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# The PCMC Journal

An Official Publication of the Philippine Children's Medical Center

## EDITORIAL STAFF



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# FUNCTIONAL RESIDUAL CAPACITY IN HEALTHY INFANTS AGED 1-24 MONTHS USING THE BABY BODY PLETHYSMOGRAPHY (CAREFUSION TM)

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**ABSTRACT**

**BACKGROUND:** The only lung volume that can be measured reliably in infants is the functional residual capacity (FRC). Published reference values vary, thus, there is a need to determine values for healthy infants using the available equipment.

**OBJECTIVES:** To determine the normal values of FRC in healthy infants using the baby body plethysmogram (CareFusion) and to determine the correlation between FRC and weight, length, age, and gender.

**METHODS:** FRC was measured using the CareFusion MasterScreen baby body plethysmogram in 62 healthy infants aged 1-24 months old. FRC was measured after sedation with Chloral hydrate at 50 mg/kg body weight. Three measurements were performed from which the mean (SD) FRC was calculated. To depict the change in FRC with growth, regression analysis between FRC as dependent variable and weight, length, and age as independent variables was done.

**RESULTS:** Among 62 infants, mean age was 10.97 months (range=1-24 mos), mean weight was 7.93 kg (range=4.5-12 kg) and mean length was 71.91 cm (range=56-90 cm). Thirty-one (50%) are males and 31 (50%) are females. Mean FRC (SD) was 24.56 ml/kg (4.41). Correlation of FRC with age was significant (R=0.820), as well as the weight (R=0.830) and the length (R=0.758). There was no difference in FRC between males and females.

**CONCLUSION:** The FRC values obtained in this study is 24.56 ml/kg (4.41). There is a direct correlation of FRC with age, weight, and length. The result of this study was comparable to other studies and may be used as a reference value for healthy infants.

**KEY WORDS:** Baby body plethysmography; Functional residual capacity; Pulmonary function test; Infant

## INTRODUCTION

Measurement of lung volumes in infants are important for assessing growth and development of the lungs and for interpretation of volume-dependent lung function parameters. There are various uses of respiratory function tests in infancy. They are used as diagnostic aids to help determine the nature of the lung function disorder; to quantify its magnitude; to assist in determining prognosis or perioperative risk; to assess the effects of medical interventions or diagnostic tests; to evaluate innovative therapies aimed at improving prognosis, quality of life, and lung function; to study the natural course of respiratory disease; and to study the growth and development of the lungs and airways and evaluate early determinants of airway function. (1)

The only lung volume that can be measured reliably and routinely in infants is the resting end-expiratory lung volume or functional residual capacity (FRC). (2) It is important for interpreting volume-dependent pulmonary mechanics such as forced expiratory flows, for defining normal lung growth and for detecting hyperinflation which is a measure of dyspnea or disease state. Published reference data for neonates have been reported, approximately 23 ml/kg when using the helium dilution technique and 32 ml/kg from plethysmographic measurements. (2) In 2001, the American

Thoracic Society and European Respiratory Society (ATS-ERS) task force published recommendations on the methodology and the equipment for infant plethysmography which measures infant lung volumes including FRC. (3) New generation of infant body plethysmographs were developed, making infant lung function readily available. However, meaningful interpretation of results will still depend on the availability of appropriate reference values.

Reference values are a set of values obtained from an individual or group in a defined state of health. Ideally, reference values must be consistent and are those expected for individuals of the same sex, similar stature, age, and other characteristics.

Published reference values for FRC varied among studies on healthy infants. In fact, during the previous years, there has been an unexplained trend toward declining values for plethysmographic assessments of FRC. There is therefore an urgent need to determine normal values for healthy infants using the available equipment, and carefully observe protocols for data collection, analysis, and quality control.

**OBJECTIVES**

**General:**

To determine the normal values of functional residual capacity (FRC) in healthy infants using the baby body plethysmogram (CareFusion)

**Specific:**

To determine the correlation between FRC and body weight, length, age, and gender

**METHODOLOGY**

This is a cross-sectional study. All healthy infants aged 1-24 months were included in the study. Infants were considered healthy if they fulfill the following criteria:

1. Well nourished based on Z scores (weight for height below Z score 3 and above Z score (-2)

2. Normal physical examination upon enrolment to the study.

Selection of healthy subjects were based on the American Thoracic Society/ European Respiratory Society recommendations.(17) Infants were excluded if they had the following: a) Recent upper respiratory tract infection or acute respiratory tract infection 3 weeks prior to the participation in the study; b) first degree family history or physician diagnosed bronchial asthma, allergic rhinitis,or atopy; c) Two or more episodes of wheezing and coughing in the past relieved by intake of and/or nebulization with B2-agonist; d) history of intake of systemic or inhaled steroids within two weeks; e) premature birth less than 37 weeks AOG, low birth weight (less than 1,500 g), history of bronchopulmonary dysplasia; f) exposure to any household member who smokes inside the house; g) infants with upper airway obstruction; h) facial/oral abnormalities; i) thoracic and chest wall deformities; j) patients on CNS depressants such as opiods, benzodiazepines, or barbiturates which may cause excessive sedation; presence of other conditions supported with laboratory tests, imaging studies, or clinical abstracts from attending physicians who made the diagnosis, such as: k) presence of congenital anomalies involving any organ system; l) cardiopulmonary or other systemic illness such as collagen disease, nephropathies, or any malignancy,; m) neuromuscular disease such as GBS, myasthenia gravis, and muscular dystrophies; n) history of thoracic or abdominal surgery within 3 months prior to participation in the study.

The sample size was calculated using the formula for estimation of population mean.

$$\underline{n= (Z )^2 (SD)^2}$$
$$E^2$$

Where:

Z = 95% confidence level

SD= Standard deviation of the variable of interest, that is, FRC= 3.4

E= measure of effect= 1

$$\underline{n= (1.96)^2 (3.4)^2}$$
$$(1)^2$$

= 44

The number of samples was computed using a 95% level of confidence. With an estimated SD of 3.4 for FRC based on reference (7), at least 44 subjects are needed. This sample size calculation was used to answer the primary objective which is to determine the functional residual capacity in healthy infants aged 1-24 months old.

The formula used for the specific objective was the formula on One Correlation Power Analysis. A sample size of 59 achieves 80% power to detect a difference of 0.1 between the null hypothesis correlation 0f 0.80 and the alternative hypothesis correlation of 0.90 using a two-sided hypothesis test with a significance level of 0.0500. The sample size calculated was at least 59 based on the specific objective, but 62 subjects were included in the study.

Subjects were selected randomly from Barangay PAG-ASA and AGHAM in Quezon City. Subjects were randomly selected from the list of households in the Barangay. Using odd numbers, the first household was selected, then succeeding numbers followed.

An informed consent was obtained by the principal investigator after the selected subjects fully understood the study. A checklist was used to assess the health status of each patient. Physical examination was done and anthropometric measurements were recorded. Infants with normal physical examination were then included in the study.

The infant was then prepared for the procedure. The infant's clothing was loosened so as not to restrict respiratory movements. Feeding was withheld according to the guidelines for monitoring pediatric patients who are sedated. Milk feeding was withheld 4-6 hours prior to the said procedure. (18) Timing of measurement coincided with the infant's normal sleep/waking routine to minimize any need for repeat sedation.

Functional residual capacity (FRC) was measured according to the Care Fusion instruction manual (19) and ERS/ATS recommendations (6). FRC was measured during quiet sleep after sedation with oral chloral hydrate at 50 mg/kg (max dose of 100 mg/kg) which was administered by the principal investigator. Another dose of 25 mg/kg was given in patients who were inadequately sedated. Infants who do not fall asleep after the second dose were rescheduled after a week. Infants who awakened during the procedure were reassessed for need of resedation. A repeat half dose was given to infants who vomited the syrup immediately after administration and observed for recurrence of vomiting. If vomiting persists, the infant will be admitted for hydration and monitoring.

