TABLE I CLINICAL AND LABORATORY CHARACTERISTICS IN NEONATES WITH HYPERNATREMIC DEHYDRATION (*N*=49)

Parameter	*Exclusively breastfed (n=16)*	*Exclusively top fed (n=17)*	*Mixed fed (n=16)*
*Acute kidney injury	11 (68.7%)	14 (82.3%)	8 (50%)
Serum Sodium (meq/L)	150 (148-159)	164.5 (145-165)	158 (149-163)
Serum Potassium (meq/L)	5.3 (4-6)	4.9 (4-5.9)	6.9 (5-7.1)
Serum Creatinine	2.5 (2-4.2)	4.1 (2-5.1)	3.1 (2.5-5.2)
Correction time for Hypernatremia (h)	30 (20-40)	42 (24-56)	30 (24-42)
Duration of hospitalization (d)	6 (4.75-8)	7 (4.5-8.75)	6.5 (4.1-8)
*Neonates with signs of dehydration	1 (6.25%)	2 (12.5%)	3 (17.6%)

*All values in median(IQR) except *No.%*

can be managed at level II neonatal intensive care unit [10].

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Content Analysis of Commercially Available Probiotics

We carried out content analysis of four batches each of 3 commercially available probiotic formulations of *Bacillus clausii*. Species identification was done using MALDI-TOF-MS technique while bacterial count was done using plate colony count. Only one of the three probiotic formulation analyzed was found to have homogeneous population of *B. clausii* while none was found to have the exact viable bacterial count as suggested on the label.

Keywords: *Bacillus clausii, Probiotics, Reliability, Supplements.*

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World Health Organization (WHO) defines probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [1]. The pharmaceutical market is flooded with numerous probiotic products with very few effective checks-and-balances to regulate their quality. We intended to culture probiotic products said to contain *Bacillus clausii*, and determine the probiotic species and their count and compare it with the product label.

The study was carried out between September 2016 to January 2017. After hospital's ethical committee approval, we tested a total of 12 samples (4 each – 4 different batches) of 3 popular probiotics containing *B. clausii* namely – Product 1 (Enterogermina – Expiry range: 05/18 – 08/18),

TABLE I CONTENT ANALYSIS OF THREE COMMERCIALLY AVAILABLE PROBIOTIC PREPARATIONS CONTAINING *BACILLUS CLAUSII*

Parameter	*Product 1*	*Product 2*	*Product 3*
Species on label	*Bacillus clausii*	*Bacillus clausii*	*Bacillus clausii*
Isolated species	*Bacillus clausii*	*Bacillus subtilis*	*Bacillus subtilis*
Label count	2×10^9	2×10^9	2×10^9
Isolated species count			
Batch 1	1×10^9	4×10^6	5×10^9
Batch 2	2.5×10^8	2.5×10^6	2×10^8
Batch 3	1×10^9	1.6×10^6	7×10^8
Batch 4	2×10^9	3.4×10^6	1.3×10^8

Product 2 (Tufpro – Expiry range: 01/18 – 08/18) and Product 3 (Darolac Aqua – Expiry range: 12/17 – 09/18). The expiry date of products as mentioned by manufacturer was two years for all three products. MALDI-TOF-MS identification method (Matrix Assisted Laser Desorption Ionization Method Time of Flight– Mass Spectrometry) was used to identify bacterial species. Plate count method was used and colonies were counted as Colony Forming Units (CFU)/sample using Miles and Mishra method [2,3].

Only Product 1 was found to contain a homogenous population of *Bacillus clausii*, whereas Product 2 and Product 3 showed growth of *Bacillus subtilis* species in the samples. None of the samples had uniform viable bacterial counts across all samples as mentioned on the labels (***Table* I**).

Our results are in agreement with some of the previously done studies. In a study by Elliot, *et al*. [4], only three out of nine tested probiotic supplements from South Africa were found to be containing the same bacteria as mentioned on the label [4], while studies by Berman, *et al*. [5] and Temmerman, *et al*. [6] found only 1/20 and 6/55 of the different probiotic supplements tested to be consistent with the product label, respectively. Patrone, *et al*. [7] analyzed five probiotics containing only *Bacillus clausii*, and found that bacterial species and counts varied among the samples.

MALDI-TOF-MS technique used by us is a cost-effective method, which determines species as well as strains with fair amount of accuracy [8-10]. The main advantage of colony counting method is that it can be used to count large number of organisms, and also that it counts only viable organisms. The limitation of our study is the small sample size with limited number of products analyzed.

Future research needs to focus on analyzing more probiotic products both from the pharmaceutical as well as the dairy industry and tested at various time points of their viability, including tests close to their expiry dates.

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