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Acidity and intestinal bacteria: an in-vitro assessment of the bactericidal activity of hydrochloric acid on intestinal pathogens

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Summary

The bactericidal activity of hydrochloric acid (HCl) on different intestinal bacteria was evaluated at various pH values and time intervals. The effect of HCl was highest at pH 2 and pH 3 on strains of *Campylobacter jejuni*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Yersinia enterocolitica*, *Salmonella* spp. and *Shigella* spp., producing complete killing after 60 min incubation. However, at higher pH values (pH 4 and above), these organisms not only survived but they also showed evidence of rapid multiplication. In contrast, clinical and standard strains of *Escherichia coli* and *Staphylococcus aureus* were resistant to all the pH values. Highest sensitivity to HCl was observed with strains of *Shigella* spp. and *Salmonella* spp. It is conceivable that patients with normal gastric acidity should not suffer from gastroenteritis caused by these intestinal pathogens.

Résumé

Les activités bactéricidales de l'acide hydrochlorique (HCl) sur les bactéries intestinales étaient évalués au différent pH et temps. L'effet de HCl était plus élevé à pH 2 et pH 3 sur les souches de *Campylobacter jejuni*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Yersinia enterocolitica* et les espèces de *Salmonella* et *Shigella*, tuant complètement après 60 min d'incubation. Cependant, aux pH plus élevés (pH 4 et plus), ces organismes n'ont pas seulement survivé, mais ils ont aussi montré une évidence de multiplication rapide. En contraste, les souches standardes et cliniques d'*Escherichia coli* et *Staphylococcus aureus* étaient résistantes à tous les valeurs de pH. La plus grande sensitivité à HCl était observée avec les souches et espéces de *Shigella* et *Salmonella*. C'est concevable que les patients avec une acidité gastrique normale ne souffriraient pas de gastroentérite causé par ces pathogènes intestinales.

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Introduction

Intestinal infections due to bacteria remain a major problem in both developing and developed countries. According to the WHO, there is a high incidence of gastroenteritis amongst infants, particularly in the developing countries. Among the bacteria incriminated in diarrhoea are *Escherichia coli*, *Vibrio cholerae*, *Shigella* spp. and *Salmonella* spp. Recently, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Aeromonas hydrophila* have been reported as prominent agents of gastrointestinal disorders [1–3].

So far in all diarrhoeal cases associated with these organisms, there is evidence that patients with reduced acid secretion suffered more than those with normal acid level [4–6], particularly amongst patients who have undergone gastric surgery, suffered from hormonal dysfunction, or are taking ulcer healing drugs like cimetidine or antacid [7].

The fact that subjects with normal acid secretion continue to suffer from diarrhoea has made some investigators question the role of *hydrochloric acid* (HCl) as a bactericidal factor in gastric juice. Factors such as mucosal tissue integrity, intestinal motility, gastric emptying,

consistency of ingested food, type of food, i.e. carbohydrate, protein or fat, or nature of food, i.e. solid or liquid, also appear to determine the fate of a bacterium in the stomach [8–10]. The contact time between a bacterium and HCl is important in determining the bactericidal effect of HCl [11]. None of these studies actually determined the contact time for each bacterium. This study was carried out to determine the contact time for the bactericidal action of HCl on different bacterial pathogens at different pH values and the general effect of HCl on these pathogens.

Materials and methods

Organisms

The following bacterial strains were used in this study: enteroinvasive *E. coli* (EIEC); enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); a standard *E. coli* strain (ATCC 25922/F50); *Shigella flexneri; S. boydii; S. dysenteriae*; and *S. sonnei*. Others were *A. hydrophila* (strain 65), *P. shigelloides* (strain 2339), *Y. enterocolitica* (strain Y03), *C. jejuni* (strain 99), *Staphylococcus aureus* (strain ATCC 25923/F9), *Salmonella typhi* and *Salmonella* spp (untyped strain), all kindly supplied by Professor Tolu Odugbemi of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos.

Media

The following media were used throughout the experiments: MacConkey agar (Oxoid: Basingstoke, U.K.), Butzler's type medium [12], blood agar (blood agar base, Oxoid; and 7% human blood) and Mueller–Hinton broth (Oxoid).

Preparation of acid broth

Mueller–Hinton broths (Oxoid), adjusted to pH 2, 3, 4, 5 and 6 with 0.2 M HCl, were prepared and labelled in universal bottles; the pH values were ascertained using a pH meter (model 7; Corning Medical, Sudbury, U.K.). The acid broth was then dispensed in 9-ml amounts into appropriately labelled half-ounce bottles and sterilized at 15 lb/in^2 and 121°C for 15 min. Mueller–Hinton broth, pH 7.3, was included as a control.

