS; the number of adherent bacterial cells were determined as described before. The control was similarly treated except that the tested agent was omitted.

Studying the Mechanism of Action of some Antiadherent Agents

This was carried out by investigating the possible nonspecific effect of the agents on bacterial surface hydrophobicity in addition to its possible interaction with specific mammalian cell surface receptors.

Effect of the Agents on Hydrophobicity of the Isolates

The cell surface hydrophobicity was measured using xylene partition test described by Balague et al. [17]. Briefly, 18-h nutrient broth culture of each isolate was centrifuged, washed twice with PBS, and resuspended to an optical density of 0.8 at 540 nm using Eagle's MEM containing different concentrations of the tested agent. One milliliter xylene was added to 2.5 ml of bacterial suspension, and the mixture was vortexed for 2 min at a constant power and then left for 20 min for phases separation. The lower aqueous phase was aspirated, and the optical density was determined at 540 nm (Ax); the absorbance of the aqueous phase without treatment with xylene (Ac) was also measured. Based on these absorbance values, the partition index was calculated using the following formula:

$$\mathrm{PI} = (\mathrm{Ac} - \mathrm{Ax})/\mathrm{Ac}.$$

A control experiment was done in parallel where the antiadherent agent was omitted. All experiments were repeated six times, and the results are expressed as a mean ± standard deviation.

Effect of the Agents on Adherence of Isolates to Immobilized Receptor Analogues

Analogues of mammalian cell receptors that were found to play a role [13] in the adherence of different isolates to Vero and Hep-2 cells were immobilized to wells of tissue culture plates as described by Dziewanowska et al. [18]. Briefly, aliquots of 40 μl of receptor analogue solution (100 nmol/ml in sterile distilled water) were transferred to wells of microtiter plate. The plate was incubated overnight at 25°C to allow drying. The wells were then washed three times with PBS, and any residual nonspecific binding sites were blocked by adding 0.2% bovine serum albumin (BSA) and incubation for further 1 h at room temperature. The wells were then washed once with MEM Eagle's MEM supplemented with 0.1% BSA. The effect of the agents on adherence of isolates to immobilized receptor analogues was determined as mentioned before regarding the effect of mucolytics on the

adherence of the isolates to mammalian cells except that the wells were coated with receptor analogue instead of mammalian cell monolayer.

Statistical Analysis

Statistical significance between means was tested by analysis of variance and Student *t* test using Instate software. The differences between means were considered statistically significant when the test yielded a value $P<0.05$. In addition, correlation between two independent variables was assessed by calculating Pearson correlation coefficient (r) using SPSS software. If r value is greater than 0.75, this indicates strong correlation; r value ranging from 0.5 to 0.75 indicates intermediate correlation, while r value less than 0.5 indicates no correlation.

Results

A number of mucolytic agents were tested for their ability to prevent adherence using test models M1 (pretreatment of tissue with the tested agent) and M2 (pretreatment of bacteria

