Original Article

Action of Colloidal Bismuth Hydroxide Gel and its Commercial Cream on Enteropathogens and Colonizers of the Gastrointestinal Tract

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Abstract

Background: Acute diarrheal diseases constitute a world public health problem because they are the second cause of death in children under 5 years of age. Colloidal bismuth hydroxide gel (CBHG) is an active ingredient in low-cost, antidiarrhetic drugs for oral use; it does not inhibit intestinal motility, and it features very low intestinal absorption of <1%. **Materials and Methods:** We analyzed the sensitivity by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC); the effect on bacterial growth by studying the specific growth velocity and the generation time in growth curves; and bacterial attachment by counting viable plaques, of enteropathogenic *Escherichia coli*, shigatoxigenic *E. coli* O157:H7, *Klebsiella pneumoniae, Salmonella* spp., and *Shigella flexneri* in the commercial cream (Chobet® bismuth cream with pectin [CBCHP]), its active ingredient (CBHG), and its excipients (E) separately. **Results:** CBCHP: MIC 6–10 mg/ml and MBC 7.5–15 mg/ml of bismuth; CBHG: MIC 6–10 mg/ml of bismuth. E: No inhibition was observed at the concentration studied in this study. At very low subinhibitory concentrations of CBCHP and CBHG, there was already evidence of a significant decrease in growth, which could not be recorded for E. CBCHP and CBHG presented an elevated capacity for bacterial displacement, significantly greater than E. **Conclusions:** We believed that the results obtained in this study are very promising from the treatment standpoint, as a possible treatment for cases of diagnosis or suspicion of bacterial gastroenteritis. The antimicrobial and attachment effects of CBCHP are exclusively due to its active ingredient CBHG; these effects are promoted in the presence of E.

Keywords: Colloidal bismuth, diarrhea, enteropathogens

Introduction

Acute diarrheal diseases constitute a world public health problem, especially in developing countries, and they are the second cause of death in children under 5 years of age. It is calculated that 1.5 billion episodes of acute diarrhea occur each year, causing the death of between 1.5 and 2 million children under 5 years of age.[1]

In Latin America, epidemiological data showed 13.5 million episodes of persistent diarrhea each year with 15% mortality. Enteric infection can be attributed to different infectious agents, and the epidemiology depends on the host, season of the year, and sanitation conditions.[2]

The reported rates of acute diarrhea in Argentina during the year 2010 were 3,034/100,000 inhabitants.[3] The bacterial etiological agents most often involved in infectious diarrheas include the bacteria *Escherichia coli* (*E. coli*), *Salmonella, Shigella, Campylobacter, Yersinia,* and *Aeromonas*.[4] In Argentina, according to data published in 2010, in 18.13% of 23,747 patients studied by stool culture, it was possible to identify more often: *Shigella* spp.(*n* = 2,728) and *E. coli* (*n* = 671) with predominance of *Shigella flexneri* and enteropathogenic *E. coli* (EPEC).[5,6] *Shigella flexneri* is the predominant species in the world, mainly in developing countries.[7] The high incidence in these countries is due to the lack of drinking water, poor sanitation, malnutrition, and the high cost of antibiotic treatment.[8]

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On the other hand, the colonization of the gastrointestinal tract after a primary infection by microorganisms such as β-lactamases and carbapenemase-producing *Klebsiella pneumoniae* (*K. pneumoniae*) is a risk factor for presenting a second infection, and furthermore, the carriers may serve as an important reservoir for dissemination of these microorganisms in health-care facilities.[9,10]

The indiscriminate use of antibiotics in cases of diarrhea can increase the risk of adverse events, as in the case of Hemolytic Uremic Syndrome due to the shigatoxigenic *E. coli* (STEC),[11] or it can produce bacterial resistance.[12] The development and propagation of resistance to antibiotics are considered as a threat to world public health.[1]

Medications that contain bismuth have been used extensively in gastroenterology.[13,14] Colloidal bismuth hydroxide is not salt, so it contains no associated radicals such as salicylate, which is responsible for the adverse effects and contraindications of bismuth subsalicylate.[15] Recently, studies have demonstrated that the use of colloidal bismuth hydroxide exhibits antibacterial activity, and also inhibits the activity of the pathogenic factor, like Shiga toxin in STEC.[14-18]