To avoid unnecessary and potentially dangerous transfer of the deeply sedated infants, sedation was administered at the pulmonary laboratory where the procedure was done. Rescuscitation equipments such as bag mask and oxygen, as well as a functioning suction apparatus were available and the principal investigator, as well as the co-investigators, or respiratory therapists who were all trained in pediatric advanced life support were present. The sedated infant was monitored at baseline by the primary investigator. Oxygen saturation and heart rate were monitored. Respiratory rate, blood pressure, and expired CO2 levels were also monitored at every 10-15 minutes. The infant's head position was continuously assessed to ensure airway patency. The sleeping infant was placed inside the plethysmograph and a face mask attached to a pneumotachograph and shutter was sealed around the nose and mouth. The seal was tested by recording at least five tidal breaths before occlusion to establish a stable end-expiratory level and then briefly closing the shutter at end expiration. If the seal is tight, flow will be zero throughout the occlusion and the volume recorded will return to the expiratory baseline after the release of the shutter. The mask was held in place with strapping to support the cheeks and reduce risk of shunting to the upper airways during occlusion. After eliminating leaks, the plethysmograph was closed. A minimum of three end-expiratory occlusions was performed from which the mean FRC was calculated. After the procedure, the infant was monitored and sent home once fully awake for at least 20 minutes and feeding. The infant's skin was evaluated for any rashes after the putty has been applied during the test. The infants were followed up the next day through a phone call for any symptoms such as vomiting and abdominal discomfort after sedation.

Demographic data was described using means and standard deviation. To depict the change in FRC with growth, regression analysis was done using the Pearson's Product Moment Coefficient of Correlation between FRC as dependent variable and weight, length, and age as independent variables.

Since the procedure entails quiet breathing, all subjects were sedated. Chloral hydrate has been the preferred medication for infant pulmonary function test. All existing healthy infant PFT reference data have been derived from studies using Chloral hydrate. It is an oral medication and thus avoids the need for an intravenous catheter. Its duration of action matches well with the duration of the infant pulmonary function test and it provides the appropriate level of sedation. An informed consent from the parents were obtained without coercion. Anonymity and confidentiality of the research correspondents were respected. The hospital's Ethics committee and Investigational Review Board approved the study prior to recruitment of subjects. There is no conflict of interest to disclose as to relationship with the distributor of the equipment.

## RESULTS

### Demographic Profile of Patients

There were 82 infants randomly recruited from Barangay PAG-ASA and AGHAM in Quezon City. Eighteen (n=18) subjects were excluded due to the following reasons: no consent (n=10), malnourished (n=3), history of recent upper respiratory tract infection (n=2), and failure of sedation (n=3). There were 64 infants who completed the procedure, but 2 were classified as outliers after the preliminary analysis. Sixty-two patients were then included in the final analysis (Figure 1).

Among the 62 subjects who completed the procedure, mean age was 10.97 months (range=1-24 mos), mean weight was 7.93 kg (range=4.5-12 kg) and mean length was 71.91 cm (range=56-90 cm). Thirty-one (50%) are males and 31 (50%) are females. (see Table 1)

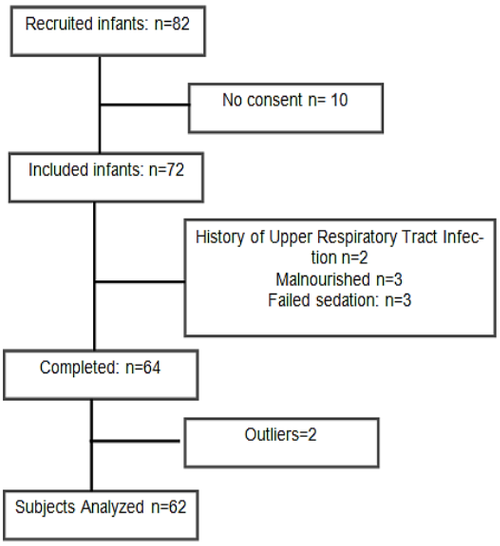


**Figure 1.** Study Population

### Functional Residual Capacity (FRC) Results

In the actual data collection, although the calculated sample size was at least 59, 64 subjects were considered. From the preliminary assessment of the data, 2 observations were classified as outliers and were excluded in the calculation of the normal values inorder to produce a more stable estimate of the normal values. Table 1 summarizes subject details and results. Mean (SD) FRC was 195.89 (58.34) ml and mean (SD) FRC per kg body weight was 24.56 ml/kg (4.41).

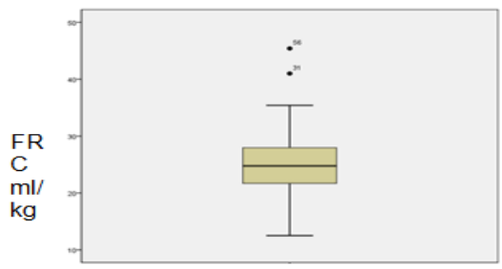


**Figure 2.** Preliminary graphical description of FRC per kilogram body weight showing 2 outliers

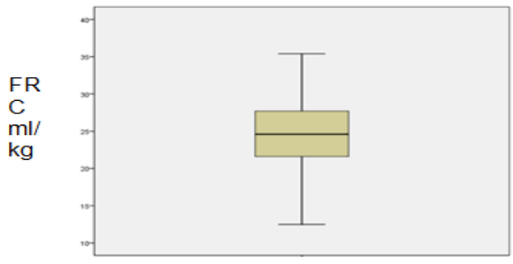


**Figure 3.** Graphical description of FRC per kilogram body weight after outliers have been eliminated

**Table 1.** FRC in Healthy Infants: Background Characteristics and Results

| Attributes | Mean (sd) | 95% CI for Mean | Range | 5%ile | 95%ile |
|---|---|---|---|---|---|
| n | 62 | | | | |
| % Male<br>% Female | 50<br>50 | | | | |
| Age, mos | 10.97 (6.33) | (9.36, 12.58) | 1 – 24 | 2.00 | 24.00 |
| Weight, kg | 7.93 (1.82) | (7.48, 8.40) | 4.5-12 | 5.00 | 11.80 |
| Length, cm | 71.91 (8.34) | (69.79, 74.03) | 56 – 90 | 60.00 | 86.85 |
| Mean FRC, ml | 195.89 (58.36) | (181.07, 210.71) | 75.13 – 349.70 | 93.85 | 288.36 |
| Mean FRC, ml/kg | 24.56 (4.41) | (23.44, 25.68) | 12.5 – 35.4 | 15.92 | 31.77 |

Correlation of FRC with age was found to be significant at the 0.05 level (R=0.820), as well as the weight (R=0.830) and the length (R=0.758). Comparison of mean FRC ml/kg using the T-test showed no difference in FRC between males and females (p=0.49). (See Table 2) Significant correlation between FRC and age, weight, and length was reinforced when regression resulted to the equation:

