Changing Characteristics of Rotavirus Diarrhea in Children Younger than Five Years in Urban Bangladesh

Mohammad Habibur Rahman Sarker1, Sumon Kumar Das1,2*, Shahnawaz Ahmed1, Farzana Ferdous1, Jui Das1, Fahmida Dil Farzana1, Abu S. M. S. B. Shahid1, K. M. Shahunja1, Mokibul Hassan Afrad1, Mohammad Abdul Malek1, Mohammad Jobayer Chisti1, Pradip Kumar Bardhan1, Md Iqbal Hossain1, Abdullah Al Mamun2, Abu S. G. Faruque1

1 International Centre for Diarrhoeal Disease Research (icddr,b), Dhaka, Bangladesh, 2 School of Population Health, The University of Queensland, Brisbane, Australia

Abstract

Background: Childhood rotavirus diarrhea is still one of the major public health challenges. The present study aimed to determine changing characteristics of rotavirus diarrhea in under-5 children at two periods of time.

Methods: We enrolled 5,357 under-5 children with rotavirus positive in two different time periods; i) 1993-1997 (n = 2,493), and ii) 2008–2012 (n = 2,864) considering beginning and ending of two decades. These children were enrolled in the urban Dhaka Hospital of icddr,b.

Results: Overall, proportion of rotavirus was about 25% in 1993–97, which was 42% in 2008–12 (68% rise; p < 0.001). Significant higher proportion of children were stunted [38% vs. 22%; aOR-1.33 (95% CI-1.09-1.62)], had vomiting [87% vs. 74%; aOR-2.58 (95% CI-2.02-3.28)], fever [10% vs. 8%; aOR-1.31 (95% CI-0.96-1.78)], family members > 5 [38% vs. 35%; aOR-1.32 (95% CI-1.10-1.58)], required more intravenous fluid [9% vs. 3%; aOR-4.93 (95% CI-3.19-7.63)], had higher co-infection with Shigella [3% vs. 1%; aOR-3.36 (95% CI-1.61-7.03)], Vibrio cholerae [4% vs. 1%; aOR-3.70 (95% CI-2.12-6.46)] and ETEC [13% vs. 7%; aOR-2.21 (95% CI-1.65-2.97)]; however, significantly lower proportion of them used sanitary toilets [54% vs. 78%; aOR-0.66 (95% CI-0.54-0.80)], boiled drinking water [16% vs. 38%; aOR-0.60 (95% CI-0.48-0.74)], used antimicrobial at home [63% vs. 82%; aOR-0.56 (95% CI-0.46-0.69)] and had some or severe dehydration [18% vs. 34%; aOR-0.15 (95% CI-0.12-0.20)] in 1st observation period compared to that of 2nd.

Conclusion: Proportion of episodes of under-5 rotavirus diarrhea increased over the period. Concomitant changes in host, socio-demographic and clinical characteristics, and co-infections were also observed. Thus, vaccination campaign which is prevailing in private sector should also be introduced in public sector.

Introduction

Childhood diarrhea is still contributing as the second leading cause of under-5 mortality and morbidity [1]. Rotavirus is one of the major pathogens responsible for yearly 111 million episodes of diarrhea and over 400,000 deaths among under-5 children globally [2]. It is also a leading cause of infantile gastroenteritis accounting 20% of diarrhea-associated deaths [3]. Literatures also documented rotavirus diarrhea attributing an estimated 39% of hospitalized under-5 childhood deaths and majority of them happening in resource poor settings of low and middle income countries [4,5].