Many epidemiological studies have confirmed the efficacy of treatments with bismuth compounds for the prevention of traveler's diarrhea, particularly for the various virotypes of *E. coli*, *Salmonella* spp., and *Shigella* spp;[19,20] for the treatment of acute diarrhea from rotavirus and enterotoxigenic *E. coli* in children;[21-23] and as a supplement in the treatment of gastric and duodenal ulcers caused by *Helicobacter pylori*,[24,25] among other uses. Despite this vast and extensive background in the use of bismuth compounds for the prevention and treatment of diarrhea, there is still controversy regarding their complete mechanism of action. Some studies have indicated that bismuth compounds inhibit the intestinal secretion caused by toxins of *Vibrio cholerae* and enterotoxigenic *E. coli*[26] and decrease the cell invasion of enteroinvasive *E. coli*.[27] Furthermore, bismuth enhances the opsonophagocytosis of *K. pneumoniae*, thereby reducing the expression of the capsule,[28] and reversibly represses the expression of fimbriae in enteropathogenic and uropathogenic *E. coli*.[29] A study by Brogan *et al*. showed dithiol bismuth to be an inhibitor of the Rho protein of *E. coli*, an essential protein that controls the expression of several genes in Gram-negative bacteria. Thus, the inhibitory and antibacterial activity of bismuth compounds is believed to be the result of multiple mechanisms.[30]

The aim of this study was to seek potential therapeutic strategies for the treatment of bacterial enterophatogens and colonizers of the gastrointestinal tract by studying the effect of colloidal bismuth hydroxide gel (CBHG) and its commercial creman CBCHP in clinical and subclinical concentrations on the viability and on the removal of the most common enteropathogenic strains in our population.

The aim of this study was to seek potential therapeutic strategies for the treatment of bacterial enterophatogens and colonizers of the gastrointestinal tract by studying the effect of clinical and subclinical concentrations of CBHG and its commercial creman CBCHP on the viability and the removal of the most common enteropathogenic strains in our population.

Materials and Methods

Bacterial strain and bismuth compounds

The strains of EPEC, STEC O157:H7, *K. pneumoniae, Salmonella* spp, and *S. flexneri* that are used in the present study were isolated at Provincial Hospital of Rosario and characterized phenotypically and genetically at our laboratory. In addition, we used the reference strain *E. coli* ATCC 25922 as a control.

We studied Chobet® bismuth cream with pectin (CBCHP) (SOUBEIRAN CHOBET, S. R. L., Buenos Aires, Argentina) because this is the only medicinal specialty with bismuth hydroxide gel (CBHG) as active ingredient with a concentration equivalent to 30 mg/ml of metallic bismuth. In addition, we recorded their excipients (E) whose composition consists of pectin, glycerin, methylparaben, propylparaben, essence of raspberries, vanillin, sodium saccharin, red amaranth, and water (SOUBEIRAN CHOBET, S. R. L., Buenos Aires, Argentina).

Determination of sensitivity to bismuth compounds

We determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CBHG, CBCHP, and E of the strains mentioned above.[31,32] We started from 0.5 McFarland bacterial suspensions. For the MIC test, MH-agar plates with different concentrations of bismuth were seeded by drop. We worked with dilutions of 1/50, 1/25, 1/15, 1/10, 1/5, and 1/3, which correspond to bismuth concentrations of 0.6, 1.2, 2, 3, 6, and 10 mg/ml, respectively. Meanwhile, in the case of MBC, MH-broth tubes with serial dilutions of bismuth were inoculated, incubated overnight at 37°C and grown in MH-agar plates in the absence of bismuth. The dilutions used were 1/32, 1/16, 1/8, 1/4, and 1/2, which are equivalent to bismuth concentrations of 0.94, 1.88, 3.75, 7.5, and 15 mg/ml, respectively. The E was evaluated at the same dilutions as for bismuth compounds.

Effect on bacterial growth

The bacterial culture was incubated with and without bismuth compounds at different subinhibitory concentrations. The dilutions used were 1/500, 1/250, 1/100, and 1/50, which are equivalent to bismuth concentrations of 0.06, 0.12, 0.3, and 0.6 mg/ml, respectively. We measured the optical density at 630 nm, at different periods of growth (0, 2, 3, 4, 6, and 24 h), and in comparison to control samples without viable bacteria.

