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RAPID COMMUNICATION

# *Helicobacter pylori* isolated from gastric juice have higher pathogenic potential than biopsy isolates

Persistent gastritis induced by *Helicobacter pylori* (*H. pylori*) is the strongest known risk factor for gastric cancer (GC). *H. pylori* is prevalent in about 50% world's population, while it causes cancer in less than 2% of exposed individuals.[1] Our studies found that *H. pylori* infection can induce oncogenic properties in AGS cells by deregulating multiple factors associated with the cell cycle, apoptosis, and other important events associated with cancer progression.[2] Another study, in which we isolated *H. pylori* from the gastric biopsy and juice sample of suspected gastritis patients, found differential growth patterns of these bacteria.[3] Previous studies have reported the difference between the microbiota in gastric juice and mucosal lining (biopsy). However, none of the reports analyzed the variation between the *H. pylori* residing in biopsy and juice samples and their morphological, physiological, and pathogenic differences. In this study, we characterized the *H. pylori* isolates from the two different physiological locations (gastric epithelium and gastric juice) of the same subjects. We assessed the morphological features of the biopsy and juice isolates. The expression profile of GC marker genes, inflammatory genes, metalloproteinases, and the expression of regulatory genes NFκB and β-catenin at 6, 12, and 24 h post-*H. pylori* infection was also evaluated along with oncogenic property resistance to anoikis.

In our previous study, we showed that biopsy isolates (HB1, HB10, and HB14) grew faster as compared to the juice isolates (HJ1, HJ10, and HJ14) of the same subjects.[3] The morphological difference between all the isolates determined by scanning electron micrograph (SEM) showed juice isolates were longer ($P < 0.05$) and thinner ($P < 0.05$) than the biopsy isolates (Fig. 1AI, II). The size of *H. pylori* isolates measures about 1.4–2.5 μm * 0.34–0.62 μm (Fig. 1AI, II). The size is similar to the size of previously reported *H. pylori* (2–4 μm in length and 0.5–1 μm in width). This is the first study in which *H. pylori* isolated from two different niches of the same subjects has been characterized for its morphological and pathogenic characteristics.

Further to determine the host interaction of these isolates, we observed the expression pattern of *H. pylori*-associated pathogenic genes (*16s-rRNA*, *CagA*, and *BabA*), host inflammatory genes (*CCL11*, *IL13*, *IL5*, *IFN-γ*, and *CCL8*), cell-cycle regulatory genes (*CCND1*, *ABL1*, *TFF2*, *DAPK3*, *APC*, *AKT* ), and cell migratory genes (*MMP3*, *MMP7*, and *MMP9*); GC marker (*GASTRIN* ); and cancer-initiating transcription factors (*CDX2* and *RUNX3*) at 6, 12, and 24 h post-infection (hpi) (Fig. S1). We determined the multiple-fold increased expression of *16S-rRNA* with all the isolates at all the time points which shows successful *H. pylori* infection (Fig. S1A). However, the expression of pathogenic genes *CagA* and *BabA* was up-regulated with the studied isolates at different time points (Fig. S1A). This variable pattern of expression in these isolates needs further detailed investigation.

Inflammatory markers associated with GC progression were decreased at the early time of infection except for *IFN-γ* at 6 hpi and *IL8* at 12 hpi (Fig. S1B). Interestingly, inflammatory genes were significantly up-regulated with HJ1 and HJ14 at 24 hpi, which shows higher inflammation potential of the juice isolates (Fig. S1B). *IFN-γ*, an important Th1 cytokine, was significantly up-regulated in the juice isolate (HJ1, HJ10, and HJ14) at 24 hpi.