Mass campaigns for home based management with oral rehydration salt solution (ORS), and use of zinc as adjunct therapy [6,7] in addition to vaccination against rotavirus not only reduce the diarrheal episodes but also decrease mortality, hospital stay and help improved case management [8–10]. Several host and socio-demographic factors have been thought to contribute high global prevalence of childhood rotavirus diarrhea such as personal and family hygiene practices as documented elsewhere [11,12]. However, changing trend of all these indicators have not been well documented in medical literatures. The Diarrheal Disease Surveillance System (DDSS) of International Centre for
Diarrhoeal Diseases Research, Bangladesh (icddr,b) systemically enrolls individuals with diarrhea irrespective of age, sex, socioeconomic status and disease severity. For us, its data base paved the way to study changing characteristics of rotavirus diarrhea among under-5 urban children between two 5-year time periods of last two decades such as 1993-1997 and 2008-2012.
Materials and Methods

Study site

Dhaka Hospital of icddr,b; established in 1962 in Dhaka, the capital city of Bangladesh, provides care and treatment to people with diarrheal diseases mostly from urban and peri-urban areas. Over 140,000 diarrheal patients receive such services each year. A DDSS has been operating since 1979 [13,14], which systematically sampled 4% of all patients up to 1995, however currently samples 2% since 1996. DDSS collects information on the clinical, epidemiological and demographic characteristics, feeding practices, particularly of infants and young children irrespective of age, sex and disease severity or socioeconomic status by administering a structured questionnaire. A trained research assistant interviews either the patient himself or the caregiver in case of young children following the questionnaire (detail was described elsewhere [13,14]).

Study population

A total of 55,173 patients were enrolled into the DDSS from 1993–2012 who were admitted in Dhaka Hospital of icddr,b. Of them 28,948 (52%) were under-5 children. They were divided into two time periods (i) 1993–1997 (9,879), and (ii) 2008–2012 (6,575) to determine the changing characteristics of these young childhood population between the starting and ending of two different five-year periods of last two decades. A total of 5,357 under-5 children presented with rotavirus diarrhea; of them, 2,493 (25%) under-5 children during 1993–97, and 2,864 (43%) during 2008–2012 constituted analyzable study sample size (Figure 1).

Specimen collection and laboratory procedure

A fresh stool specimen is routinely collected from the surveillance patients for screening of common diarrheal pathogen such as rotavirus,

Shigella

spp.,

Vibrio cholerae

, Enterotoxigenic Escherichia coli (ETEC) following standard laboratory method [15,16].

Definition

Diarrhea was defined as passage of 3 or more abnormally loose or liquid stools per day, or more frequently than normal for the individual. Well nourished children were defined as individual with weight-for-age z-score (WAZ), height-for-age z-score (HAZ), and weight-for-height z-score (WHZ)$\leq 2.00$ SD. However, malnutrition was defined as children with any of the indicators of malnutrition WAZ, HAZ, WHZ$\leq 2.00$ SD.

Ethics

The DDSS of icddr,b has been approved by the Research Review Committee and the Ethical Review Committee of icddr,b which currently enrolls every 50th patient attending for the treatment to the Dhaka Hospital of icddr,b. At the time of enrollment, verbal consent was taken from the caregivers or guardians on behalf of the patients. The information was stored in

### Table 1. Changing socio-demographic, clinical and host characteristics, and co-infection among under-5 children with rotavirus diarrhea in two observation periods (1993–1997, and 2008–2012).