Standardization of bacterial inoculum

Overnight broth culture of test strain was diluted in 0.25-strength Ringers solution to give approximately 10^8 c.f.u. (colony-forming units)/ml by adjustment to no. 2 MacFarland opacity standard. This was the standard inoculum used to inoculate each acid and control broth in the experiments.

Inhibitory test of the acid broth at different pH

One millilitre of the standardized bacterial suspension was inoculated into 9 ml of Mueller–Hinton broth at pH values of 2, 3, 4, 5, 6 and 7.3 (control) contained in universal bottles which were labelled 0, 10, 20, 30, 40, 50, 60, and 70 min for each pH value. These were then incubated as appropriate at 37°C. At the end of each incubation period viable counts were performed for each test organism by the modified method of Miles and Misra [13]. The viable count was expressed as c.f.u./ml. The inhibitory action of HCl in broth at different pH values was demonstrated graphically by plotting $\log_{10}$ viable count against incubation time.

Results

Effect of acidity on E. coli

As demonstrated in Fig. 1a, there was no appreciable inhibitory effect of acid pH (pH 2–5) on the *E. coli* strain (ATCC 25922/F50) tested from 0 to 70 min. After an initial slight decrease in bacterial count in the first 30 min, particularly in pH 2 and 3, the organism then multiplied. Similar effects of the various pH values were observed with the EPEC, ETEC and EIEC strains as shown in Fig. 1b–d.

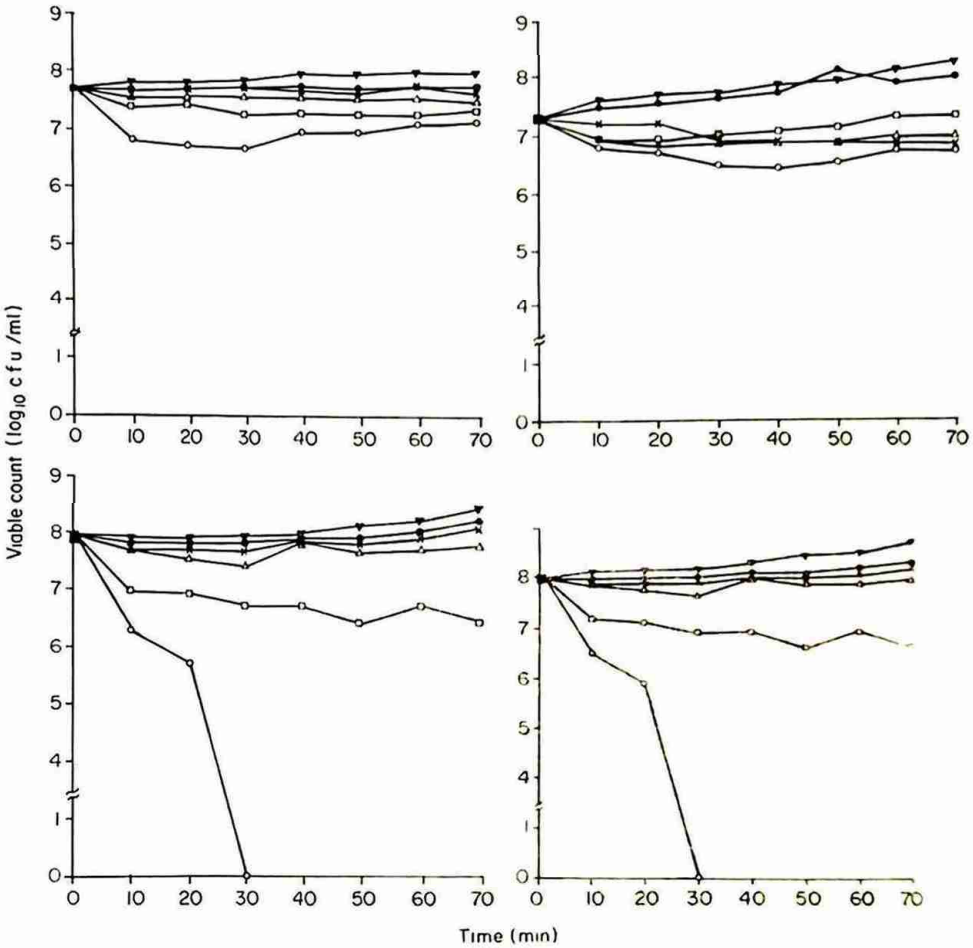

Fig. 1. The effect of HCl on the viable count of (a) *E. coli*, (b) enteropathogenic *E. coli*, (c) enteroinvasive *E. coli* and (d) enterotoxigenic *E. coli* at different pH values: (○) pH 2, (□) pH 3, (△) pH 4, (×) pH 5, (●) pH 6, (▼) pH 7.3.

However, EIEC and ETEC were found to be susceptible at pH 2 only after 20 min exposure (see Fig. 1c and d).

