**Probiotic Lactobacillus casei Shirota improves efficacy of amoxicillin-sulbactam against childhood fast breathing pneumonia in a randomized placebo-controlled double blind clinical study**

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(Received 2 November, 2017; Accepted 19 December, 2017; Published online 8 June, 2018)

The aim of the present study was to investigate the efficacy of oral administration of probiotic Lactobacillus casei Shirota and amoxicillin-sulbactam in treating childhood fast breathing pneumonia. 518 children diagnosed of fast breathing pneumonia were enrolled and randomly assigned to be administered either amoxicillin-sulbactam + Lactobacillus casei Shirota or amoxicillin-sulbactam + placebo. Primary outcome was defined as treatment failure before day 3, and secondary outcome was defined as treatment failure during follow-ups on day 6 and 12. Serum levels of tumor necrosis factor-α and interferon-γ were both significantly reduced in amoxicillin-sulbactam + Lactobacillus casei Shirota group compared to amoxicillin-sulbactam + placebo group. Serum levels of tumor necrosis factor-α and interferon-γ were both significantly reduced in amoxicillin-sulbactam + placebo group on day 3. On day 6 and 12, although treatment failure rates were higher than on day 3 in both groups, it was still significantly reduced in amoxicillin-sulbactam + Lactobacillus casei Shirota group compared to amoxicillin-sulbactam + placebo group. No severe adverse effects were observed in either treatment group. In conclusion, Probiotic Lactobacillus casei Shirota, in combination with amoxicillin-sulbactam, is more effective in treating childhood fast breathing pneumonia, which supports the potential clinical application of Lactobacillus casei Shirota as a safe supplement to amoxicillin-sulbactam therapy against childhood fast breathing pneumonia.

Key Words: childhood pneumonia, fast breathing pneumonia, amoxicillin-sulbactam, probiotics, Lactobacillus casei Shirota

Pneumonia is a deteriorating complication that systemically affects multiple organs. It is defined by the World Health Organization (WHO) as a sum of clinical symptoms ranging from fast breathing to more dangerous signs. Since the lung of a child is more susceptible to infections than that of an adult, childhood pneumonia is regarded as a leading cause of childhood morbidity and mortality around the world, particularly in under-developed and developing countries. It has been estimated that approximately 150,000–500,000 infants under one year old die of pneumonia each year.

Prescription of antibiotics is recommended by WHO for most childhood pneumonia-like symptoms. In particular, amoxicillin-sulbactam (AS), a novel combination of aminopenicillinsulbactamase inhibitors, has been reported to produce satisfactory therapeutic outcomes against pneumonia in children. However, prescriptions with antibiotics to treat childhood pneumonia can become expensive. For instance, in Pakistan, the average outpatient treatment cost per child per episode of childhood pneumonia was 13 US dollars in 2006, equal to 82% of health expense per person in the same year.[13] Furthermore, in South Asia and sub-Saharan Africa, providing all children with antibiotic treatment against pneumonia is estimated to cost approximately 200 million US dollars.[16] Hence, despite recently decreased childhood mortality from pneumonia, pneumonia still remains as a heavy burden and distressing challenge to the health system in under-developed and developing countries.

Probiotics refer to live microbial ingredients in food that usually exert beneficial effects on human health.[10,11] Consumption of probiotics, often in drinks or capsules as dietary supplements, is safe for healthy individuals as well as patients with various diseases,[12–14] including children who were critically ill.[15] Probiotics have also been reported to exhibit beneficial effects in clinical treatment against pneumonia. For example, Bo et al. presented evidences suggesting that use of several probiotics was associated with reduced incidence of ventilator-associated pneumonia (VAP).[16] Similarly, in a randomized controlled multicenter trial among critically ill patients, therapies using the probiotic bacteria B. Subtilis and E. faecalis were found to be both effective and safe against VAP.[17] A recent pilot trial also supported the use of probiotics in preventing against severe pneumonia.[18] Of particular interest to the current study, in a recent open-label randomized controlled clinical trial conducted among critically ill children on mechanical ventilation, administration of probiotics, such as Lactobacillus casei Shirota (LcS), was also shown to reduce incidence of VAP, without complications.[15] Provided with the reported beneficial effects of probiotics against pneumonia, we aimed to examine the clinical efficacy of probiotic LcS to improve the treatment outcome of AS against childhood fast breathing pneumonia in a randomized placebo-controlled double blind study.
Breaths/min in children aged 2–11 months and respiratory rate equal or higher than 50 breaths/min in children aged 2–11 months and equal or higher than 40 breaths/min in children aged over 12 months.