| Variables | 1993-1997; n = 2,493 (%) | 2008-2012; n = 2,864 (%) | OR (95% CI) | aOR (95% CI) |
|-----------|--------------------------|--------------------------|-------------|-------------|
| Male      | 1538 (62)                | 1813 (63)                | 0.93 (0.83–1.04) | -           |
| Maternal literacy | 1312 (53)                | 2387 (83)                | 0.22 (0.20–0.25)* | 0.31 (0.26–0.38)* |
| Use sanitary latrine | 1348 (54)                | 2228 (78)                | 0.34 (0.30–0.38)* | 0.66 (0.54–0.80)* |
| Family size (>5 members) | 954 (38)                | 1018 (35)                | 1.12 (1.00–1.26)* | 1.32 (1.10–1.58)* |
| Slum residence | 283 (11)                 | 107 (4)                  | 3.30 (2.61–4.18)* | 1.32 (0.92–1.89) |
| Boiled drinking water | 402 (16)                 | 1078 (38)                | 0.32 (0.28–0.36)* | 0.60 (0.48–0.74)* |
| Use of antimicrobial at home | 1558 (63)                | 2339 (82)                | 0.37 (0.33–0.42)* | 0.56 (0.46–0.69)* |
| History of measles within 6 months | 225 (9)                  | 73 (2)                   | 3.79 (2.87–5.01)* | 1.76 (1.12–2.78)* |
| Vomiting | 2164 (87)                | 2125 (74)                | 2.29 (1.98–2.65)* | 2.58 (2.02–3.28)* |
| Fever | 262 (10)                 | 233 (8)                  | 1.33 (1.10–1.60)* | 1.31 (0.96–1.78) |
| Abdominal pain | 491 (20)                 | 463 (16)                 | 1.27 (1.10–1.47)* | 2.06 (1.66–2.55)* |
| Duration of diarrhea (>24 hours) | 1883 (76)                | 2227 (78)                | 0.88 (0.78–1.00) | -           |
| Number of stool in last 24 hours (>10 times) | 1058 (42)                | 1525 (53)                | 0.65 (0.58–0.72)* | 0.67 (0.56–0.80)* |
| Watery stool | 2408 (97)                | 2796 (98)                | 0.69 (0.49–0.96)* | 1.24 (0.71–2.17) |
| Some or severe dehydration | 457 (18)                 | 985 (34)                 | 0.43 (0.38–0.49)* | 0.15 (0.12–0.20)* |
| Use of intravenous fluid | 216 (9)                  | 79 (3)                   | 3.33 (2.54–4.37)* | 4.93 (3.19–7.63)* |
| Hospital stay ≥24 hours | 1048 (43)                | 1196 (42)                | 1.01 (0.91–1.13) | -           |
| Underweight (WAZ<–2) | 1110 (45)                | 747 (26)                 | 2.26 (2.01–2.54)* | -           |
| Stunted (HAZ<–2) | 940 (38)                 | 626 (22)                 | 2.15 (1.90–2.43)* | 1.33 (1.09–1.62)* |
| Wasted (WHZ<–2) | 611 (23)                 | 530 (19)                 | 1.42 (1.24–1.62)* | -           |
| Vibrio cholerae | 94 (4)                   | 32 (1)                   | 3.47 (2.28–5.31)* | 3.70 (2.12–6.46)* |
| Shigella | 76 (3)                   | 21 (1)                   | 4.26 (2.56–7.14)* | 3.36 (1.61–7.03)* |
| ETEC | 112 (13)                 | 193 (7)                  | 2.06 (1.60–2.65)* | 2.21 (1.65–2.97)* |

*p<0.05; aOR-Adjusted Odds ratio; ETEC- Enterotoxigenic Escherichia coli.

doi:10.1371/journal.pone.0105978.t001
Data analysis

The hospital database and used for conducting researches. Although the DDSS of icddr,b is a scheduled activity on the hospital patients, and performed after taking verbal consent from the parents or guardians of the patients following the hospital policy. This verbal consent was documented by keeping a check mark in the questionnaire which was again shown to the patient or the parents. Parents or guardians were assured about the non-disclosure of information collected from them, and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of their children. ERC was satisfied with the voluntary participation, maintenance of the rights of the parents or guardians of the patients following the hospital policy. This verbal consent was documented by keeping a check mark in the questionnaire which was again shown to the patient or the parents. Parents or guardians were assured about the non-disclosure of information collected from them, and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of their children. ERC was satisfied with the voluntary participation, maintenance of the rights of the participants and confidential handling of personal information by the hospital physicians and has approved this consent procedure.