**FRC= 3.9(age in months) + 15.4(wt)- 0.053(length) + k ,where k= 34.32**

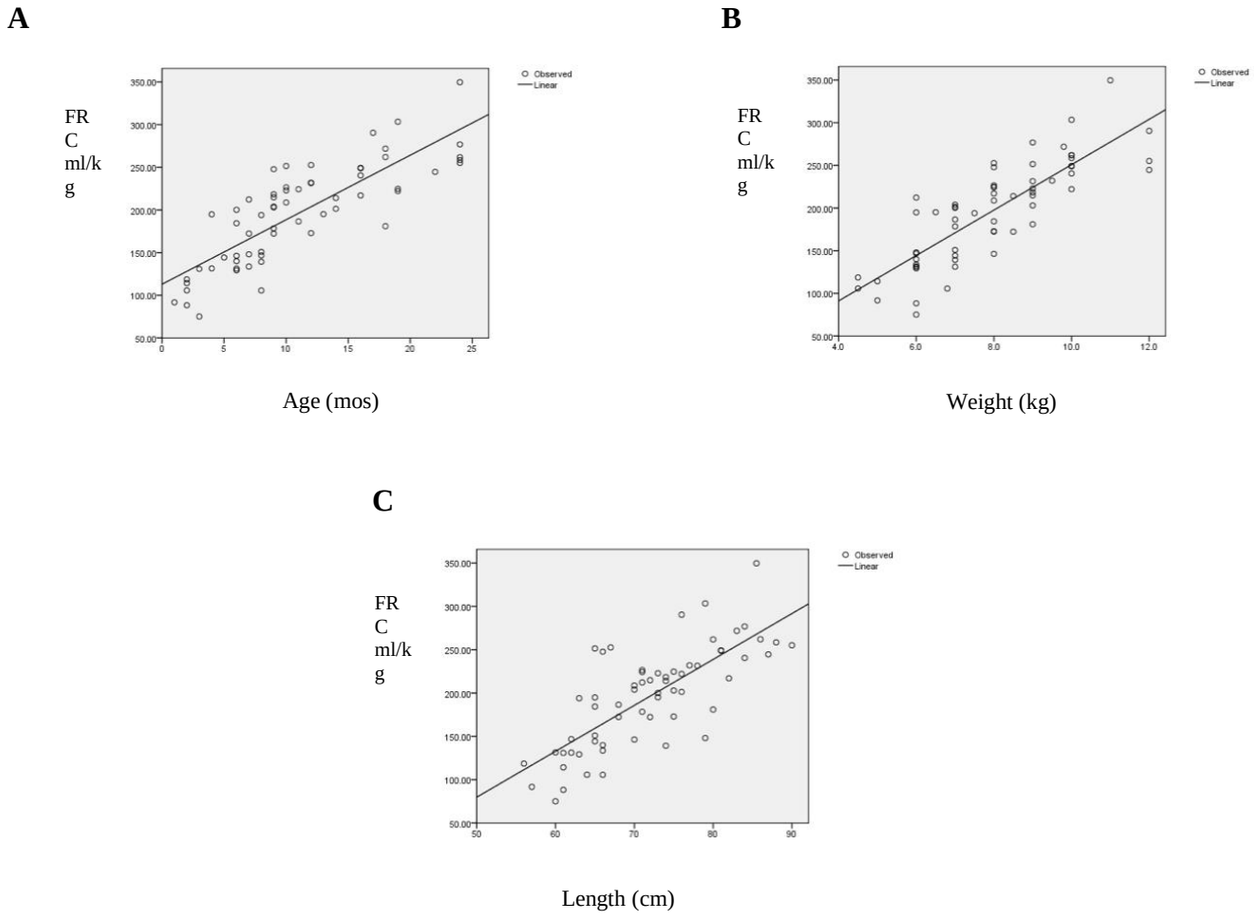


**Figure 4.**
Scatter plot of FRC against A. Age B. Weight C. Length
Solid line indicating the line of best fit of the scatter of the points

**Table 2.** FRC in Healthy Infants: Correlation with Background Characteristics

| Characteristics | | Mean FRC (DS subtracted), ml/kg R‡ or Mean (sd) | p-value |
|---|---|---|---|
| Gender | Male (n = 31) | 24.95 (4.58) | 0.49¶ (NS) |
| | Female (n = 31) | 24.16 (4.27) | |
| Age, mos (n = 62) | | 0.820 | 0.00§ |
| Weight, kg (n = 62) | | 0.830 | 0.00§ |
| Length, cm (n = 62) | | 0.758 | 0.00§ |

‡ – Pearson's Product Moment Coefficient of Correlation
¶ – using t-test for comparing means
§ – using test for the significance of R
[NS] – not significant
* - significant at the 0.05 level of significance
** - significant at the 0.01 level of significance

## DISCUSSION

Objective measurement of pulmonary function in infants are very limited since this age group are uncooperative and usually requires sedation. One of the procedures that can be applied in this age group is the determination of Functional Residual Capacity (FRC) which requires infants to be in quiet sleep.

Several studies have reported values for Functional Residual Capacity (FRC) in healthy infants using plethysmography.(2,8) This is the first local data gathered to establish reference values of FRC using the recently available equipment at the Philippine Children's Medical Center and the first in this country.

To produce a reliable data, possible causes of discrepancies between the reported values of FRC as discussed by Hulskamp et al. were considered.(8) Equipment protocol was strictly observed, including subtraction of compressible deadspace according to the ATS-ERS guidelines.(3)

When adjusted for weight, reported mean (SD) FRC was 24.56 ml/kg (4.41), with a range from 12.5-35.4 ml/kg; 95th CI 23.44-25.68 ml/kg. This was comparable to results from previous compilation of reference data. (8) The study by Hulskamp, however had lower values compared to this study which was reported at 19.6 ml/kg (3.4).This discrepancy may be partially attributed to variations in age with relatively few infants being studied during the first few months of life in this present study. Although Hulskamp's study had almost the same age range which is 1-21 months, the scatter of age groups were not reported in detail. In this study, the bulk of the age group falls under ages 9-12 months. Other subject characteristics may be taken into consideration such as the selection of healthy infants. This study recruited subjects from the community and represented an unbiased sample of healthy infants in the Philippines. Inclusion of infants in this study was based on the ATS/ERS guidelines as compared to the study by Hulskamp et al. where infants with history of wheezing were not excluded thus, may explain the difference in the values obtained.

According to the ATS/ERS guidelines on standards for infant respiratory function testing, dead space of the mask should be measured by water displacement and 50% of this value subtracted to take into account the space occupied by the infant's face and the putty film. (3) Compared to the dead space accounted for in the study by Hulskamp, 11.8-14.3 ml were subtracted from the actual FRC compared to the

7.5-10 ml in our study. The higher values of dead space subtracted in their study may explain the lower FRC per kg body weight reported. Dead space from the tubings connecting the pneumotachometer and mouth pressure port to the transducers were all accounted for in the recent software, thus only the dead space from the mask were substracted. Another factor which might have affected the variation in reported values of FRC is feeding prior to sedation. Although the ATS/ERS recommend that the infant may not be placed on NPO since tests tend to be more successful if the infant is fed (9), feeding was witheld 4-6 hours in accordance to the guidelines for monitoring pediatric patients who are sedated. (7) Previous studies have not mentioned withholding feeding prior to sedation. There is limited data that discusses the influence of feeding in pulmonary function tests in infants, but this factor may be considered. A study by Pitcher-Wilmott et al. (20) reported a significant decrease in FRC fifteen minutes after feeding.

Previous studies have established the correlation between FRC and age, weight, and length. Among the mentioned variables, length has been reported to be most correlated with FRC. In this study however, weight (R=0.830) was found to be most correlated compared to age (R=0.820) and length (R=0.758). Previous prediction equations have not included age as a variable, which was found to be the variable more correlated to FRC than length in this study. This study showed that as age, length, and weight increases, FRC also increases. Of all the studies of FRC in infants and preschool children, only those by Taussig et al. (21) and Wall et al. (22) have demonstrated significant differences due to gender where FRC in males exceeded that in females at any given length.

## CONCLUSIONS/RECOMMENDATIONS

The FRC values obtained in this study is 24.56 ml/kg (4.41). There is a direct correlation of FRC with age, weight, and length. The result of this study was comparable to other studies and may be used as reference value for healthy infants. The findings support the assumption that decreased values of FRC in healthy infants reflect technological advances and improvement in equipment ptotocols.

Although the computed sample size achieved 80% power, similar studies may be explored in the future using a larger sample size in order to increase the power of the study.

## REFERENCES:


1. Hofhuis W. Clinical Applications of Infant Lung Function Testing.Uylenburgh Pers, Amsterdam.2004

2. Stocks J, Quainter H. Reference values for residual volume, functional residual capacity and total lung capacity. ATS Workshop on lung volume measurements. Official statement of the European Respiratory Society. Eur Respire J, 1995,8, 492-506.

3. Stocks J, Godfrey S, Beardsmore C,Bar-Yishay E, Castile R. Standards for infant respiratory function testing: plethysmographic measurements of lung volume and airway resistance. Our Respire J 2001; 17:302-312.

4. Merkus P.J.F.M, Jongste J.C., Stocks J. Respiratory function measurements in infants and children. Eur Respir Mon, 2005, 31,166-194.

5. Criee CP, Sorichter S, Smith HJ, Kardos P, Merget R, Heise D, et al. Body plethysmography-Its principles and clinical use. Respiratory Medicine 2011, 105, 959-971.

6. Coates AL, Peslin R, Rodenstein D, Stocks J. Measurement of lung volumes by plethysmography. Eur Respir J, 1997,10, 1415-1427

7. Stocks J., Godfrey S. Plethysmographic measurements of lung volume and airway resistance. Standards for infant respiratory function testing: ERS/ATS Task force.

8. Eur RespirJ 2001; 17: 302-312

9. Hulskamp G., Hoo AF., Ljungberg H., Lum S., Pillow J., Stocks, J. Progressive Decline in Plethysmographic Lung Volumes in Infants. Physiology or Technology?. Am J Respire Crit Care Med, 2003.Vol 168, 1003-1009.

10. Gaultier C, Fletcher M, Beardsmore C, Motoyama E, Stocks J.Infant respiratory function testing, 1st ed. New York: John Wiley & Sons, Inc.; 1996.29-44.