Besides the inflammatory genes, cell cycle regulators, cancer-initiating transcription factors, and metastasis-associated genes play important roles in the progression of GC. All these genes showed down-regulation at 6 hpi except *AKT* (Fig. S1C). At 12 hpi, these genes were heterogeneously expressed (Fig. S1C). At 24 hpi, we recorded the increased expression of *CCND1*, *ABL1*, *TFF2*, *DAPK3*, *AKT*,

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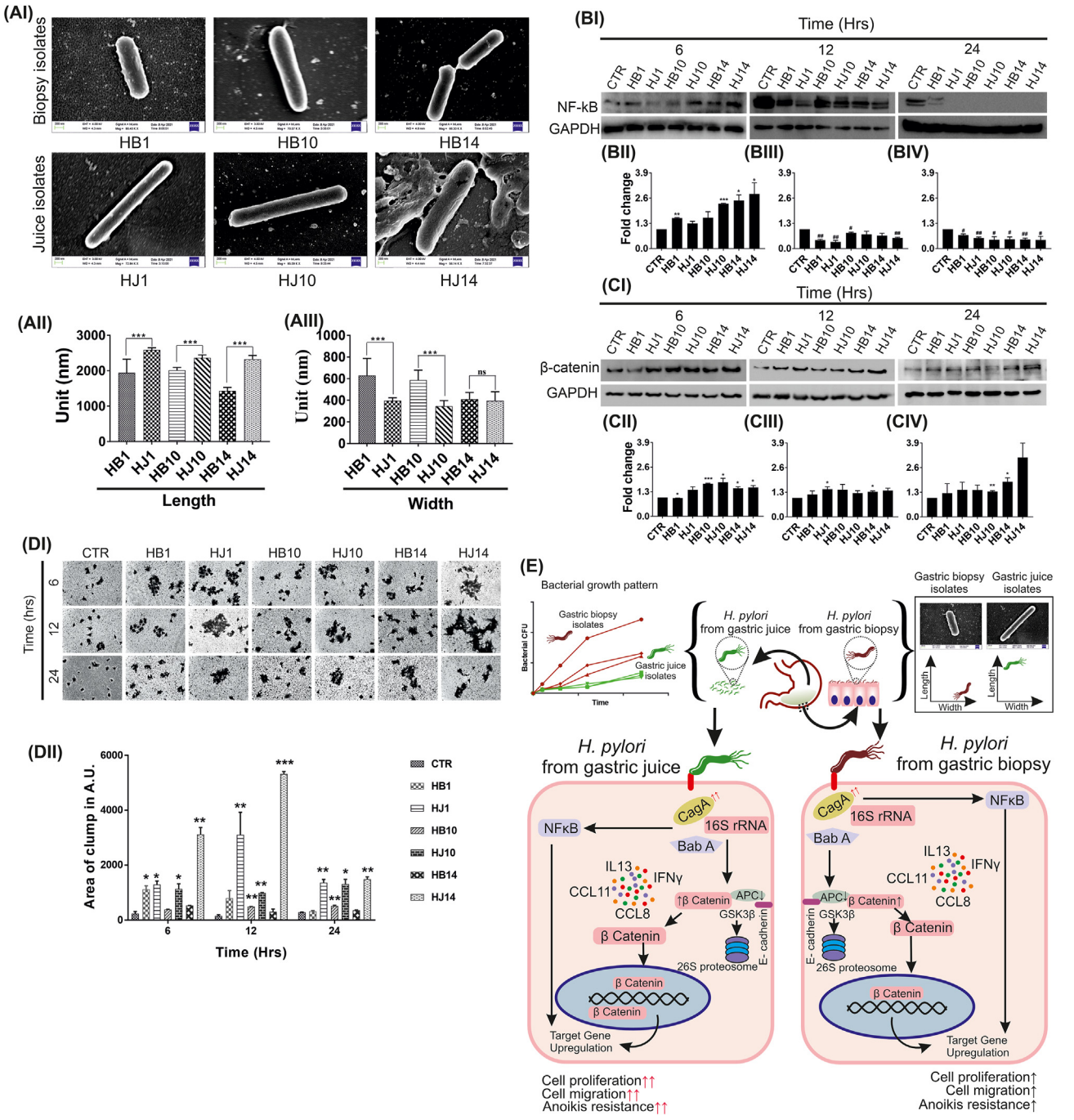