### Results

Overall isolation rate of rotavirus was 20% in 1993 which increased to more than double (45%) in 2012. Among the patients of rotavirus diarrhea, proportion of malnourished children was 57% in 1993, which decreased to 37% (35% reduction) in 2012. On the other hand, among well nourished under-5 children, it increased from 43% to 63% from 1993 to 2012 (46% increase) On the other hand, among well nourished under-5 children, it increased from 43% to 63% from 1993 to 2012 (46% increase) On the other hand, among well nourished under-5 children, it increased from 43% to 63% from 1993 to 2012 (46% increase) On the other hand, among well nourished under-5 children, it increased from 43% to 63% from 1993 to 2012 (46% increase).

All categorical variables were compared across the periods using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test. The period for categorical variables were compared using the Chi-square test.
pain, vomiting, intravenous fluid use, hospital stay (≥24 hours) and malnutrition (underweight, stunting, and wasting) significantly decreased from 1st to 2nd observation period (Table 1). Considering co-pathogens such as *Vibrio cholerae*, *Shigella* spp. and ETEC significantly decreased from 1st period of observation to 2nd period (Table 1).

In multivariate analysis, significant changes were also observed between two observation periods. The variables associated were maternal literacy, use of sanitary toilets, family members (>5 members) and drinking of boiled water. Usually, rotavirus diarrhea is considered as a disease of well nourished children due to higher presence of receptors for rotavirus at binding site in healthy lining epithelium of intestinal mucosa [18]. On the other hand, in malnourished children there are pathological changes of intestinal mucosa due to several micronutrient deficiencies, compromised immunity and repeated diarrhoea [19].

### Discussion

One of the major observations of the present study was the increasing trend of rotavirus diarrhea cases among under-5 children which is an alarming public health concern and reiterates the demand of mass vaccination. This might be a parallel effect of increasing diarrhea patient population in the hospital; however, Das SK et al. reported the changing patient population in the Dhaka Hospital of icddr,b with increasing trend among adult and elderly [17]. Over the period, the proportion of bacterial infection such as *Vibrio cholerae*, *Shigella* spp. and ETEC have been reduced which might be explained the increased rotavirus diarrhea [17]. Another interesting observation was association of rotavirus diarrhea with nutritional status of the children. For example, higher proportion of well-nourished children during 2nd observation period (2008–2012) had rotavirus diarrhea which was found to be higher among malnourished children during 1st observation period (1993–1997). Usually, rotavirus diarrhea is considered as a disease of well nourished children due to higher presence of receptors for rotavirus at binding site in healthy lining epithelium of intestinal mucosa [18]. On the other hand, in malnourished children there are pathological changes of intestinal mucosa due to several micronutrient deficiencies, compromised immunity and repeated diarrhoea [19].

### Table 3. Changing socio-demographic, clinical and host characteristics, and co-infection among under-5 children between rotavirus and non rotavirus diarrhea in the period of 2008–2012.