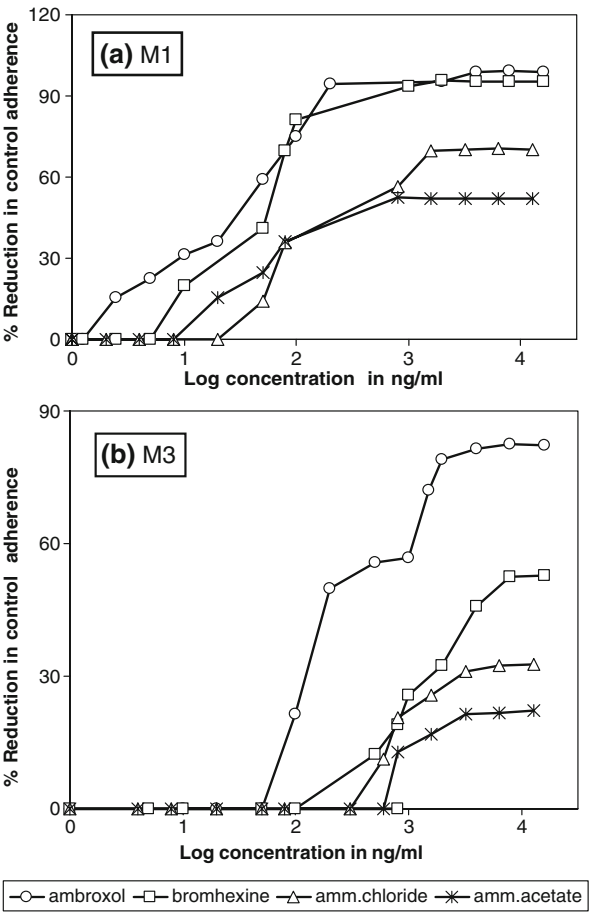

Fig. 1 Effect of mucolytics on adherence of *S. aureus* isolates to Vero cells using M1 (**a**) and M3 (**b**) test models

with the tested agent) as well as their ability to eradicate preadhered bacteria using model M3. The mucolytics were screened preliminarily for any possible antiadherent activity at three concentration levels (0.1×, 1×, and 10× of therapeutic plasma concentration of each agent). The results showed that ambroxol, bromhexine, ammonium chloride, and ammonium acetate significantly reduced the adherence of the clinical isolates to cultured mammalian cells ($P<0.05$). The antiadherent effect of such agents was only observed using the test models M1 and M3, while nearly no effect was observed upon using M2 model. On the other hand, neither guaiphenesin nor carbocysteine showed an antiadherent activity with any of the used models.

The effect of concentration on the antiadherent activity of ambroxol, bromhexine, ammonium chloride, and ammonium acetate was investigated. As shown in Figs. 1, 2, 3, 4, 5, 6, 7, and 8, the mucolytics reduced the adherence of the isolates in a dose-dependent manner. The minimum antiadherent concentrations of ambroxol, bromhexine, ammonium chloride, and ammonium acetate using model M1 were 2.5, 5, 50, and 20 ng/ml, respectively, whereas significantly higher mucolytic concentrations were required to show antiadherent activity using M3 model.

The results also demonstrated that upon using M1, the maximum reduction in adherence of the isolates attained by ambroxol, bromhexine, ammonium chloride, and ammonium acetate were 99%, 98%, 75%, and 54% to that of control, respectively. However, these

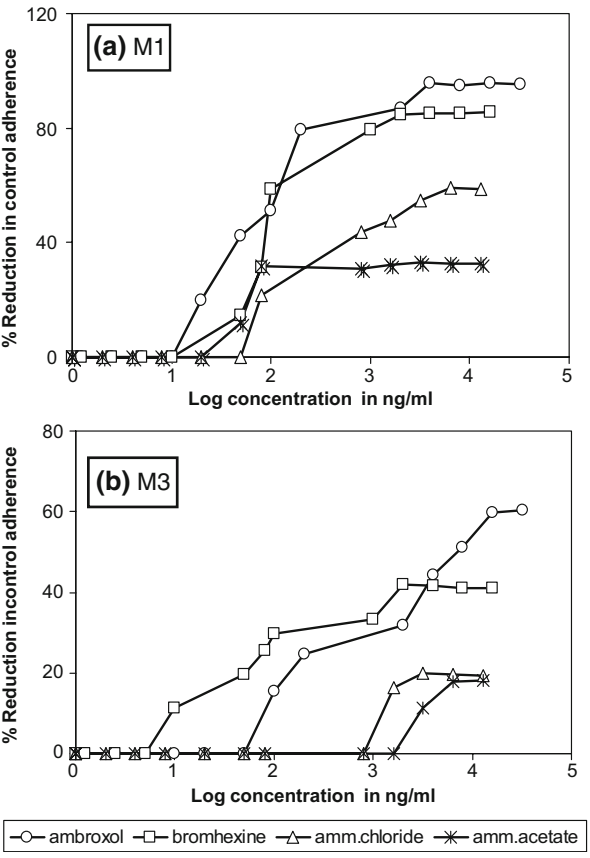

Fig. 2 Effect of mucolytics on adherence of *S. epidermidis* isolates to Vero cells using M1 (**a**) and M3 (**b**) test models