11. Gaultier C, Fletcher M, Beardsmore C, Motoyama E. Respiratory function measurements in infants: measurement conditions. Report of working group/ ERS-ATS Statement. Eur Respir J, 1995, 8,1057-1066

12. Ren C, Robinson P, Ranganathan S. Chloral hydrate sedation for infant pulmonary function testing. Pediatr Pulmonol.2014 Dec; 49(12): 1251-1252.

13. Kraus B, Green SM. Sedation and analgesia for procedures in children. N Engl J Med 2000;342: 938-945

14. Lipshitz, M, Marino BL, Sanders ST. Chloral hydrate side effects in young children: Causes and management. Heart, Lung.1993 Sep-Oct;22(5):408-414.

15. Greenberg SB, Faerber EN, Aspinall CL, Adams RC. High dose Chloral hydrate sedation for children undergoing MR imaging: Safety and efficacy in relation to age. AJR Am J. Roent 1993 Sept;161(3):639-641.

16. Valenzuela DG, Kumar DS, Atkins CL, Beers A, Kozak FK, Chadna NK. Chloral hydrate sedation for auditory brainstem response (ABR) testing in children: Safety and effectiveness. Int J Pediatr Otorhinolaryngol.2016 April;83: 175-178Valenzuela Feb 12, 2016.

17. Wilson ME, Karaoui M, Al Djasim L, Edward DP, Al Shamrani M, Friedman DS. The safety and efficacy of Chloral hydrate sedation for pediatric ophthalmic procedures: a retrospective review. J Pediatr Ophthalmol Strabismus. 2014 May-June;51(3):154-159.

18. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Disease 1991; 144:1202-18

19. Cote CJ, Wilson S. Guidelines for monitoring and management of pediatric patients before, during, and after sedation for diagnostic and therapeutic procedures: Update 2016. American Academy of Pediatrics 2016.

20. CareFusion Germany. Instructions for use MasterScreen Paed/Baby Body 2013.

21. Pitcher-Wilmott R, Shutack, Fox W. Decreased lung volume after nasogastric feeding of neonates recovering from respiratory disease. J Pediatr 1979;95:119-121.

22. Taussig LM, Harris TR, Lebowitz MD. Lung function in infants and young children. Am Rev Respir Disease 1977;116: 233-239.

23. Wall MA, Misley MC, Dickerson D. Partial expiratory flow volume curves in young children. Am Rev Respir Disease 1884; 129:557-562.

# A STUDY ON THE ACCURACY OF PEN CLICK TEST AS A HEARING SCREENING TOOL AMONG NEWBORNS SEEN IN TWO TERTIARY GOVERNMENT HOSPITALS

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**ABSTRACT**

**BACKGROUND:** Hearing impairment has a great impact on the functional, social and emotional aspects of a child. Thus, early detection and management is crucial for optimal development of the child. The Newborn Hearing Screening Act was approved in the Philippines to "institutionalize measures for prevention and early diagnosis of congenital hearing loss among newborns". A simple, accurate and readily available hearing screening tool is necessary in less privileged communities.

**OBJECTIVES:** To determine the accuracy of Pen Click Test as compared to otoacoustic emission test as a hearing screening tool among newborns seen in two tertiary government hospitals. The accuracy of Pen Click Test was measured for its sensitivity, specificity, positive and negative predictive value.

**METHODS:** The study is an experimental design consisting of three phases: Phase I is a randomized complete block design; Phase II involves inter-rater and intra-rater variability randomized block design and Phase III is a cross sectional design. The study was done in two government tertiary hospitals. The subjects are term newborns with both ears analyzed independently from each other.

**RESULTS:** Phase I of the study identified Acroball retractable pen as the study stimulus based on its accessibility and its capability to produce high decibel. In phase II, all health workers produced a sound stimulus of more than 70 decibels. Majority of the health workers had no significant difference among each other which means there is minimal deviation from the mean. Phase III showed that pen click test has a high specificity of 98% and a sensitivity of 43%. Based on disease prevalence, the test showed a positive predictive value of 77% and negative predictive value of 93%. Kappa agreement showed moderate result with a Kappa coefficient of 0.54.

**CONCLUSION:** The study showed high specificity in identifying hearing impairment and a positive association of Pen Click test to the standard hearing audiometer. The application of this test in the community may be done as a hearing screening tool. This study provides an accessible, easily reproducible and accurate tool for hearing screening that may be applied in communities without facilities.

**KEYWORDS**: Newborn Hearing Screening, Hearing impairment, Accuracy

## INTRODUCTION

The World Health Organization (WHO) reported increasing incidence of hearing impairment worldwide from 42 million in year 1985 to 360 million people in year 2012. Approximately 32 million (9%) of which were children with disabling hearing impairment.[1]

Two thirds of the population with hearing impairment come from Southeast Asian countries.[1] The most common causes associated with hearing impairment were congenital hearing impairment, otitis media and noise induced hearing loss. In a study by Naeem and Newton, sensor-neural hearing loss was prevalent among Asian children with rates ranging from 5.09 to 9.61per 1000. The relative risk of having hearing impairment among Asian children was two to three times greater than non-Asian children.[1] Of these children, one to two of 1000 newborns have hearing impairment that warrants observation or treatment.

In the Philippines approximately 1,443 thousand persons of the 92.1 million household

population (1.57 percent) had disability. The Philippine disability survey was categorized as follows: moving disability (39%); hearing (33%); speaking (10%); mental (10%) and seeing (8%). The survey revealed that hearing disability ranked as second highest form of disability with 1.10% prevalence rate.[2]

Hearing impairment has great impact on the functional, social and emotional aspects of a child which may include speech delay, poor academic performance and poor personal-social development. Thus, early detection and management is crucial for optimal development of the child. The National Institute of Health in 1993 and the Joint Committee on Infant Hearing in 2000 recommended that the diagnosis of hearing impairment must be done before three months of age to provide early intervention before six months of age. This age is considered as the critical period for the development of central auditory pathway.[4]

There are various audiology tests used for hearing screening that is age appropriate. The most commonly used procedure is the Otoacoustic emission test (OAE) which measures cochlear response to stimuli. It is appropriate for all ages and may be done in a few minutes. It is independent on the sleep-wake status of the patient. Automated Auditory Brainstem Response (ABR) is another audiologic tool that may be used for newborns. This test measures auditory nerve and brainstem activity. The OAE and ABR tests are considered only as a primary screening tool since they cannot assess cortical sound processing. In older children, play audiometry and conventional audiometry may be done. These tests assess auditory perception and would require participation of the child.[4]

The universal hearing screening program was fully implemented. However, a major problem in adopting the program is the insufficient facilities of hearing screening tool such as OAE and ABR especially in developing countries. Various methods on improvised hearing screening were done among children using tuning fork and pen. These methods are easily reproducible. However, the accuracy of each test may vary as a hearing screening tool. It is important to establish a hearing screening tool that is simple, readily available, affordable and accurate. This study will answer the research question "How accurate is pen click test as a hearing screening tool among full term newborns seen in two tertiary government hospitals?

## METHODOLOGY

The study is an experimental design consisting of three phases: randomized complete block design, randomized block design (inter and intra-rater variability) and cross-sectional design. The study was conducted among newborns seen in two tertiary government hospitals.

The study included 97 newborns, with a total of 194 ear subjects that underwent hearing screening in compliance with the Universal Hearing Screening Program. One hundred forty-six ear subjects were enrolled from two tertiary government hospitals in Quezon City. The sample size was computed based on the formula shown in Figure 1 with a total of 194 ear subjects.

$$n = \frac{2\, Z^2\alpha/2\, pq}{\theta^2}$$

n= desired sample size
$z^2$= critical value for confidence interval
$\alpha$ = alpha
p = specificity or sensitivity
q = 1- specificity or 1- sensitivity
$\theta^2$ = margin of error

Thus,
For number of cases $z^2$=1.96; p= 0.71; q=0.21; $\theta^2$ = 0.01
For number of controls $z^2$=1.96; p= 0.95; q=0.05; $\theta^2$ = 0.02

**Figure1**. Formula to compute for the sample size

## STUDY PROCEDURE

### Phase I. Randomized Complete Block Design

Phase I of the study determined the variability of the pen click test. Three different types of retractable pens were used as the experimental factor and trials as the block. Phase 1 measured the inter-rater variability which was conducted by the principal investigator. Three types of retractable pens (Pilot Acroball, BIC and HBW) which are all easily accessible were used as the study stimulus. Three pens were tested per brand of pen included. The Pen click test was tested with a sound level meter (TES-1350A). The investigator produced the pen click stimulus thirty times at two inches distance from the sound level meter. The pen click stimulus was repeated for three trials with fifteen minutes interval. All sound measurements from

the selected pens were recorded in decibels(dB) and were written in a data form (Appendix 2). The type of ballpen with the highest mean decibel was used in Phase II and Phase III of the study.