In all cases, to evaluate the growth, the following parameters were analyzed: specific growth velocity (μ) = $\ln(At_2/At_1)/T$ and time of generation (G) = $\ln 2 * T/\ln(At_2/At_1)$, where At_1 is the absorbance in time 1, At_2 is the absorbance in the subsequent time during the logarithmic phase and T is the time in hours.[33]

Determination of binding

The bacterial culture (10^{10} bacteria/ml) was incubated with and without bismuth compounds at a subinhibitory concentration (2 mg/ml) in LB medium at 37°C with shaking. Samples were then collected at specific time intervals (6, 24, and 168 h) to determine the total number of viable organisms present in the suspension. Additional samples were removed and centrifuged at 190 × g for 5 min, and they were counted on agar plates.[32] Preliminary experiments indicated that this centrifugation did not pellet bacteria free in suspension, but these conditions were sufficient to pellet CBHG. Bacteria remaining in the supernatant were considered unbound, and those present in the pellet were considered bound to CBHG.

Statistical analysis

All tests were performed independently three times, and the data were statistically evaluated by variance analysis, followed by the Tukey-Kramer multiple comparison test.

Results

Sensitivity to bismuth compounds

When we performed the inhibition assays with CBCHP, we observed a MIC corresponding to 6 mg/ml of bismuth for the *Salmonella* spp., *S. flexneri,* EPEC and STEC and 10 mg/ml for the *K. pneumoniae.* We observed an MBC corresponding to 15 mg/ml of bismuth for the *Salmonella* spp., *K. pneumoniae,* EPEC and STEC and 7.5 mg/ml for the *S. flexneri.* In the case of CBHG, we observed a MIC corresponding to 10 mg/ml of bismuth for the *Salmonella* spp., *K. pneumoniae,* EPEC and STEC and 6 mg/ml for the *S. flexneri.* However, MBC was higher than 15 mg/ml. We did not observe a MIC or an MBC in any strain when we used the E alone at the studied concentrations. For the control strain *E. coli* ATCC 25922 we only observed a MIC of 6 mg/ml of bismuth with CBCHP [Table 1].

Effect on bacterial growth

Then, we evaluated the effect of the drugs on bacterial growth. For this purpose, we worked at low, subinhibitory concentrations (0.06, 0.12, 0.3, and 0.6 mg/ml) of CBCHP, CBHG, and E to determine the scope of the inhibition observed in the previous section [Figures 1 and 2].

For all strains, a significant decrease in bacterial growth was observed in the presence of bismuth compounds (CBCHP y CHBG) in comparison to the untreated, which could be appreciated due to a significant decrease in the specific growth velocity ($P < 0.05$), and an increase in generation time ($P < 0.05$). No significant difference was observed in the presence of E in comparison with the untreated.

The inhibitory effect was increased with the bismuth concentration tested. Almost all strains demonstrated the same behavior toward bismuth, and significant inhibition of growth was observed starting from the lowest concentration (0.06 mg/ml), with the exception of *S. flexneri*, which showed a significant inhibition starting from 0.3 mg/ml [Figures 1 and 2c].

The inhibitory effect was significantly superior ($P < 0.05$) with CBCHP in comparison with CHBG in almost all strains, with the exceptions of *S. flexneri* and EPEC, which did not show significant differences between compounds.

Table 1: Sensitivity to Chobet® bismuth cream with pectin, colloidal bismuth hydroxide gel and excipients

Bacterial strains	Concentration of bismuth (mg/ml)					
	CBCHP		CBHG		Excipients	
	MIC	MBC	MIC	MBC	MIC	MBC
Salmonella spp.	6	15	10	>15	>10	>15
K. pneumoniae	10	15	10			
S. flexneri	6	7.5	6			
STEC	6	15	10			
EPEC	6	15	10			
E. coli ATCC 25922	6	>15	>10			

CBCHP: Chobet® bismuth cream with pectin, CBHG: Colloidal bismuth hydroxide gel, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. flexneri*: *Shigella flexneri*, STEC: Shigatoxigenic *E. coli*, EPEC: Enteropathogenic *E. coli*, *E. coli*: *Escherichia coli*

Bacterial binding to colloidal bismuth

Finally, we tested the *in vitro* binding capacity that compounds showed on enterobacteria. CBHG and CBCHP exhibited a high bacterial attachment, from 94% to 97% and from 86% to 90%, respectively [Table 2]. These values were not modified significantly over time ($P > 0.05$) and they were significantly greater ($P < 0.05$) than that shown with excipients (36%–59%) in all studied strains.