**Exclusion criteria.** All 518 patients initially enrolled were further evaluated by the exclusion criteria as follows: 1) danger signs, including inability to drink, seizure, somnolence, central cyanosis, grunting in a calm child and nasal flaring; 2) lower-chest wall indrawing; 3) malnutrition; 4) chronic debilitating diseases, such as chronic pulmonary illness besides asthma, anaesthetic abnormalities of the respiratory tract, cancer, progressing neurological disorders, immunological defects, heart disease with clinical repercussion, psychomotor retardation, haemoglobinopathy and liver or kidney disease; 5) other concurrent infections; 6) amoxicillin allergy; 7) history of aspiration; 8) hospitalization during the previous 7 days; 9) any dietary and/or medicinal probiotic supplement during the previous 6 months; 10) amoxicillin or similar antibiotic use during the last 48 h. 64 patients were excluded from the study based on the above criteria.

**Randomization and treatments.** The remaining 454 patients after exclusion were assigned, in a random manner with a permuted-block design stratified to their body weight, into two groups (227 in each group): 1) AS + LcS group, who received intravenous administration (i.v.) of AS at a dose of 100 mg kg/day (up to 3 g/day) every 8 h (Q8H), plus oral administration of skimmed milk containing a minimum of 6 x 10^5 colony-forming units (CFU) of LcS; 2) AS + placebo group, who received i.v. AS at a dose of 100 mg kg/day (up to 3 g/day) Q8H, plus skimmed milk as placebo. All patients were treated on a daily basis for 3 consecutive days. Drugs including both types of skimmed milk were prepared by investigators blind to the notified not to consume any food supplement or medication containing probiotics, except those supplied by the investigators throughout the study. 12 patients from the AS + LcS group and 14 patients from the AS + placebo group did not comply to such instruction and were thus dropped out from the study. Their data were not included from the final analysis.

**Serum cytokine measurements.** Blood samples (2–5 ml) were collected from all patients at the end of day 3 into test tubes with 0.1% EDTA, and then immediately centrifuged and stored at −80°C until further use. Serum concentrations of interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) were assessed with commercial assay kit from Harbin Pharmaceutical Group (Harbin, China) following manufacturer’s instructions.

**Treatment outcomes.** The primary outcome was defined as treatment failure up to the third day, with patients displaying any of the following symptoms: 1) persistent axillary temperature >37.5°C; 2) persistence of tachypnea, with respiratory rate ≥50 breaths/min in children aged 2–11 months and respiratory rate ≥40 breaths/min in children aged ≥12 months; 3) development of danger signs, including chest indrawing, inability to drink, grunting, cyanosis, nasal flaring, seizure and somnolence; 4) development of serious adverse reactions. Secondary outcome was defined as treatment failure at day 6 and 12 upon follow up, with patients displaying all of the above complications, pluspersistent cough and recurrent fever.

**Statistical analysis.** Sample size of treatment groups was estimated using established statistical analysis method. A sample size of 454 patients, 227 in each arm, is sufficient to detect a clinically important difference of 24% between two treatment groups in terms of primary outcome using a two-tailed z-test of proportion between two groups with 80% power and a significance of 0.05. This 24% difference represents an 80% reaching primary outcome in AS + LcS group and 56% reaching primary outcome in AS + placebo group. Two tailed Students t test was utilized to determine statistical differences between the two treatments, and p value less than 0.05 was considered statistically significant. Data analysis was performed using SPSS 18.0 software package (SPSS Inc., Chicago, IL).

**Results.**

Design of the current study was illustrated in Fig. 1. A total of 518 children (2–48 months old) were diagnosed of fast breathing pneumonia, including 64 patients who were later excluded based on the exclusion criteria. The remaining 454 patients were subsequently assigned into two treatment groups in a random fashion: 227 patients in the AS + LcS group and 227 patients in the AS + placebo group. 12 and 14 patients from AS + LcS group and AS + placebo group, respectively, were dropped out from the study before end of study.