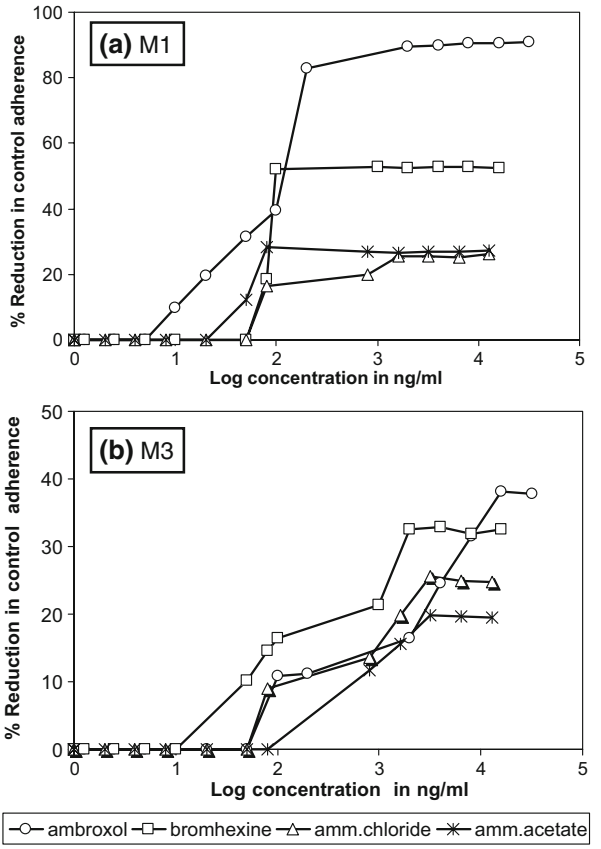

Fig. 3 Effect of mucolytics on adherence of *S. saprophyticus* isolates to Vero cells using M1 (**a**) and M3 (**b**) test models

maxima became 82%, 64%, 33%, and 30% to that of control, respectively, when M3 model was applied. The statistical analysis of the obtained results showed that at any concentrations, the antiadherent effect of ambroxol, bromhexine, ammonium chloride, and ammonium acetate was significantly higher using M1 test model than that using M3 model ($P \leq 0.05$).

Among the tested mucolytic agents, ambroxol had the highest antiadherent activity followed by bromhexine, then ammonium chloride, and finally ammonium acetate. However, the statistical analysis of the obtained results revealed that insignificant difference existed between the antiadherent activities of ambroxol and bromhexine, while both agents had significantly higher effect than ammonium chloride and ammonium acetate ($P \leq 0.05$).

Studying the Mechanism of Antiadherent Action of some Mucolytics

In order to clarify the mechanism of antiadherent activity of the tested mucolytic agents, two different approaches were adopted. The first was concerned with studying the nonspecific effect that is related to bacterial surface hydrophobicity, while the second approach involved investigating the possible specific interaction between the mucolytic agent and mammalian cell surface receptors or bacterial adhesins. The results revealed that none of the tested mucolytics had an effect on bacterial surface hydrophobicity, and this

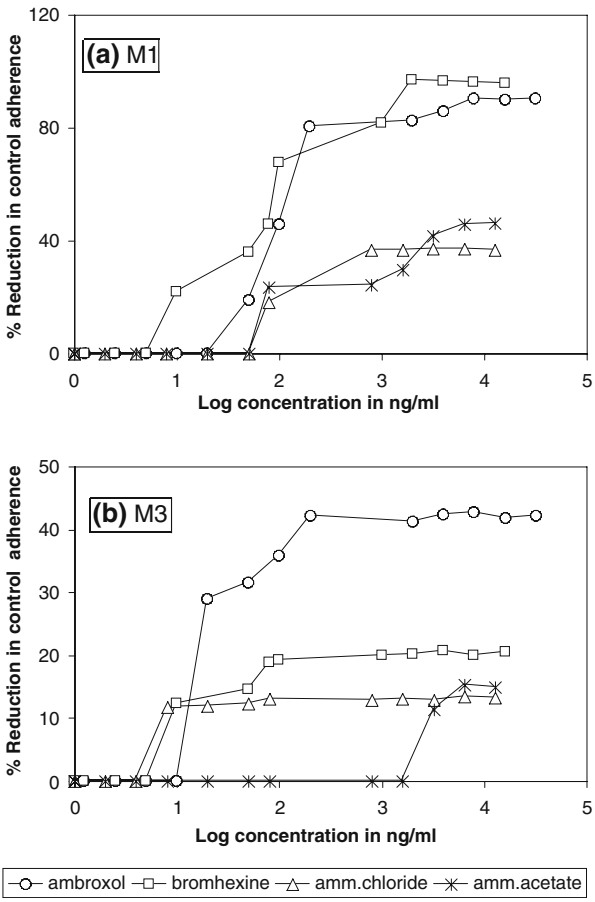

Fig. 4 Effect of mucolytics on adherence of *P. aeruginosa* isolates to Vero cells using M1 (**a**) and M3 (**b**) test models