| Variables                  | 2008–2012; rotavirus: n = 2,493 (%) | 2008–2012; non rotavirus: n = 3,711 (%) | OR (95% CI) | aOR (95% CI) |
|----------------------------|------------------------------------|----------------------------------------|-------------|--------------|
| Male                       | 1813 (63)                          | 2276 (61)                              | 1.09 (0.98–1.20) | -            |
| Maternal literacy          | 2387 (83)                          | 2904 (78)                              | 1.39 (1.22–1.58)* | 1.24 (1.07–1.44)* |
| Use sanitary latrine       | 2228 (78)                          | 3025 (82)                              | 0.79 (0.70–0.90)* | 0.88 (0.76–1.01) |
| Family size (>5 members)   | 1018 (35)                          | 1145 (31)                              | 1.23 (1.11–1.37)* | 1.15 (1.03–1.29)* |
| Slum residence             | 107 (4)                            | 220 (6)                                | 0.62 (0.48–0.78)* | 0.79 (0.60–1.04) |
| Boiled drinking water      | 1078 (38)                          | 1715 (46)                              | 0.70 (0.63–0.78)* | 0.75 (0.67–0.84)* |
| Use of antimicrobial at home| 2339 (82)                          | 2556 (69)                              | 2.01 (1.78–2.26)* | 1.60 (1.40–1.84)* |
| History of measles within 6 months | 73 (2)                            | 111 (3)                                | 0.85 (0.62–1.16) | -            |
| Vomiting                   | 2125 (74)                          | 2452 (66)                              | 1.47 (1.32–1.64)* | 1.52 (1.35–1.71)* |
| Fever                      | 233 (8)                            | 252 (7)                                | 1.21 (1.01–1.47)* | 1.30 (1.06–1.60)* |
| Abdominal pain             | 463 (16)                           | 787 (21)                               | 0.72 (0.63–0.81)* | 0.77 (0.66–0.88)* |
| Duration of diarrhea (≥24 hours) | 2227 (78)                          | 2623 (71)                              | 1.45 (1.29–1.62)* | 1.04 (0.91–1.19) |
| Number of stool in last 24 hours (>10 times) | 1525 (53)                          | 1663 (45)                              | 1.40 (1.27–1.55)* | 1.28 (1.14–1.43)* |
| Watery stool               | 2796 (98)                          | 3512 (95)                              | 2.31 (1.73–3.08)* | 1.96 (1.44–2.65)* |
| Some or severe dehydration | 985 (34)                           | 1371 (37)                              | 0.89 (0.81–0.99)* | 0.99 (0.88–1.12) |
| Use of intravenous fluid   | 79 (3)                             | 416 (11)                               | 0.22 (0.17–0.29)* | 0.25 (0.19–0.32)* |
| Hospital stay ≥24 hours    | 1116 (42)                          | 1020 (28)                              | 1.91 (1.71–2.12)* | 2.20 (1.95–2.48)* |
| Underweight (WAZ < −2)     | 747 (26)                           | 1265 (35)                              | 0.67 (0.60–0.75)* | -            |
| Stunted (HAZ < −2)         | 626 (22)                           | 1055 (29)                              | 0.69 (0.62–0.78)* | 0.72 (0.63–0.81)* |
| Wasted (WHZ < −2)          | 530 (19)                           | 856 (24)                               | 0.74 (0.66–0.84)* | -            |
| *Vibrio cholerae*          | 32 (1)                             | 369 (10)                               | 0.10 (0.07–0.15)* | 0.15 (0.10–0.22)* |
| *Shigella*                 | 21 (1)                             | 186 (5)                                | 0.14 (0.09–0.22)* | 0.17 (0.10–0.27)* |
| ETEC                       | 193 (7)                            | 441 (12)                               | 0.54 (0.45–0.64)* | 0.57 (0.47–0.69)* |

doi:10.1371/journal.pone.0105978.t003
infections resulting in destruction of binding sites [19]. Over the period, as there is significant reduction of childhood malnutrition which might explain our present observation of increased frequency of rotavirus cases due to reduced malnourished children presenting with diarrhea; although, achievement in substantial reduction of childhood malnutrition to reach MGD 4 still lagging behind [20].

The present study also noted several changes, specially socio-demographic and clinical indicators among under-5 rotavirus diarrhea children between two observation periods. For example; increase in maternal literacy, more use of sanitary toilets, higher boiled water drinking, frequent use of antimicrobial at home, and lesser slum dwelling. These might be due to overall gross national improvements in maternal level of education; and health and hygiene practices [21]. All these indicators had significant association with childhood diarrhea as reported elsewhere [22].

On the other hand, clinical presentations such as frequent vomiting, and higher frequency of stool (>10 times/24 hours) might contribute to increased proportion of dehydrating diarrhea. Interestingly, use of intravenous saline for initial correction of dehydration significantly decreased which implies optimal acceptance of the use of ORS at home soon after the onset of diarrhea [23]. These, once again are related with increased literacy as well as acquiring related health education from mass media [24,25]. However, increased use of antimicrobial at home for rotavirus diarrhea is an alarming public health challenge as antimicrobials have no role at all in rotavirus diarrhea [26].