**Phase II. Randomized Block Design: Intra-rater and Inter-rater Variability**

Phase II of the study determined the variability of the pen click using the type of ballpen with the highest mean decibel produced (Pilot Acroball). In Phase II, the healthcare worker was the experimental factor and trials as the block. Phase 2 measured the inter-rater and intra-rater variability among the health care workers. This phase of the study showed the reproducibility of the test among healthcare workers in the community at any given time.

A total of ten participants were chosen from the qualified healthcare workers through fishbowl sampling. Phase II was conducted in an audiology room. The health care workers clicked the pen thirty times at two inches distance from the sound level meter. Three trials were done with fifteen minutes interval per trial. All sound measurements from the selected pens were recorded in a data form. The difference in the values obtained by the health care workers were determined.

**Phase III. Cross Sectional Design or Validation study**

Phase III was a hospital-based trial. Inter and intra rater variability tests were conducted among health care workers selected to do the pen click test. Three videos of an actual pen click test were presented to three health care workers to determine inter rater variability and one video of an actual pen click test was shown twice to a healthcare worker for the intra-rater variability. This part of the study showed the reliability of health workers from the two government institutions in arriving with a correct assessment.

The principal investigator oriented the parents or guardians of the newborn on the importance, objectives and the benefits of the study. A written informed consent (Appendix 1) was obtained from the parents or guardians prior to the commencement of the study. Inclusion criteria were all healthy newborns with normal otoscopic examination findings. Excluded in the study were preterm newborns, sick neonates and those with ear deformities.

Phase III of the study involved the pen click as the sound stimulus. The Acroball pen had the highest mean decibel and was used as the sound stimulus based on the results generated in phase I. Acroball pen is readily available, accessible and produced the least variable level of sound intensity. A trained health care worker facilitated the pen click test. The study was done at the audiology room of two tertiary government hospitals. The audiology room was quiet with a comfortable environment.

The newborns included in the study were on their 2$^{nd}$ day to 30$^{th}$ day of life. The newborns were in a comfortable condition prior to the study. A comfortable condition was described as asleep, in a fed state or awake but has no abrupt movements and is not crying. The healthcare worker stood on the side of the patient's ear to be examined. An ear plug was applied on the contralateral ear prior to the test. The same procedure was done to the other ear. The health care worker positioned the pen two inches away from the test ear. Pen click test was administered to the newborns for three trials with fifteen minutes interval per trial. The results of the test were recorded as "pass" if blinking, startling or crying was observed from the newborn. The test was labelled "fail" in the absence of the blinking, startling and crying in two out of three trials. The pen click test was recorded with a video camera. An assistant investigator determined or observed the reaction of the newborns. An assistant investigator unknowledgeable of the study objective performed an otoacoustic emission test at the hearing unit of the two tertiary government hospitals. The pens were replaced every 10 patients. All pens used in this study were calibrated every two days.

Descriptive statistics were used to summarize the clinical characteristics of the patients. Frequency and proportion were used for nominal variables and range for ordinal variables. Coefficient of variation was used to determine inter-rater agreement. Intra-class coefficient was used to determine reliability of intra-rater agreement. Sensitivity, specificity, Negative Predictive Value, Positive Predictive

Value, and likelihood ratios were used to determine the diagnostic quality and accuracy of the Pen Click Test compared to otoacoustic emission test as a hearing screening tool among newborns in two tertiary government hospitals. Kappa agreement was also used to test inter-rater reliability.

All valid data was included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05 α-level of significance. STATA 15.0 was used for data analysis.

The study protocol was submitted and approved by the Institutional Review Board – Ethics Committee of two tertiary government hospitals. A written informed consent was obtained from the parents prior to inclusion to the study. The primary investigator explained in Filipino the nature, objectives and possible risks of the study to the parents or guardians of the newborns in Filipino language. The parents or guardians were informed that a video of the pen click test was obtained during the procedure. Personal information of the participants was kept confidential. The results of the tests were disclosed to the parents or guardians. The babies with their mother were requested to stay for forty-five more minutes from the usual procedure of hearing screening. The research assured that no harm was inflicted to the participants. Throughout the procedure, the investigator avoided unnecessary noise, pain or stimuli that can disturbed the patient. Normothermia was always maintained .

## RESULTS

### Phase I. Randomized Complete Block Design

Phase I of the study determined the variability of the pen click test using Pilot Acroball, BIC and HBW. Acroball pen showed the highest decibel obtained from TES sound level meter with the mean of 79.1 decibels. In comparison, HBW ball pen showed the lowest decibel with a mean of 77.4.

We sought to compare the intra-rater agreement using three pen types. In all pens, the intra-rater coefficient of variations was all below 5%, which is deemed acceptable for repeatability testing. However, the 95% Confidence Interval for Coefficient of Variation for Acroball 2, Acroball 3, BIC 1 included values slightly higher than 5%. (Table 1)

| | %CV (95% CI) | ICC (95% CI) | P-Value |
|---|---|---|---|
| Pilot Acroball | | | |
| Acroball 1 (n=3) | 1.76% (0.35% - 3.16%) | 0.167 (0.07 – 0.89) | 0.390 |
| Acroball 2 (n=3) | 3.4% (0.68% - 6.12%) | | |
| Acroball 3 (n=3) | 3.0% (0.6% - 5.42%) | | |
| HBW | | | |
| HBW 1 (n=3) | 1.94% (0.39% - 3.49%) | 0.432 (0.05 – 0.78) | 0.283 |
| HBW 2 (n=3) | 2.27% (0.45% - 4.09%) | | |
| HBW 3 (n=3) | 1.0% (0.2% - 1.85%) | | |
| BIC | | | |
| BIC 1 (n=3) | 3.97% (0.79% - 7.14%) | 0.443 (0.23 – 0.69) | 0.278 |
| BIC 2 (n=3) | 2.13% (0.43% - 3.83%) | | |
| BIC 3 (n=3) | 1.0% (0.2% - 1.8%) | | |

%CV – Coefficient of Variation percentage; ICC – Intraclass correlation coefficient

**Table 1.** Intra-rater and inter-rater agreement using three pen types in Phase 1 of the study

### Phase II. Inter-rater and Intra-rater Randomized Block Design

Phase II of the study measured the inter-rater and intra-rater variability among health workers regardless of pen type. This phase of the study showed the reproducibility of the test among health care workers in the community. Table 3 outlines the 95% confidence interval of decibels produced by each health worker. The mean decibel of each health worker was within the 95% Confidence Interval. In most of the health workers, the intra-rater coefficient of variations were all below 5%, which is deemed acceptable for repeatability testing. However, the 95% Confidence Interval for Coefficient of Variation for Health Worker 6 and Health Worker 9 included values slightly higher than 5%.

In the inter-rater agreement, the Intraclass Coefficient (ICC) point estimate was at 0.543 ($p = 0.031$), halfway between 0 to 1, which indicated that neither intra-rater nor inter-rater variation dominates. Result showed a wide 95% CI ranges for ICC (ranging from 0.04 to 0.87), However, the relatively low coefficients of variation indicated a good intra-rater agreement (Table 2).

| | %CV (95% CI) | ICC (95% CI) | P-Value |
|---|---|---|---|
| Health worker 1 | 1.87% (0.37% - 3.37%) | | |
| Health worker 2 | 0.64% (0.13% - 1.15%) | | |
| Health worker 3 | 2.14% (0.43% - 3.86%) | | |
| Health worker 4 | 1.84% (0.37% - 3.3%) | | |
| Health worker 5 | 1.81% (0.36% - 3.25%) | 0.543 (0.04 – 0.87) | **0.031** |
| Health worker 6 | 3.03% (0.61% - 5.46%) | | |
| Health worker 7 | 1.89% (0.38% - 3.39%) | | |
| Health worker 8 | 2.28% (0.46% - 4.11%) | | |
| Health worker 9 | 3.11% (0.62% - 5.59%) | | |
| Health worker 10 | 1.95% (0.39% - 3.51%) | | |

%CV – Coefficient of Variation percentage; ICC – Intraclass correlation coefficient

**Table 2.** Intra-rater and inter-rater agreement among ten health workers in Phase II of the study

### Phase III. Cross Sectional Design or Validation study

A total of 97 newborns, 2 to 30 days of age with a mean age of 6 days was enrolled in this study. The male to female ratio was 1:1.3. A total of 194 ear subjects were tested with pen click test and OAE.