No significant differences were observed in binding between CBHG and CBCHP. *E. coli* ATCC 25922 exhibited less attachment for all compounds tested (from 45% to 55%), and it was the only strain that did not show significant difference between bismuth compounds and the excipients. No significant difference was observed in the number of viable microorganisms attached without bismuth compounds under applied conditions.

Discussion

Bismuth salts have been used for more than two centuries to treat different gastrointestinal pathologies. In spite of this, their mechanisms of therapeutic action continue to be a controversial subject. CBCHP is a low-cost, antidiarrhetic drug for an oral use that demonstrates multiple benefits in treating gastrointestinal infections. The most important benefit is that bismuth is available in colloidal form (CBHG) and pectin as active ingredients and not in the form of salts such as salicylate, subsalicylate, or subcitrate.[21-23,25,28,29] CBHG does not inhibit intestinal motility and demonstrates very low intestinal absorption at <1%.

In previous studies of CBGH, we demonstrated an antimicrobial activity toward STEC and its main factors of virulences,

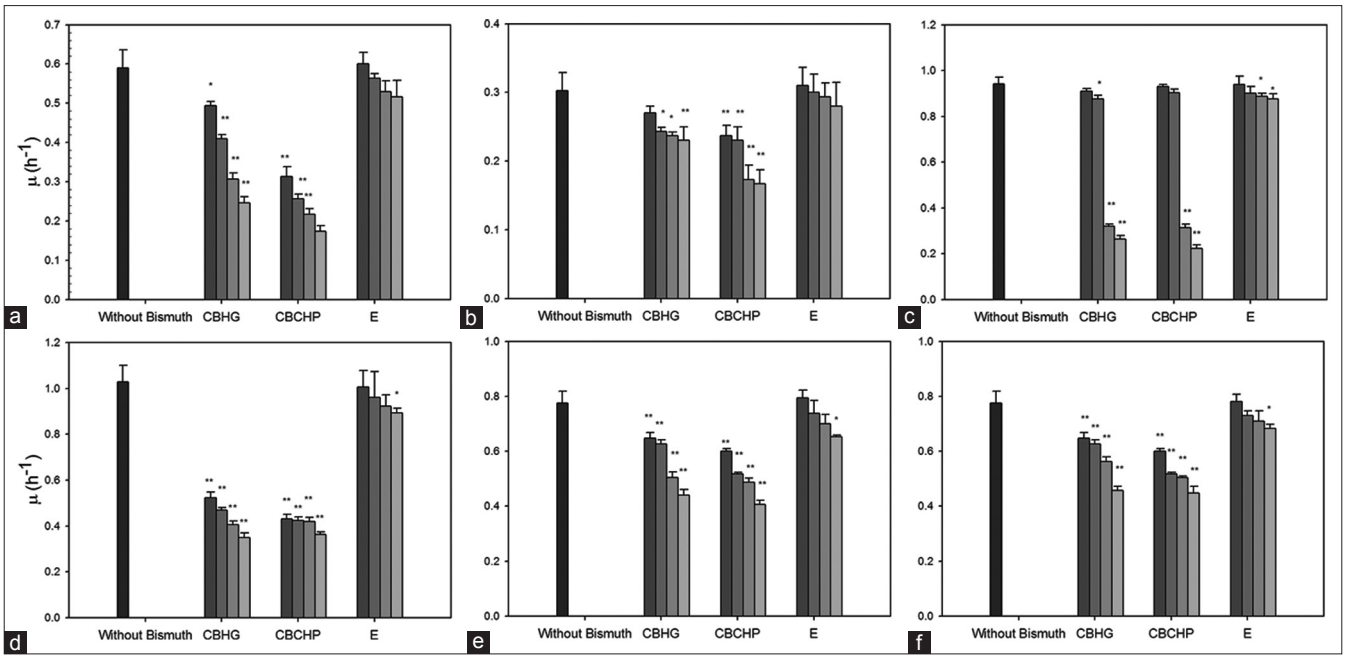

Figure 1: Specific growth velocity (μ) as a function of treatment with the compounds at different concentrations. Bacterial strains: (a) *Salmonella* spp., (b) *Klebsiella pneumoniae*, (c) *Shigella flexneri*, (d) Shigatoxigenic *Escherichia coli*, (e) Enteropathogenic *Escherichia coli,* and (f) *Escherichia coli* ATCC 25922. Compounds: CBCHP: Chobet® bismuth cream with pectin, CBHG: Colloidal bismuth hydroxide gel, E: Excipients. Concentration of bismuth (grayscale): Without bismuth (■); 0.06 mg/ml (■); 0.12 mg/ml (■); 0.3 mg/ml (■) and 0.6 mg/ml (■). *($P < 0.05$), **($P < 0.01$)