Comparison of characteristics of all eligible patients from the two treatment groups was shown in Table 1. No significant differences were observed with regard to age, gender or symptoms at enrollment including tachypnea, fever, reduced pulmonary expansion, chest retraction, rhonchi, wheezing and crackles.

Treatment failure up to day 3 was defined as the primary outcome, and the specific causes for failures were listed in Table 2. There was a significantly lower number of patients in the AS + LcS group manifesting fever, chest indrawing and tachypnea than those in the AS + placebo group. No serious adverse reaction to either treatment was observed.

As pro-inflammatory cytokines TNF-α in IFN-γ have been reported to be involved in childhood severe and non-severe community acquired pneumonia, we were curious whether these two cytokines were potentially implicated in childhood fast breathing pneumonia. In this context, at the end of treatment day 3, blood samples were collected to analyze serum levels of TNF-α and IFN-γ. As shown in Fig. 2, both TNF-α and IFN-γ levels in the serum were significantly reduced in AS + LcS group patients compared to those in AS + placebo group.

Patients were followed by clinic revisits on day 6 and 12 after the start of study, and treatment failures on both revisits were defined as secondary outcome (Table 3). Number of patients in the AS + LcS group manifesting recurrent fever and persistent cough on day 6 was significantly lower than those in the AS + placebo group. In addition, although the number of treatment failure increased in patients from both treatments on day 12, the incidence of treatment failures in the AS + LcS group was nevertheless significantly lower than that in the AS + placebo group.

**Discussion.**

Pneumonia is a serious disorder systemically affecting a variety of organs. Several risk factors have been implicated in childhood pneumonia, such as lack of exclusive breastfeeding, cigarette use and in particular exposure to air pollution in rapidly developing economies. A recent investigation revealed that exposure to particulate matter such as PM2.5 was associated with hospitalizations for respiratory diseases, indicating air pollutants contribute to multiple respiratory diseases including pneumonia.

With the recent vast economic growth in China, air pollution, for instance from vehicle exhaust, is thought to contribute to the
increased incidence rate of pneumonia.\(^{(28,29)}\) Hence, it is urgent to improve the current clinical management of pneumonia, particularly among children, because lungs of children appear more susceptible to air pollution and/or infections than adults, and even air pollution from cooking with solid fuels was reportedly associated with pre-mature deaths from pneumonia for children under 5 years old.\(^{(30)}\)

In the current clinical study, we focused on the milder form of childhood pneumonia termed childhood fast breathing pneumonia, for which WHO recommended oral amoxicillin as a priority treatment.\(^{(31)}\) Unfortunately, based on recent clinical reports among children with fast breathing pneumonia, the efficacy of oral amoxicillin was still far from satisfactory.\(^{(32)}\) On the other hand, the wide anti-microbial property of AS makes it an effective therapy against childhood pneumonia.\(^{(4,33)}\) However, its clinical efficacy against childhood fast breathing pneumonia has not been investigated, and a safer and more effective agent to supplement AS is also needed. To this end, we examined the probiotic LcS, that has been widely used as a dietary supplement and reportedly exerts beneficial effects in various models of respiratory disorders,

### Table 1. Comparison of baseline characteristics between analyzed patients of the two treatment groups

|                     | AS + LcS (n = 215) | AS + placebo (n = 213) |
|---------------------|--------------------|------------------------|
| Gender (male/female)| 104/111            | 107/106                |
| Median age, range (months) | 27.3 (2–48)       | 26.8 (2–48)            |
| Fever               | 67                 | 71                     |
| Tachypnea           | 128                | 133                    |
| Chest retraction    | 12                 | 10                     |
| Reduced pulmonary expansion | 47              | 51                     |
| Rhonchi             | 154                | 149                    |
| Wheezing            | 86                 | 91                     |
| Crackles            | 124                | 112                    |

### Table 2. Primary outcome: comparison of treatment failure up to day 3 between analyzed patients of the two treatment groups

|                     | AS + LcS (n = 215) | AS + placebo (n = 213) | p     |
|---------------------|--------------------|------------------------|-------|
| Fever               | 10                 | 22                     | <0.05 |
| Tachypnea           | 19                 | 54                     | <0.05 |
| Chest indrawing     | 4                  | 9                      | <0.05 |
| Adverse drug reaction | 0                 | 0                      | N.A.  |
| Total number reached primary outcome | 182             | 128                    | <0.05 |
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