The accuracy of pen click test as compared to OAE was determined through computation of the sensitivity and specificity of the test. Results showed that 43.48% of patients with hearing impairment have a positive pen click test. In comparison, 98.25% of patients without hearing impairment have a negative pen click test. (see Table 3)

The estimation of the probability of the presence or absence of the disease was determined through the computation of the positive predictive value and negative predictive value. When pen click test shows a positive result, we have 76.92% probability that a positive result will be obtained in OAE. In comparison, we have 92.82% probability that the patient will have a negative OAE result when pen click test is negative. (Table 3)

A useful measure in the interpretation of diagnostic test is the likelihood ratio. Patients who are OAE positive are 24.78 times more likely to yield a positive pen click test compared to patients who are OAE negative (LR+) and are 58% less likely to yield a negative pen click test result (LR-) (Table 3). Overall, the accuracy of the pen click test, when compared against OAE in screening for hearing loss, is 91.75% (Table 3)

| Pen click test | Otoacoustic Emission test | | Total |
|---|---|---|---|
| | Positive/Refer | Negative/Pass | |
| Positive/Refer | 10 (5.15) | 3 (1.55) | 13 (6.7) |
| Negative/Pass | 13 (6.7) | 168 (86.6) | 181 (93.3) |
| Total | 23 (11.86) | 171 (88.14) | 194 (100) |

| | | | |
|---|---|---|---|
| Sensitivity (Sn) | 43.48% (23.2% – 65.5%) | Positive LR | 24.78 (73.36 – 83.5) |
| Specificity (Sp) | 98.25% (95% – 99.6%) | Negative LR | 0.58 (0.4 – 0.82) |
| PPV | 76.92% (49.7% – 91.8%) | Accuracy | 91.75% (87% – 95.2%) |
| NPV | 92.82% (90% – 94.9%) | | |

PPV, positive predictive value; NPV, negative predicted value; LR, likelihood ratio

**Table 3.** Diagnostic accuracy of Pen Click Test as compared to Otoacoustic emission test as a hearing screening tool among newborns in two tertiary government hospitals (n = 194)

The kappa values were interpreted according to the guidelines adapted from Landis and Koch. As shown in Table 4, Pen Click Test and OAE has moderate agreement based on Kappa Coefficient.

| | Kappa Coefficient | | | |
|---|---|---|---|---|
| | Agreement | (95% CI) | Interpretation | P-Value |
| Pen click test | 91.75 | 0.514 (0.31 – 0.72) | Moderate | **<0.001** |

**Table 4.** Kappa agreement (n = 194)

## DISCUSSION

Newborn Hearing Screening Act or Republic Act 9709 was approved in the Philippines with the purpose to "institutionalize measures for prevention and early diagnosis of congenital hearing loss among newborns". [9] However, the availability of an accessible and cost-effective tool is a major problem among distant communities.

Hearing impairment refers to hearing loss greater than 30 decibels among children. Hearing loss may be described as mild, moderate or severe depending on how a person can hear the loudness or intensity of a sound stimulus. Determining the proper stimulus is very important for a hearing screening tool as being presented in the Phase I of the study. Criteria for the hearing screening stimulus in this study

includes the highest decibel produced and its availability in the community. Acroball recorded the highest decibel with a mean of 79 (ranges from 77.6 to 83.9 decibels). A normal ear is able to hear at a range of 25 to 75 decibels. With the use of Acroball pen as the study stimulus, we are able to identify ear subjects that may have hearing impairment. Hearing impairment is present if the ear subject is unable to identify the sound stimulus greater or equal to 77 decibels.

The reproducibility of the study is also essential in finding a diagnostic test such as a hearing screening tool. In Phase II of the study, health workers were randomly selected and were asked to produce the sound stimulus using the Acroball pen obtained from Phase I. The mean decibels ranging from 73 to 77.9 were computed from the three trials done by each health worker. The results showed that all health workers were able to produce a sound stimulus of more than 70 decibels which is a requirement in the study. Results in the study showed most of the health workers had no significant difference among each other which means that there is minimal deviation from the mean decibel. The test can be reproduced by most of the health care workers and may be applied in the community.

Validation study allows evaluation of an index test in comparison to the reference test in determining subjects with the target disease. Test validation involves the basic measure in quantifying a diagnostic accuracy of a test such as the sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratios. [10]

The pen click test has a high specificity of 98. 25%, which would indicate those ears that have no hearing impairment is correctly identified by the index test. This means that 98.25% of ears tested without hearing impairment will have a negative result in pen click test. The sensitivity of the test measures the capability in identifying subjects with the disease.[9] The pen click test has a sensitivity of 43.48% which means that we would expect 43.48% of ears tested with hearing impairment to have a positive pen click test result. Pen click test is a highly specific test which can be used as a screening tool to rule in patients with hearing impairment.

The positive and negative predictive values were also computed. The positive predictive value is the probability that the disease is truly present given a positive result is obtained. [9] The test showed a positive predictive value of 76.92% and negative predictive value of 98.25%. Based on prevalence of hearing impairment, we would expect 76.92% of ears with positive results to have hearing impairment while 98.25% of ears with negative results to have no hearing impairment. Using the 2x2 table, the accuracy of the study was computed to be at 91.75% which means that the results obtained from pen click test is close to the results from the standard hearing screening test (OAE).

The likelihood ratio of positive results tells us how well the test performs in the study population. [16] The likelihood ratio for a positive test in the present study is 24.78. This means that a child with hearing impairment is 24.78 times more likely to have a positive result than a child without hearing impairment.

Kappa agreement showed moderate result with a Kappa coefficient of 0.541. This measures interrater variability or the consistency among individuals who underwent data collection.[14]

Based on the results of validity and association test, the Pen click test can be an accurate hearing screening tool in identifying ear subjects of newborns with high suspicion of hearing impairment. The test is readily available for communities without facilities for OAE or ABR as hearing screening tool. The procedure done in the pen click test is easily reproducible and health workers may be trained to standardize the sound stimulus. This screening may encourage more health workers to be involved in community work should this test be considered in the community. As a primary health care worker, early recognition and timely intervention if necessary is of outmost important.

## CONCLUSION AND RECOMMENDATION

The study showed high specificity of 98.25% which signifies that Pen Click test is a good screening tool in ruling in patient with hearing impairment. Pen click test has a sensitivity of 43.48%. There is a positive

association of Pen Click test to the standard hearing audiometer with a positive predictive value of 76.92% and negative predictive value of 98.25% as evident in the study. Thus, a child with hearing impairment is 24.78 times more likely to have a positive result than a child without hearing impairment. Kappa agreement which measures the inter-rater variability showed moderate result with a Kappa coefficient of 0.541. Pen click test has a high accuracy rate of 91.75%. Therefore, application of this test in the community may be done as a hearing screening tool in comparison with the standard hearing screening tool.

This study provides an accessible and easily reproducible tool for hearing screening that may be applied in communities without facilities. This hearing screening tool will help in early recognition and intervention to prevent profound hearing impairment.

This study recommends involvement of a larger sample size and a longer study period. More health workers should be involved in phase II in assessing the reproducibility of the hearing screening tool. Hearing screening test should be implemented in all communities.

**REFERENCES**


1. World Health Organization. Newborn and infant hearing screening current issues and guiding principles for action.2010;7-23

2. Better Hearing Philippines, Prevalence of Hearing Impairment in the Philippines, Retrieved from http://bhphil.org/downloads/prevalence_of_ear.pdf

3. Ptok,M. Early detection of hearing impairment in newborns and infants. Dutsch Arztebl International.2011;108(25):426-31

4. Naeem Z and Newton V. Prevalence of sensorineural hearing loss in Asian children. 1996; 30(5):332-339

5. Gloria-Cruz T, Abes G, Abes F. The voice test for newborn hearing screening. Acta Medica Philippina.2012; 46(3): 46-51

6. Pirozzo S , Papinczak, T, Glasziou P. Whispered voice test for screening for hearing impairment in adults and children: systematic review. BMJ.2003.327:967s

7. Garcia M, Chiong C, Abes G, Carrillo R. Accuracy of reflexive behavioral (“Baah”) test in the screening for hearing impairment in infants six months old and below. Philippine Journal of Otolaryngology-Head and Neck Surgery.2012; 27(1): 6-11

8. Subroto O.et al, A simple ballpen click as a tool for screening of hearing impairment in Filipino school children in Metro Manila”.The Philippine Journal of Pediatrics. 1995; 44(4): 249-252.