implies that the antiadherent activity of such agents is not mediated through a nonspecific mechanism (data not shown). Therefore, the possible interaction of these agents with mammalian cell surface receptors or bacterial adhesins was investigated. The results in Tables 1 and 2 demonstrate that the adherence of all tested isolates to immobilized mucin was effectively reduced when immobilized mucin was pretreated with ambroxol, bromhexine, ammonium chloride, or ammonium acetate. The maximum reduction was observed with ambroxol and bromhexine, which had significantly higher antiadherent activity as compared to other mucolytics ($P \leq 0.05$). Statistically, a strong correlation was observed between the antiadherent activity of ambroxol, bromhexine, ammonium chloride, or ammonium acetate in case of mammalian cells and their similar effect on bacterial adherence to immobilized mucin. Moreover, pretreatment of immobilized chondroitin sulfate-B (CS-B) and heparin with the abovementioned agents significantly reduced the adherence of *S. aureus*, *P. aeruginosa*, and *E. coli* isolates to such immobilized glycoproteins. On the contrary, the results revealed that pretreatment of immobilized carbohydrates, CS-A, CS-C, heparan sulfate (HS), fibronectin, as well as protein receptor analogues with mucolytics did not lead to significant changes in adherence of the isolates to such receptor analogues. Similar results were observed when the bacteria adhered to the immobilized abovementioned receptors were treated with the mucolytic agents (Tables 3 and 4).

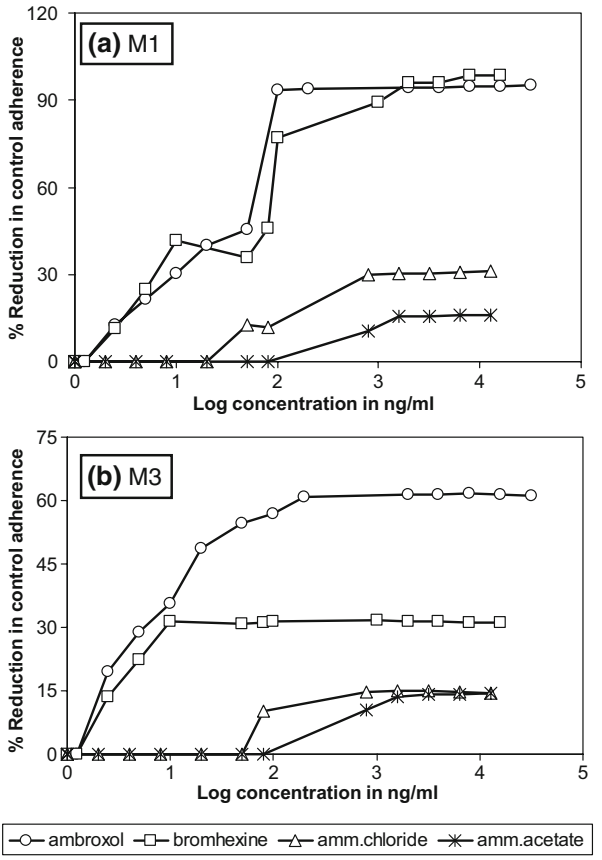

Fig. 5 Effect of mucolytics on adherence of *E. coli* isolates to Vero cells using M1 (**a**) and M3 (**b**) test models