**Figure 1** Gastric juice-derived *H. pylori* possess a higher carcinogenic ability. **(A)** The SEM image of the juice (HJ1, HJ10, and HJ14) and biopsy isolates (HB1, HB10, and HB14) was acquired at 4 kV and with a working distance of 4.4 mm. (AI) The representative SEM image of the isolates used in the study. Scale bar = 200 nM. (AII) The graph denoting the length of the isolates in nm. (AIII) The graph showing the width of the isolates in nm. **(B, C)** The expression level of transcription factors NF$\kappa$B (65 kDa), β-catenin (85 kDa), and GAPDH (36 kDa) was determined by Western blot. The relative expression level of the molecules was determined using Image J software and represented as a histogram. (BI) Western blot image of NF$\kappa$B 6, 12, and 24 h post-infection. Relative expression of NF$\kappa$B 6 h (BII), 12 h (BIII), and 24 h (BIV) post-infection. (CI) Western blot image of β-catenin 6, 12, and 24 h post-infection. Relative expression of β-catenin 6 h (CII), 12 h (CIII), and 24 h (CIV) post-infection. *X*-axis: samples infected with specific *H. pylori* isolates; *Y*-axis: fold change with respect to control. The experiment has been performed in triplicates, and the results are shown as the mean ± standard deviation. Unpaired *t*-tests were applied to determine the statistical significance. $P < 0.05$ was considered significant in all the cases. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.0001$ for significant up-regulation; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.0001$ for significant down-regulation. **(D)** Anoikis assay was performed to assess the ability of AGS cells for anchorage-independent growth. (DI) Representative image of AGS cell clumps (representing resistance to anoikis) 6, 12, and 24 h post-*H. pylori* infection, followed by 48 h anoikis induction and staining with 0.5% crystal violet. Images were acquired by inverted light

*GASTRIN*, and *CDX2* and reduced expression of *APC* and *runx3* (except HB1) in all the infected samples (Fig. S1C). Here, the differential expression of cell cycle regulatory genes such as *CCND1*, *ABL1*, *TFF2*, *DAPK3*, *APC*, and *AKT* reflects progression in oncogenesis.

Studies have suggested the activation of NFκB pathway in *H. pylori* mediated GC progression and further activation of multiple signaling pathways like β-catenin.[4] We determined the increased protein expression of NFκB with all the isolates at 6 hpi through immunoblotting (Fig. 1BI, II) and immunofluorescence study (Fig. S2A, B). Surprisingly, the expression of NFκB was diminishing at 12 hpi and 24 hpi (Fig. 1BI, III, IV). We noticed the significantly decreased expression of NFκB with immunofluorescence at 24 hpi similar to the Western blot (Fig. S2E, F).