9. Patrick M. Bossuyt, Johannes B. Retisma, David E. Bruns. The STARD Statement for Reporting Studies of Diagnostic Accuracy: Explanation and Elaboration. 2003; 49:7-18.

10. Hwee Bee Wong and Gek Hsiang Lim. Measures of Diagnostic Accuracy: Sensitivity, Specificity, PPV and NPV. 2011;20 (4): 316-318.

11. 11.Wrightson,Stevens. Universal Newborn Hearing Screening.American Family Physician.2007; 75(9); 1349-1352

12. Republic Act 9709.Republic of the Philippines, Fourtheen Congress, Second Regular Session, Metro Manila. Senate and House of Representatives of the Philippines.

13. Sedighi,Iran.Interprretation Diagnostic Tessts: Likelihood ratio vs Predictive value. 2013. Kowsar Medical Institute; 23(6): 717

14. McHugh, Mary. Interrater reliability: The Kappa Statistic. 2012. Biochem Med; 22(3): 276-282

# IMMEDIATE RESCUE REVERSAL OF ROCURONIUM-INDUCED INTENSE NEUROMUSCULAR BLOCKADE USING SUGAMMADEX IN PEDIATRIC SURGICAL PATIENTS

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**ABSTRACT**

**BACKGROUND:** The dose of Sugammadex for rescue reversal of intense neuromuscular block has not been studied in children. The only recommended dose of Sugammadex in children is 2mg/kg to reverse a shallow block.

**OBJECTIVES:** To assess the efficacy and safety of Sugammadex 2mg/kg and 4mg/kg as immediate rescue reversal of intense rocuronium-induced neuromuscular block in pediatric patients

**METHODS:** 80 children, aged 2 to 11 years old, requiring general anesthesia were enrolled in this randomized prospective study. Group 1 given Sugammadex 2mg/kg (40 subjects) while Group 2 received Sugammadex 4mg/kg (40 subjects), at the end of the procedure if PTC=0. The Recovery Time was recorded (TOF ratio ≥0.9) (Primary Outcome). Discharge readiness in the PACU was assessed using Modified Aldrete Scale (Secondary Outcome). Monitoring of adverse effects in the ward continued until 24 hours postoperatively.

**RESULTS:** There were significantly more patients in the Sugammadex 4mg/kg that had a recovery time of ≤2min as compared to those given Sugammadex 2mg/kg (p=0.012). There was no significant difference in the Aldrete score between the two groups (p=0.2776). All patients achieved a very satisfactory discharge score in the PACU. The adverse effects experienced by the patients in the two doses of Sugammadex in the PACU and up to 24 hours postoperatively were not significantly different.

**CONCLUSION:** Sugammadex 4mg/kg can be considered safe and effective as an immediate reversal agent for rocuronium-induced intense neuromuscular blockade in children.

**RECOMMENDATION:** Clinicians should identify if Sugammadex 6mg/kg, compared with 4mg/kg, would translate to a shorter Recovery time to a TOF ratio of 0.9. The time from TOF ratio of 0.9 to the time of extubation should be measured to increase the efficacy and safety assessment of Sugammadex in this age group.

**KEYWORDS:** Sugammadex, Immediate Rescue Reversal, Intense Neuromuscular Blockade

## INTRODUCTION

The difficulty of airway management is usually due to either difficulty in performing adequate mask ventilation or in successfully achieving tracheal intubation[1]. Difficulty with endotracheal intubation may occur unexpectedly even under controlled situations such as during induction of anesthesia in the operating room[2]. Although some difficult airways can be predicted, even the most thorough assessment of the airway may not detect the possibility of a difficult intubation and associated problems with ventilation of the patient[2]. Failure to oxygenate by face mask or supraglottic device occurring in conjunction with failed tracheal intubation defines a failed oxygenation, "cannot intubate, cannot oxygenate" situation (CICO)[3]. CICO or CICV ("cannot intubate, cannot ventilate") situations are rare anesthetic emergencies[4], but if they happen respiratory complications[5,6] and catastrophic outcomes including cerebral anoxia and death can occur[2].

In a study by Heinrich et al[7], the incidence of difficult direct laryngoscopy

(Cormack-Lehane grade 3 or 4 views) was 4.7% in children less than one year of age and 0.7% in children older than one year. Meanwhile, Murat et al[6] cited that the frequency of unanticipated difficult tracheal intubations was 0.24% in children less than one year of age and 0.07% in children older than one year.

For more than 50 years, acetylcholinesterase inhibitors have been used to speed up the recovery from non-depolarizing neuromuscular blockade[8]. However, acetylcholinesterase inhibitors like neostigmine are ineffective against profound block[8].
The modified γ-cyclodextrin Sugammadex can reverse any degree of block induced by rocuronium, in a dose-dependent manner[8,9].

Sugammadex rescue reversal is recommended to be retained for use in unanticipated difficult airways[9,10]. When facing a CICV scenario following rocuronium induction, anesthesiologists need to have an appropriate dose of sugammadex immediately available[9].

In pediatric patients with unanticipated difficult airway requiring immediate neuromuscular blockade reversal, what is the effective dose of sugammadex? Is this dose safe for the pediatric population? While sugammadex can be relied upon for immediate reversal of rocuronium-induced blockade in adults, the age-related change in efficacy of sugammadex and an adequate dose of sugammadex in pediatric patients have not been completely investigated[11]. Although a few studies have shown successful off-label use of sugammadex in younger patients[12,13,14] no recommendation is made until further data become available[15].

## OBJECTIVES OF THE STUDY

### General Objective

1. To evaluate the efficacy and safety of Sugammadex as an immediate reversal agent for Rocuronium-induced intense neuromuscular blockade in children ages 2-11 years old undergoing Surgical Procedures under General Anesthesia in Philippine Children's Medical Center

### Specific Objectives

1. To describe the clinical profile of the patient population

2. To identify the Recovery Time (RT) from administration of Sugammadex to a TOF ratio of 0.9 in both groups (2mg/kg and 4mg/kg) (Primary Outcome)
    a. RT to TOF 0.9 ≤ 2 minutes
    b. RT to TOF 0.9 > 2 minutes

3. To assess the level of consciousness, vital signs, neuromuscular function, and pain control using the Modified Aldrete Scale in both groups

4. To investigate the adverse effects of Sugammadex during the postoperative stay in the recovery room and during the 24-hour postoperative visit (Secondary Outcome) in both groups

## METHODOLOGY

This is a double-blind randomized control trial conducted in the Philippine Children's Medical Center, a specialized pediatric government hospital. The study protocol was evaluated by the Institutional Review Board and was conducted in accordance with the International Conference on Harmonization Guidelines / Good Clinical Practice, and current regulatory requirements. The subjects were screened a day prior to the procedure. History-taking and Physical examination by the investigator included a Review of Systems Checklist. A baseline PT, PTT, SGPT and creatinine were drawn from the patient (5ml blood sample). Written Assent Form and Informed Consent were obtained from the subjects and their parents by the investigator prior to the procedure and before obtaining the blood sample. The 80 subjects were randomized into two groups as follows: Group 1 – Sugammadex 2mg/kg (40 subjects); and Group 2 – Sugammadex 4mg/kg (40 subjects). Random assignment of study subjects was done using computer-generated random numbers which were prepared prior to start of recruitment. Sealed envelopes containing the assignment were placed sequentially in a box. The anesthesiologist (other than the investigator) who opened the envelope prepared the drug and administered it to avoid bias. The study dose and rescue dose of Sugammadex were prepared respectively in similar-looking tuberculin

syringes and were concealed from the investigator.

The trial comprised 4 periods, namely:

1) a screening period
2) a perianesthetic period
3) a postanesthetic period that consisted of an assessment prior to recovery room discharge
4) a postoperative visit by the safety assessor within 24h after the study drug administration

Adverse events (AEs) and serious AEs were monitored and recorded by the assessor during the said postoperative period. The investigator was the assessor in all 4 periods.