Discussion

It became widely accepted that bacterial adherence to tissues is a prerequisite step in the infectious process [19, 20]. Different attempts have been made to prevent infection by interfering with adherence of the pathogen to the tissue by using substances that interact with the bacterial adhesin and the target cell receptor [21]. The results of the present study revealed that ambroxol, bromhexine, ammonium chloride, and ammonium acetate significantly reduced the adherence of the tested clinical isolates to cultured mammalian cells ($P<0.05$). The observed antiadherent effect of these mucolytics comes in accordance with that of Riise et al. [10] who reported that the mucolytic *N*-acetyl cysteine reduced the adherence of *S. pneumoniae* and *H. influenzae* to oropharyngeal epithelial cells. Similar observation was reported by Braga et al. [22] regarding the adherence of *S. aureus* to human mucosal cells. Moreover, it agrees with that reported by Zheng et al. [11] who proposed that one of the mechanisms of mucoregulating drugs to decrease the episode of respiratory infections is by inhibiting the attachment of bacteria to the upper respiratory tract. Conversely, the marked antiadherent activity of ambroxol found in this study is inconsistent with that of Ndour et al. [23] who reported that ambroxol had no potential antiadherent effect against *H. influenzae*. On the other hand, the present study revealed that neither guaiphenesin nor carbocysteine showed an antiadherent activity with any of the test

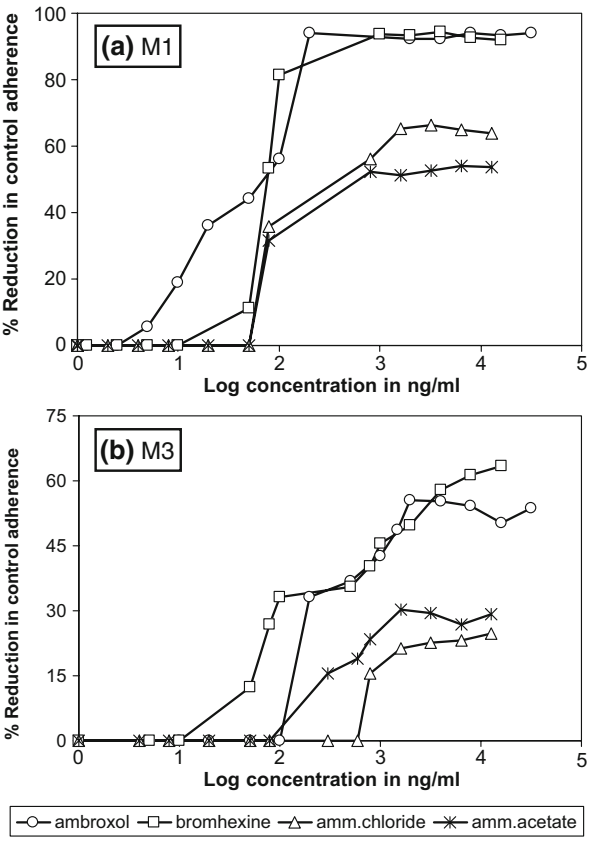

Fig. 6 Effect of mucolytics on adherence of *S. aureus* isolates to Hep-2 cells using M1 (**a**) and M3 (**b**) test models

models used. This finding is inconsistent with that of Ndour et al. [23] who reported a significant reduction in the adherence of *H. influenzae* to oropharyngeal cells in the presence of carbocysteine. It also disagrees with that of Zheng et al. [11] who observed a significant decrease (35–45%) in the attachment of *M. catarrhalis* to pharyngeal cells after oral administration of carbocysteine. The conflict between the reported antiadherent effect of carbocysteine and the lack of such effect in the present study may be attributed to the difference in the type of mammalian cells used in each case. This interpretation is in agreement with that reported by Ofek and Doyle [24] who stated that the type of mammalian cell is an important determining factor in adherence events. It is also in accordance with that of Hazlett et al. [25] and Ramphal et al. [26] who reported that the receptors involved in the adherence of *P. aeruginosa* to bronchial epithelium is different from those involved in the adherence of the same pathogen to the corneal cells. Similar observations were previously reported with *S. aureus* [27, 28], *S. epidermidis* [29, 30], *S. saprophyticus* [24, 31], and *E. coli* [32, 33]. Accepting the idea that pathogens use different receptors to adhere to different types of mammalian cell will pave the road for accepting the conflict regarding antiadherent effect of carbocysteine with different cell lines.