In addition to NFκB, the expression of β-catenin was also determined by Western blot. β-Catenin was up-regulated in all the isolates at 6 hpi (Fig. 1CI, II). However, a heterogeneous expression pattern was determined at 12 hpi (Fig. 1CI, III) and 24 hpi (Fig. 1CI, IV). Importantly, immunofluorescence of β-catenin showed its elevated expression and nuclear localization in AGS cells infected with all the selected *H. pylori* isolates at 6 hpi (Fig. S3A, B). Additionally, after 12 hpi (Fig. S3C, D) and 24 hpi (Fig. S3E, F), we observed increased expression of β-catenin in all the infections. At 24 hpi, the nuclear localization of β-catenin is significantly higher ($P > 0.01$) in juice isolate HJ1 and HJ14 infected cells. The levels of these regulatory molecules indicate an interplay in which the high levels of NFκB in the early time point might be inducing β-catenin which further regulates the aggressiveness and metastatic properties of GC in the later part. A recent study also demonstrated that *H. pylori* infection activates NFκB which promotes CDK1 expression and activates β-catenin. Previous studies by Deng et al have demonstrated that in colorectal cancer and breast cancer β-catenin help in tumor progression by repressing the expression of NFκB.[5] Also, in this study, the nuclear localization of β-catenin was higher in juice isolates (HJ1 and HJ14) infected gastric epithelial cells at a later time point. Anoikis resistance is a hallmark of cancer. We demonstrated that *H. pylori*-infected AGS cells make larger clumps compared to the uninfected control at 6, 12, and 24 hpi (Fig. 1DI, II). Meanwhile, the clumps in HJ1 and HJ14 infected samples are significantly larger ($P < 0.05$) than the uninfected cells (Fig. 1DI, II). Moreover, the juice isolates (HJ1 and HJ14) showed higher inflammation potential, carcinogenic ability, and anoikis resistance (Fig. 1E). Besides, another juice isolate HJ10 infected sample shows a differential pattern of regulation. It is intriguing to mention that the same isolate HJ10 showed a different pattern of antibiotic resistance compared to others (data not shown). This indicates genetic variability more than the other isolates and provides a basis for its differential regulation pattern.

The study is one of the earliest to characterize *H. pylori* based on their physiological site of isolation from gastric epithelium or gastric juice. Here we showed differences in size, growth pattern, and pathogenic ability of *H. pylori* isolated from biopsy and gastric juice of the same subjects. Overall, the results showed that the studied juice isolates possess higher carcinogenic potential than the biopsy isolates (Fig. 1E). Additionally, the host—pathogen interaction and underlying molecular mechanism can decipher the specific role of epithelial and juice *H. pylori* in GC occurrence and progression. This study will help to open a new horizon of *H. pylori*-mediated gastric cancer research.

## Author contributions

Conceptualization: Hem Chandra Jha, Budhadev Baral, and Dharmendra Kashyap; Methodology: Budhadev Baral, Dharmendra Kashyap, Ajay Kumar Jain, and Debi Chatterji; Formal analysis and investigation: Budhadev Baral, Dharmendra Kashyap, and Nidhi Varshney; SEM analysis and optimization: Budhadev Baral and Vinod Kumar; Writing - original draft preparation: Budhadev Baral; Writing - review and editing: Budhadev Baral, Dharmendra Kashyap, Amit Mishra, Awanish Kumar, and Hem Chandra Jha; Writing — preparation of figures: Tarun Prakash Verma and Budhadev Baral; Funding acquisition: Hem Chandra Jha; Resources: Hem Chandra Jha and Ajay Kumar Jain; Supervision: Hem Chandra Jha.

## Conflict of interests

The authors have no conflict of interests to disclose, relevant to this article's content.

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microscopy (Leica Ltd.) using a 20× objective lens. (DII) Graphical representation of the clump area (arbitrary unit). The area of the clump was measured using Image J software from the acquired images. 50 clumps were measured for every sample. The experiment was performed in triplicates, and the results are shown as the mean ± standard deviation. Unpaired *t*-tests were applied to determine the statistical significance. $P < 0.05$ was considered significant in all the cases. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.0001$. **(E)** A model illustrating the difference in growth, morphology, and pathogenic ability of *H. pylori* isolates from gastric biopsy and juice. Although both juice and biopsy isolates accelerate the occurrence of cancerous properties, the juice isolates possess a higher carcinogenic ability. There is increased β catenin expression and nuclear localization at the later time points which may reduce the expression of NFκB, which is up-regulated at the earlier time points. The inflammatory genes also get highly up-regulated in juice isolate infected samples. Our study showed the difference in the juice and biopsy *H. pylori* isolates from the same subjects. This is a preliminary report and further study is warranted in this aspect.

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## Data availability

All the data are present in the manuscript or the supplementary file. More supporting data can be found in the data article "Data on differential pathogenic ability of *Helicobacter pylori* isolated from distinct gastric niches" (https://doi.org/10.1016/j.dib.2023.108981).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.03.003.

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