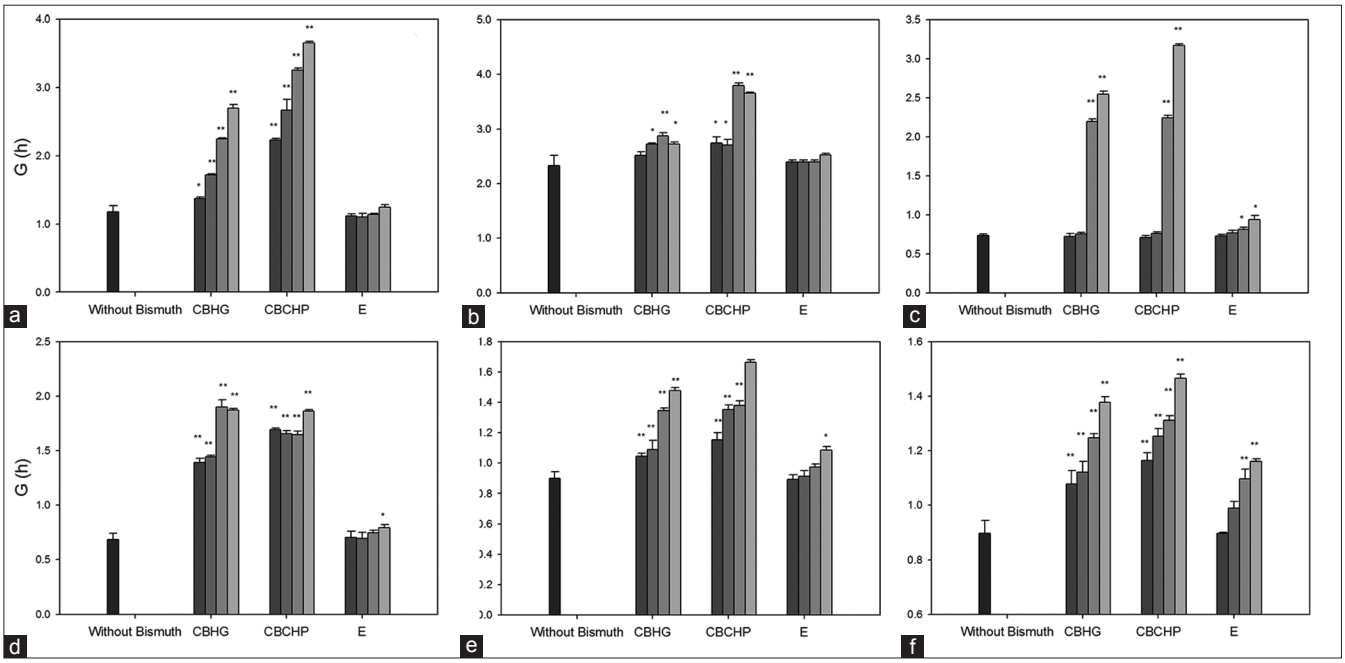

Figure 2: Generation time (G) as a function of treatment with the compounds at different concentrations. Bacterial strains: (a) Salmonella spp., (b) *Klebsiella pneumoniae*, (c) *Shigella flexneri*, (d) Shigatoxigenic *Escherichia coli*, (e) enteropathogenic *Escherichia coli* and (f) *Escherichia coli* ATCC 25922. Compounds: CBCHP: Chobet® bismuth cream with pectin, CBHG: Colloidal bismuth hydroxide gel, E: Excipients. Concentration of bismuth (grayscale): Without bismuth (■); 0.06 mg/ml (■); 0.12 mg/ml (■); 0.3 mg/ml (■) and 0.6 mg/ml (■). *($P < 0.05$), **($P < 0.01$)

very promising results in the prognosis of hemolytic uremic syndrome.[18] As a result of this work, we base our study on the analysis of the effect of CBHG, within the usual dosage, on Gram-negative enterobacteria strains frequently involved in gastrointestinal infections in our population.