The present study demonstrated that the antiadherent action of ambroxol, bromhexine, ammonium chloride, and ammonium acetate was only observed using the M1 and M3 test models where the mammalian cells come in contact with the mucolytic agent. Conversely, no interference with adherence was observed using M2 test model, in which only bacteria

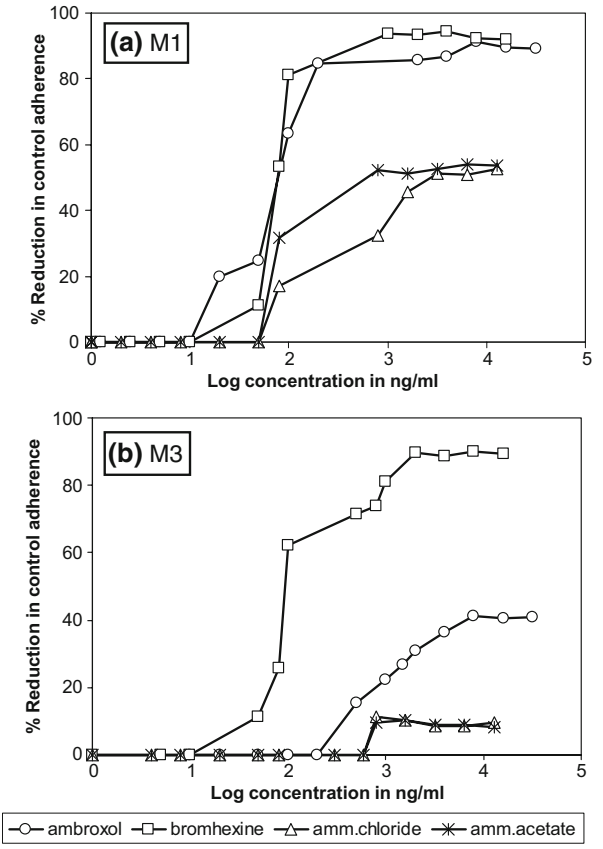

Fig. 7 Effect of mucolytics on adherence of *P. aeruginosa* isolates to Hep-2 cells using M1 (**a**) and M3 (**b**) test models

were treated with the mucolytic agent. This finding implies that the antiadherent action of the abovementioned mucolytics may be mediated through inference with mammalian cell surface components rather than bacterial adhesions. This proposal is in agreement of that of Zheng et al. [11] who found that *N*-acetyl cysteine and carbocysteine exert their antiadherent action through affecting some moieties on mammalian cell surface. It also agrees with that of Ndour et al. [23] who suggested that the decrease of attachment of *H. influenzae* with epithelial cells after treatment with carbocysteine was possibly due to the decrease of the mammalian cell surface charge. Conversely, the results of the present study is inconsistent with that of Braga et al. [22] who found that the mucoactive drug erdosteine interferes with bacterial adherence via inducing stereochemical conformational changes in the structure of pilin, a protein of bacterial fimbriae. It is noteworthy that the antiadherent effect of some mucolytics observed in this study was significantly higher upon using M1 (only mammalian cells come in contact with the drug) as compared to M3 test model (both bacteria and mammalian cells come in contact with the drug simultaneously; $P \leq 0.05$). This confirms our postulate that such antiadherent action take place via interfering with mammalian cells rather than bacteria.

The antiadherent activity of some mucolytics observed in this study represents a valuable alternative approach for treatment or prevention of infectious diseases. However, a question remains regarding the exact mechanism by which such agents impede the adherence of the tested pathogens. To answer such question, in the present study, the