Effect of acidity on Shigella *spp.*

The typical effect of HCl on the viable count of the *Shigella* spp. is demonstrated by its effect on *S. dysenteriae* as shown in Fig. 2. The four species of *Shigella*, i.e. *S. sonnei*, *S. boydii*, *S. flexneri* and *S. dysenteriae*, were readily killed at all the pH values tested by the end of 50 min incubation. There was, however, slight variation in the time of killing. For instance, at pH 2 and 3 no *Shigella* species survived after 20 min. At pH 4 it took longer contact time (40 min) to kill the *Shigella* spp.

Effect of acidity on C. jejuni

Figure 3 demonstrates the effect of HCl on *C. jejuni*. By far the most susceptible of all the pathogens with regard to contact time before killing at very low pH was *C. jejuni*, which was completely inhibited at pH 2 and 3 within 10 min of incubation. However, at pH 4 there was only a slight reduction in the viable count from 2.4×10^7 to 5.0×10^6 c.f.u./ml at 30 min contact time. Thereafter, a steady increase in count for another 40 min was noted. Above pH

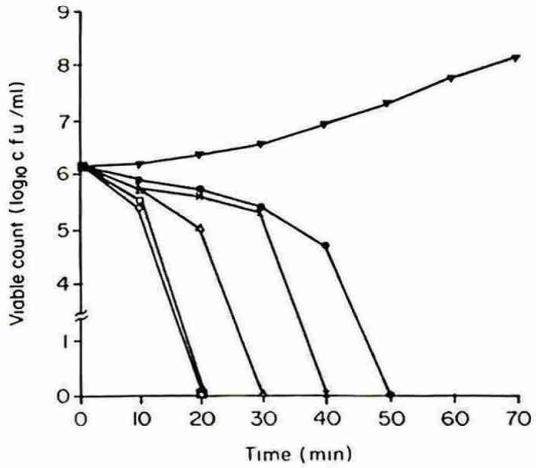

Fig. 2. The effect of HCl on the viable count of *Shigella dysenteriae* at different pH values: (○) pH 2, (□) pH 3, (△) pH 4, (×) pH 5, (●) pH 6, (▼) pH 7.3.

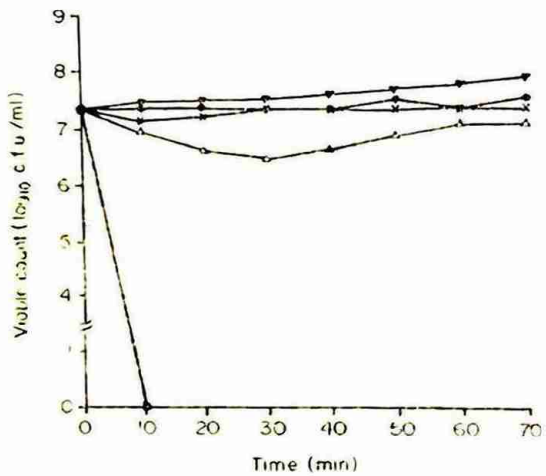

Fig. 3. The effect of HCl on the viable count of *Campylobacter jejuni* at different pH values: (○) pH 2, (□) pH 3, (△) pH 4, (×) pH 5, (●) pH 6, (▼) pH 7.3.

4, no appreciable inhibitory effect of pH on the organism was observed.

Effect of acidity on other pathogens

Staph. aureus (strain 25923/F9) behaved similarly to the wild strain of *E. coli*, surviving at all the values of pH tested (Fig. 4), although there

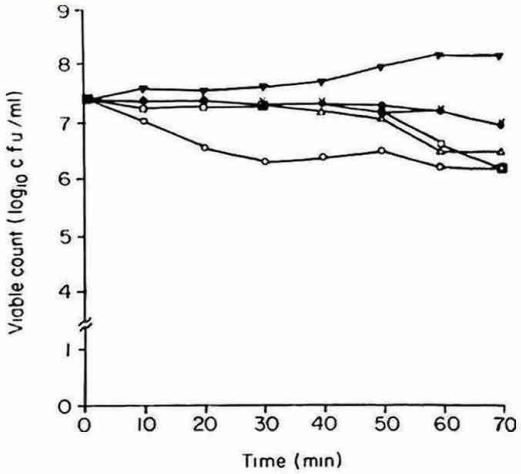

Fig. 4. The effect of HCl on the viable count of *Staph. aureus* at different pH values: (○) pH 2, (□) pH 3, (△) pH 4, (×) pH 5, (●) pH 6, (▼) pH 7.3.

was a reduction in the viable count from 3.1×10^7 to 1.1×10^6 c.f.u./ml after 30 min contact time with an acid pH (pH 2). The organism divided rapidly thereafter, attaining a count of 7.6×10^6 c.f.u./ml at the end of the experimental time, i.e. 70 min.