In the first place, we evaluated the sensitivity of the bacteria to CBCHP, to its active principle CBHG and their excipients. The MIC is the most commonly used indicator in selecting an antimicrobial treatment. In all cases tested, the values of MIC were from 6 to 10 mg/ml of bismuth concentration. It

Table 2: Percentage of bacterial attachment to Chobet® bismuth cream with pectin, colloidal bismuth hydroxide gel and excipients

Bacterial strains	Colony forming units attached (%)								
	CBCHP			CBHG			Excipients		
	Incubation time			Incubation time			Incubation time		
	6 h	24 h	168 h	6 h	24 h	168 h	6 h	24 h	168 h
Salmonella spp.	98.5	98.7	97.1	98.4	96.2	96.3	38.9	63.6	61.5
K. pneumoniae	81.3	96.3	94.8	89.9	92.7	98.0	49.0	57.3	62.0
S. flexneri	89.9	92.6	88.0	96.2	97.3	96.5	46.5	49.1	65.7
STEC	85.4	90.6	91.4	89.6	94.1	97.7	22.9	41.1	41.4
EPEC	77.1	82.0	81.3	98.3	97.6	96.8	24.3	36.3	65.1
E. coli ATCC 25922	45.4	55.1	54.9	45.0	45.3	50.1	45.0	53.0	54.3

CBCHP: Chobet® bismuth cream with pectin, CBHG: Colloidal bismuth hydroxide gel, *K. pneumoniae*: *Klebsiella pneumoniae*, *S. flexneri*: *Shigella flexneri*, STEC: Shigatoxigenic *E. coli*, EPEC: Enteropathogenic *E. coli*, *E. coli*: *Escherichia coli*

was observed that *K. pneumoniae* was the strain that showed a slightly greater resistance, and *S. flexneri* slightly less than the rest of the studied strains, a similar result to that presented in other studies.[34] The standard concentration of bismuth in CBHG and CBCHP is 30 mg/ml, 3–5 times higher than the MICs detected. Hence, the strains were sensitive to CBHG treatment in concentrations lower than those normally used in treatment and in concentrations that it reaches *in vivo* in the intestine (10.8 mg/ml of bismuth).

The inhibitory scope of CBCHP and CHBG observed was high enough since it significantly inhibited bacterial growth in all strains at very low, subinhibitory concentrations of bismuth. At a concentration of 0.3 mg/ml of bismuth, all strains showed a reduction in growth velocity, while bismuth subsalicylate alone does not have a significant inhibitory effect on growth.[34]

In addition, other authors found comparable results regarding the viability of cells of *Helicobacter pylori*[35] and *E. coli*.[36] This bactericide action of the compound could be due to the loss of function of the cell membrane followed by the inhibition of ATP.[32]

The values MIC, MBC, and bacterial growth obtained showed a greater sensitivity to CBCHP, and no inhibitory effect was observed in the presence of only the excipients (E), which demonstrated that this effect was due exclusively to the active principle CBHG and that there was a cooperative effect between CBHG and the excipients that promote this effect.

Moreover, CBHG and CBCHP have demonstrated a great capacity for binding and displacement of the studied strains in solution. Both compounds demonstrated similar capacity of binding bacterial cells and was significantly greater than the capacity of the excipients. This demonstrated that this capacity for displacement was due mainly to the colloidal form of bismuth in the active ingredient CBHG.

The reference strain *E. coli* ATCC 25922, which lacks pathogenic factors, showed a significantly decreased capacity for displacement. Hence, we could infer that the presence of superficial structures, as pathogenic surface factors present in pathogenic cells, promote the attachment and displacement by colloidal bismuth.

The virulence of the bacterial strains was directly related with their capacity to be mobilized and attach to the epithelial gastrointestinal walls.[37-39] The mechanical attachment to the compounds used probably obstruct the bacterial attachment to the gastrointestinal epithelium, thus preventing colonization and favoring the rapid expulsion of the pathogens from the area.

Conclusions

The aim of this study was to find potential therapeutic strategies for the treatment of bacterial gastroenteritis by studying the effect of CBHG in clinical and subclinical concentrations on the viability and on the removal of the most frequent enteropathogenic strains in our population.

We believe that the results obtained with CBHG are very promising from the treatment standpoint. In this respect, its wide antimicrobial spectrum on the most frequent gastrointestinal pathogens and its capacity for bacterial displacement establish it as a treatment option in cases of diagnosis or suspicion of bacterial gastroenteritis.

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Conflicts of interest

There are no conflicts of interest.

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