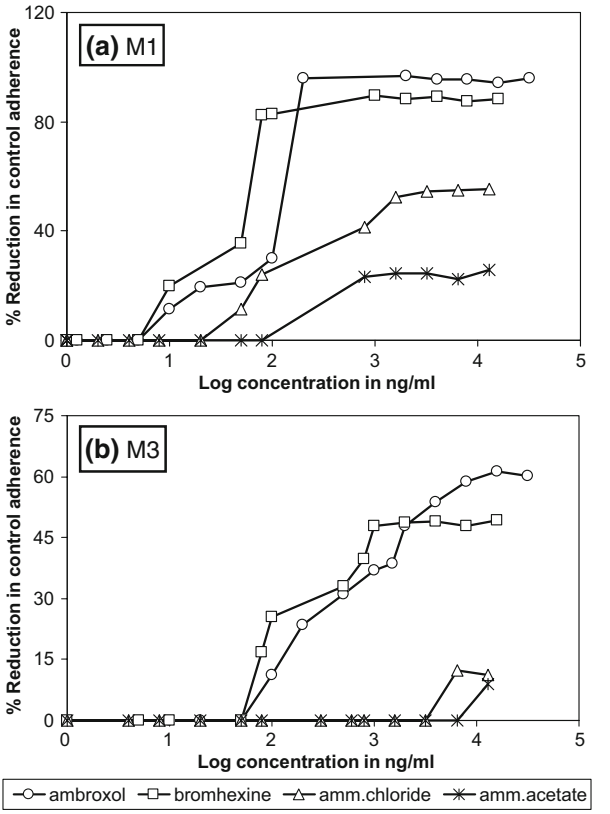

Fig. 8 Effect of mucolytics on adherence of *E. coli* isolates to Hep-2 cells using M1 (**a**) and M3 (**b**) test models

Table 1 Effect of pretreatment of immobilized receptor analogues with mucolytics on adherence of different staphylococcal isolates.

Antiadherent agent	Percentage reduction in adherence to the immobilized receptor analogues by							
	Staphylococcus aureus			*Staphylococcus epidermidis*			*Staphylococcus saprophyticus*	
	Mucin	CS-B	Heparin	HS	Mucin	HS	Mucin	HS
Ambroxol	79.8±7.1	23.5±5.6	25.9±6.3	18.9±8.5	93.5±7.6	35.6±7.2	89.7±9.7	28.7±7.6
Bromhexine	77.9±10.5	18.9±6.5	NE	NE	85.4±9.2	NE	93.6±8.9	NE
Ammonium chloride	56.3±5.7	NE			49.8±6.8		65.9±7.1	
Ammonium acetate	45.6±5.8				42.3±7.1		46.9±7.8	

For all the tested isolates, their adherence to the immobilized CS-A and CS-C were nearly unaffected by pretreatment of these immobilized agents with the tested mucolytics. For both *S. aureus* and *S. epidermidis* isolates, their adherence to the immobilized fibrinogen and fibronectin (*S. epidermidis*) and fibrinogen, fibronectin, collagen, and gelatin (*S. aureus*) were nearly unaffected by pretreatment of these immobilized agents with the tested mucolytics.

NE No effect

Table 2 Effect of pretreatment of immobilized receptor analogues with mucolytics on adherence of *Pseudomonas aeruginosa* and *Escherichia coli* isolates.

Antiadherent agent	Percentage reduction in adherence to the immobilized receptor analogues by						
	P. aeruginosa				*E. coli*		
	Mucin	Heparin	CS-B	HS	Mucin	Heparin	CS-B
Ambroxol	81.2±6.8	38.9±6.5	25.6±5.3	15.8±2.9	88.6±7.8	29.3±8.4	19.6±9.5
Bromhexine	95.5±9.4	18.5±6.5	20.3±4.3	NE	63.8±2.8	15.7±5.6	15.8±4.7
Ammonium chloride	58.7±4.6	15.6±3.8	NE		41.7±8.3	NE	NE
Ammonium acetate	41.3±5.6	17.8±5.7			24.1±5.9	NE	NE

For *P. aeruginosa* isolates, their adherence to the immobilized CS-A and CS-C were nearly unaffected by pretreatment of these immobilized agents with the tested mucolytics. For *E. coli* isolates, their adherence to the immobilized CS-A, CS-C, and HS were nearly unaffected by pretreatment of these immobilized agents with the tested mucolytics.

Table 3 Ability of mucolytics to eradicate different staphylococcal isolates preadhered to immobilized GAGs and proteinaceous receptor analogues.