Y. enterocolitica (data not shown in the tables) was killed at pH 2 in 50 min. At higher pH values, particularly pH 3 and 4, there was a decreased count of 1×10^5 c.f.u./ml at 70 min incubation but it was never completely killed.

Both *A. hydrophila* and *P. shigelloides* were killed at pH 2 and 3 after 40 min, at pH 4 after 50 min, and at pH 5 and 6 after 60 and 70 min, respectively.

Salmonella spp. and *S. typhi* behaved alike in the very acid conditions. They were both killed at pH 2 after 20 min and pH 3 at 30 min contact time. At pH 4 and above, *S. typhi* was generally resistant, as shown in Fig. 5. However, at these pHs the *Salmonella* spp. showed marked reduction in count but it was not completely eliminated after 70 min.

Discussion

In an in-vitro study by Giannella *et al.* [14], it was reported that the bactericidal barrier of gastric juice was primarily pH dependent. They also confirmed that HCl is responsible for the acidity in the gastric juice of the stomach. Since

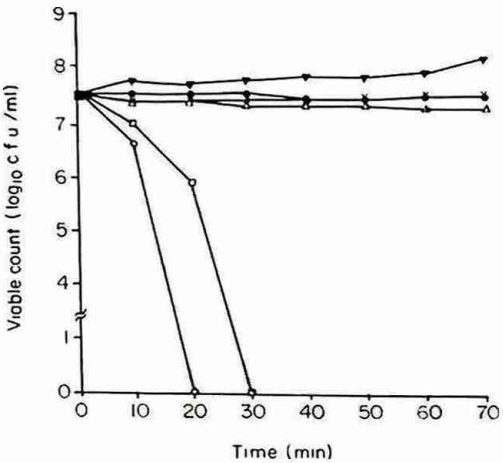

Fig. 5. The effect of HCl on the viable count of *Salmonella typhi* at different pH values: (○) pH 2, (□) pH 3, (△) pH 4, (×) pH 5, (●) pH 6, (▼) pH 7.3.

then there has been no investigation on the direct effect of HCl on a variety of intestinal pathogens like the ones investigated in this report.

In this study, two observations came out quite clearly. First, the activity of the HCl is pH dependent, and secondly, it is also dependent on the genus of the micro-organism being tested. For instance, most of the bacteria that were sensitive to the HCl died at low pH values, especially pH 2. Furthermore, while the four species of *Shigella* were very sensitive to the HCl at all the pH values tested (pH 2–6), the four different strains of *E. coli* tested were uniformly relatively resistant to the action of HCl at all the pH values used.

It is remarkable that the *Shigella* spp. showed high susceptibility to the low pHs attainable in gastric juice and yet they remain a common cause of diarrhoeal diseases in adults and children. This apparent paradox may be explained by the fact that only 10 shigellae bacteria are required to initiate the disease process [15]. So the few which survive in the gastric acidity of the stomach can cause infection upon reaching the small intestine where the pH is favourable for growth.

The relative resistance of *A. hydrophila*, *C. jejuni*, *Salmonella* spp. and *P. shigelloides* to HCl as the pH of the medium increased towards neutral pH, explains in part the higher susceptibility of patients with achlorhydric stomach to bacterial infections than those with normal acid stomach. This assertion has been confirmed by earlier reports [4,5] which claimed that patients who have gastric resection with subsequent increase in gastric pH were more susceptible to *Salmonella* enteritis. The general observation in the present study was that at low pH (pH 2 and 3) fewer bacteria survived after 60 min of contact. This would appear to be in agreement with the report of Knott [11] who referred to the stomach as a germicidal barrier under conditions of prolonged high acidity.

There was an interesting observation with *C. jejuni*, strain 99, at pH 4 in this study. Initially the viable count of *C. jejuni* at pH 4 dropped for the first 30 min; thereafter an increase in the viable count at 70 min was observed. The explanation for this is not very clear. It is possible that the organism became selectively resistant to acidity, although this suggestion is highly speculative at this time. It is conceivable that this acid resistance may be an important factor in the pathogenesis of *C. jejuni*. It may also be the case with EPEC, EIEC, ETEC, *Staph. aureus* and *Y. enterocolitica*.

In a study, which to date is the most cited reference in the literature concerning gastric acidity and bacteria, Giannella *et al.* [14] reported that 99.9% of *S. paratyphi*, *S. enteritidis*, *S. typhimurium*, *Serratia marcescens* and *E. coli* were killed in 30 min below pH 4. The result obtained in the present study with *Salmonella* spp., *A. hydrophila*, *C. jejuni*, *Shigella* spp. and *P. shigelloides*, is in agreement with this observation. However, we found the *E. coli* strains (EPEC, EIEC, ETEC and standard strains) to be relatively more resistant to acidity at the same pH values.

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