The observation of decreasing trend of co-infection of rotavirus with Vibrio cholerae, Shigella spp. and ETEC from 1st to 2nd observed period might be due to the relation with compromised immunity from malnutrition as well as history of measles within 6 months prior admission in 1st observation period [27].

References

1. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, et al. (2010) Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet 375: 1909–1951.
2. Paradar UD, Bresco JS, Glass RI (2003) The global burden of diarrhoeal disease in children. Bull World Health Organ 81: 236.
3. Lundgren O, Svensson L (2001) Pathogenesis of rotavirus diarrhea. Microbes Infect 3: 1145–1156.
4. Paradar UD, Gibson CJ, Bresco JS, Glass RI (2006) Rotavirus and severe childhood diarrhea. Emerg Infect Dis 12: 304–306.
5. Tate JE, Burton AH, Bossi-Pinto C, Steele AD, Duque J, et al. (2012) 2008 estimate of worldwide rotavirus-related mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 12: 136–141.
6. Baqui AH, Black RE, El Arefen S, Yumus M, Chakraborty J, et al. (2002) Effect of zinc supplementation started during diarrhea on morbidity and mortality in Bangladesh children: community randomised trial. BMJ 325: 1059.
7. Bhutta ZA, Bird SM, Black RE, Brown KH, Gardner JM, et al. (2000) Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. Am J Clin Nutr 72: 1516–1522.
8. Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, et al. (2011) Real-world impact of rotavirus vaccination. Pediatr Infect Dis J 30: S1–S5.
9. Joresu J, Koskenmaki E, Pang XL, Vesikari T (1997) Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. Lancet 350: 1205–1209.
10. Santoshm M, Moulton LH, Reid R, Croll J, Weatherholt R, et al. (1997) Efficacy and safety of high-dose rhesus-human reassortant rotavirus vaccine in Native American populations. J Pediatr 131: 632–638.
11. Jensen PK, Jayasinghe G, van der Hoek W, Cairncross S, Dalsgaard A (2004) Is there an association between bacteriological drinking water quality and gastrointestinal and respiratory infections in children: a public health problem. Int J Environ Res Public Health 8: 1174–1205.
12. Das SK, Faruque AS, Chisti MJ, Malek MA, Saha K, et al. (2012) Changing trend of persistent diarrhea in young children over two decades: observations from a large diarrhoeal disease hospital in Bangladesh. Acta Paediatr 101: e452–e457.
13. Qadri F, Das SK, Faruque AS, Fuchs GJ, Albert MJ, et al. (2000) Prevalence of rotavirus infection among 1 year old children. Arch Pediatr 14 Suppl 3: S169–175.

Limitations

Although unbiased systematic sampling method was used to enroll patients into surveillance system irrespective of age, sex, nutritional status, disease severity or socioeconomic background and large dataset with standard laboratory facility were supportive of the strengths of this analysis. There might be a sampling bias by enrolling every 50th patient and hospital data might not be the representative of general population.

Conclusion

The present analysis clearly indicated increasing trend of rotavirus diarrhea among under-5 children in urban Bangladesh. This observation was also correlated with several socio-demographic, clinical and host characteristics which changed significantly over the period especially water sanitation, hygiene practices, and improved nutritional status of the child. Thus, vaccination again rotavirus may be prioritized by the policy maker with improved water-hygience practices among this high risk group.

Author Contributions

Conceived and designed the experiments: MHRS SKD MJC PKB MIH AAM ASGF. Performed the experiments: MHRS SKD PKB ASGF. Analyzed the data: MAM MHRS SKD AAM ASGF SA JD FDF FF. Contributed reagents/materials/analysis tools: MHRS SKD MJ PKB MIH AAM ASGF. Wrote the paper: MHRS SKD MJC SA JD FDF ASGF. Reviewed the manuscript: SKD MHRS SA FF JD ASMSBS KMS MHA MAM MJC PKB MIH AAM ASGF.