Antiadherent agent	Percentage reduction in adherence of preadhered *Staphylococcus* isolates to the immobilized receptor analogues upon treatment with mucolytics							
	S. aureus				*S. epidermidis*		*S. saprophyticus*	
	Mucin	CS-B	Heparin	HS	Mucin	HS	Mucin	HS
Ambroxol	54.3±7.8	16.5±4.3	18.9±4.6	13.5±4.3	75.6±8.7	20.4±6.5	74.6±8.6	20.3±5.9
Bromhexine	60.4±8.1	13.2±3.2	NE	NE	68.9±6.8	NE	70.3±7.9	NE
Ammonium chloride	45.6±6.7	NE			38.7±6.4		41.3±6.7	
Ammonium acetate	32.5±7.4				33.5±6.7		30.2±6.1	

For both *S. aureus* and *S. saprophyticus* isolates, their adherence to the immobilized CS-C (*S. aureus*) and CS-A and CS-C (*S. saprophyticus*) were nearly unaffected by treatment with the tested mucolytics.

Table 4 Ability of mucolytics to eradicate *Pseudomonas aeruginosa* and *Escherichia coli* isolates preadhered to immobilized GAGs and proteinaceous receptor analogues.

Antiadherent agent	Percentage reduction in adherence of preadhered *P. aeruginosa* and *E. coli* isolates to the immobilized receptor analogues upon treatment with mucolytics				
	P. aeruginosa			*E. coli*	
	Mucin	Heparin	CS-B	Mucin	Heparin
Ambroxol	62.3±7.5	23.6±4.6	17.2±4.1	67.9±8.3	15.6±4.2
Bromhexine	74.6±8.5	13.6±3.1	13.2±3.1	47.8±7.9	NE
Ammonium chloride	41.2±6.7	NE	NE	28.9±6.7	
Ammonium acetate	27.9±6.5			24.1±5.9	

For *P. aeruginosa* isolates, their adherence to the immobilized CS-A, CS-C, and HS were nearly unaffected by treatment with the tested mucolytics. For *E. coli* isolates, their adherence to the immobilized CS-A, CS-B, CS-C, and HS were nearly unaffected by treatment with the tested mucolytics.

interference of ambroxol, bromhexine, ammonium chloride, and ammonium acetate on both bacterial surface hydrophobicity and mammalian cell surface receptors involved in the adherence of the tested isolates to both Vero and Hep-2 cells was investigated. In a previous work, we elucidated the role of mammalian cell surface receptors in bacterial adherence [17]. We found that mucin-like and glycosaminoglycans (GAG)-like receptors play a role in the adherence of staphylococcal isolates. Conversely, the role of different carbohydrate moieties of glycolipid receptors was only observed in case of *E. coli* and *P. aeruginosa* isolates. However, it is worth noting that while sialylated receptors contribute to the adherence of *E. coli* isolates, the desialylated ones (asialo GM1) has a major role in the adherence of *P. aeruginosa*. In addition, fibronectin- and fibrinogen-like receptors are important for adherence of *S. aureus* and *S. epidermidis* isolates, while other protein receptors, namely, collagen and gelatin, also contribute to the adherence of *S. aureus* isolates only [17]. Based on these data, attempt was made to clarify the antiadherent mechanism of the tested mucolytics. The results of the present study revealed that the tested mucolytics exerted no effect on bacterial cell surface hydrophobicity. This finding excludes the contribution of such nonspecific mechanism in the antiadherent activity of such agents. On the other hand, our results strongly suggested that the antiadherent effect of ambroxol, bromhexine, ammonium chloride, and ammonium acetate against the tested isolates was mainly through specific interaction with mucin-like receptors on mammalian cell surface. However, the antiadherent action of ambroxol and bromhexine against *S. aureus*, *P. aeruginosa*, and *E. coli* isolates was additionally owed to their interaction with the CS-B and heparin-like receptors on mammalian cell surface. These findings agrees with that of Zheng et al. [11] who observed by electron microscope a fine, granular, electron-dense, ruthenium red-positive layer on the surface of pharyngeal epithelial cells; this layer was absent on cell surfaces treated with mucoregulating drugs; they owed the antiadherent effect of some mucolytics to the disappearance of such molecules. However, our findings are consistent with those of Olofsson et al. [34] who attributed the antiadherent activity of the mucolytic agent *N*-acetyl cysteine to reduction of microbial exopolysaccharide production.

Taken together, the findings of the present study open up a new possibility of interfering with bacterial adhesiveness and its resulting pathogenicity by agents devoid of antibacterial activity. Further studies are required including in vivo experiments and clinical trials to put these new antiadherent agents into clinical use.

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