Patients were eligible for the trial if they fulfilled the following inclusion criteria: categorized as American Society of Anesthesiologists (ASA) class 1 or 2 (Appendix Table 7); age 2 years-11 years old, inpatient, scheduled to undergo a minor/major surgical procedure (extended 2 hours at most) under general anesthesia. Patients were excluded from participation in the study if they had anticipated difficult airway, cardiac disease, neuromuscular disease, liver and/or renal failure, had coagulopathy or bleeding disorders, or were using medication known to interact with rocuronium (toremifene, flucloxacillin, fusidic acid, magnesium, anticonvulsants), had a family history of malignant hyperthermia or allergy to any medication used during general anesthesia, or using an inadequate method of contraception.

The study was planned to compare a continuous outcome variable from independent control and experimental subjects with 1 control per 1 experimental subject. Based on previous study conducted by Sparr et al entitled, "Early Reversal of Profound Rocuronium-induced Neuromuscular Blockade by Sugammadex in a Randomized Multicenter Study", the response variable is normally distributed with a standard deviation of 0.95. If the true difference of the means between the 2 groups is 0.6, a sample size of 40 experimental subjects and 40 control subjects were needed to be able to reject the null hypothesis that the population means of the experimental and control groups were equal with 0.8 power. The type I error associated with the test was 0.05.

**Data Collection Procedure**

Upon arrival of the patient at the operating room with the parent, noninvasive automatic monitoring devices for arterial blood pressure, oxygen saturation, and electrocardiography were applied. The neuromuscular monitoring device was applied by placing two small electrocardiography (ECG) electrodes on the wrist over the ulnar nerve to stimulate the adductor pollicis muscle. The acceleration transducer was placed on the thumb to record evoked motor responses. Neuromuscular monitoring was performed in accordance with Good Clinical Research Practice with TOF-Watch®SX, Organon Ireland Ltd after administration of propofol. Alternate site for neuromuscular monitoring included the posterior tibial nerve. The negative electrode (black) was placed over the inferolateral aspect of medial malleolus while the positive electrode (red) was placed 2-3cm proximal to the negative electrode. This stimulated the flexor hallucis brevis muscle to elicit plantar flexion of the big toe. However, this was not utilized in the study since all patients were accessibly monitored on their upper extremities. Induction of General Anesthesia was performed using the following agents: atropine 0.02mg/kg, fentanyl 2mcg/kg, midazolam 0.1 mg/kg, and propofol 2mg/kg IV while patients received 100% oxygen through an anesthesia facemask. The TOF Watch was calibrated after induction and before the neuromuscular blocker was administered. Tracheal intubation was performed with rocuronium 1 mg/kg. Anesthesia was maintained with 2.5-3% end-tidal concentration of sevoflurane. Monitoring of the depth of neuromuscular block intraoperatively was obtained every 20 minutes using the TOF Watch, and an intense level of neuromuscular blockade (TOF 0, PTC 0)[80] was maintained all throughout the procedure until the end. Incremental dose of Rocuronium 0.2mg/kg was given when the first response to the PTC was detected[100]. At the end of the procedure, level of paralysis was verified. If the PTC showed 0, the subject was given one of the sugammadex treatment doses: 2mg/kg or 4 mg/kg.

If the PTC was ≥1, the subject was given top-up dose of Rocuronium 0.2mg/kg. To verify intense blockade, a repeat PTC was performed 6 min after the first take of PTC to avoid underestimation of block. If PTC was

equal to 0, then the patient was given either of the sugammadex doses based on the randomization prepared. Neuromuscular monitoring was continued after administration of the reversal agent Sugammadex. TOF monitoring was obtained every 15 seconds and the patient's airway remained intubated until the standard for safe extubation (TOF ≥0.9)[76] was achieved. Patient remained anesthetized with Sevoflurane at 2-2.5% end-tidal concentration during TOF monitoring and was discontinued after TOF 0.9 was reached. The time from sugammadex administration to recovery (RT) of neuromuscular function (TOF ratio ≥ 0.9) was recorded (Primary Outcome). If Recovery Time (RT) of ≤2 minutes was achieved, patient was extubated. If RT exceeded 2 minutes, an incremental single dose of sugammadex 2 mg/kg was given intravenously. The Recovery Time to TOF ≥0.9 was recorded, and patient was only extubated at this TOF value. The investigator facilitated the perianesthetic period, but during this time, another anesthesiologist maintained the patient so that the investigator focused on the recording of Recovery Time to TOF ≥0.9. This subject was included in the Intention to Treat Group, and results were analyzed. They were monitored in the postanesthetic period in the recovery room as well as 24 hours postoperatively.

Starting before transfer to the recovery room (after tracheal extubation), patients were assessed by the investigator every 15 minutes for an hour for clinical signs of residual and recurrence of neuromuscular blockade in the postoperative period until PACU discharge. This included an assessment of the patient's level of consciousness, vital signs, pain control, and adverse effects using the Modified Aldrete Scale[94] (Appendix Table 3) (Secondary Outcome). The Modified Aldrete Scoring was used to assess discharge readiness from the recovery room. Adverse effects such as nausea, vomiting, bleeding, flushing, urticaria, and pyrexia were noted.

Then within 24 hours post operatively, the subject was reassessed in the ward every 8 hours by the investigator. The presence of adverse events described above was investigated (Secondary Outcome). During the said post-operative visit, a physical examination was done, and vital signs were noted.

In the event of complications arising from the study (may or may not be directly related to Sugammadex) such as prolonged/recurrent curarization, bradycardia, anaphylaxis, and other adverse effects mentioned above, the Department of Pediatric Anesthesia of this institution was responsible to provide intervention.

Primary outcome was the Recovery Time (RT) defined as the time from administration of Sugammadex to recovery of neuromuscular function measured by a TOF ratio of 0.9. TOF monitoring at this time was done every 15 seconds. Secondary outcome were the clinical signs of neuromuscular recovery, pain control, level of consciousness, and vital signs. This was done every 15 minutes in the Recovery Room. The Modified Aldrete Scale was used as reference. Measurement of Secondary Outcome extended into the 24-hour postoperative visit and included an assessment of adverse drug effects every 8 hours.

Because of the unreliability of visual/tactile assessment of neuromuscular function[95], a quantitative device such as a TOF-Watch® SX, Organon Ireland Ltd was used in this study. In an article by McGrath[76], a nerve stimulator should be battery operated and be able to deliver a constant current, up to a maximum of 80 mA. At a constant voltage, current will vary depending on the resistance of the skin. The skin should always be cleansed with alcohol adequately before applying the electrodes. The nerve stimulator should be capable of delivering a variety of pattern of stimulation including: single twitch (at 1 Hz); TOF twitch stimulation (usually 2 Hz with at least a 10 second interval between trains); tetanic stimulation at 50 Hz for up to 5 s; and double-burst stimulation (DBS). The ideal stimulator also enabled monitoring of the evoked responses. In this study, a PTC value of 0 was obtained before Sugammadex administration, and a TOF ratio of 0.9 or greater was the goal prior to extubation.

Data were encoded and analyzed using Stata 14 MP. Baseline characteristics of the two groups were presented in tabular form. Continuous variables such as age and weight were reported as mean±standard deviation, and t-test was used for comparison between the two groups. On the other hand, categorical variables

such as ASA score were reported as frequency and percentages, and chi-square test was used for comparison between the two groups. The association between the two treatment groups and the recovery time as well as adverse events was analyzed using $\chi^2$ test, while the association between the Aldrete scores between the two groups was analyzed using Mann-Whitney test. A p-value of <0.05 was considered statistically significant.

## RESULTS

There were a total of 80 patients included in the study, of which 40 were given 2 mg/kg Sugammadex dose and another 40 patients were given 4 mg/kg Sugammadex dose. The mean age of the patients in the two groups was 6 ranging from a little over 2 years to 11 years of age. There were more males than females but no significant difference in sex distribution was noted between the two groups.

### Clinical Profile

More than half of the patients were classified as ASA 1 (58.8) and the rest were classified as ASA 2. The mean weight of the patient was 21 kgs in both groups. The mean surgery time was observed to be at 2.0 hours ranging from less than 1 hour to more than 5 hours. There were 95% of the patients in the Sugammadex 2 mg/kg group who were given Sugammadex rescue compared to only 75% in the Sugammadex 4 mg/kg group (*p-value*=0.012). The mean recovery time to a TOF ratio 0.9 was 321 seconds in the group given Sugammadex 2mg/kg *vs.* 229 seconds in those given Sugammadex 4 mg/kg. Recovery time in patients who received 4mg/kg was significantly faster than those who received 2mg/kg (Table 1)

**Table 1.** Clinical Profile of the Patient Population

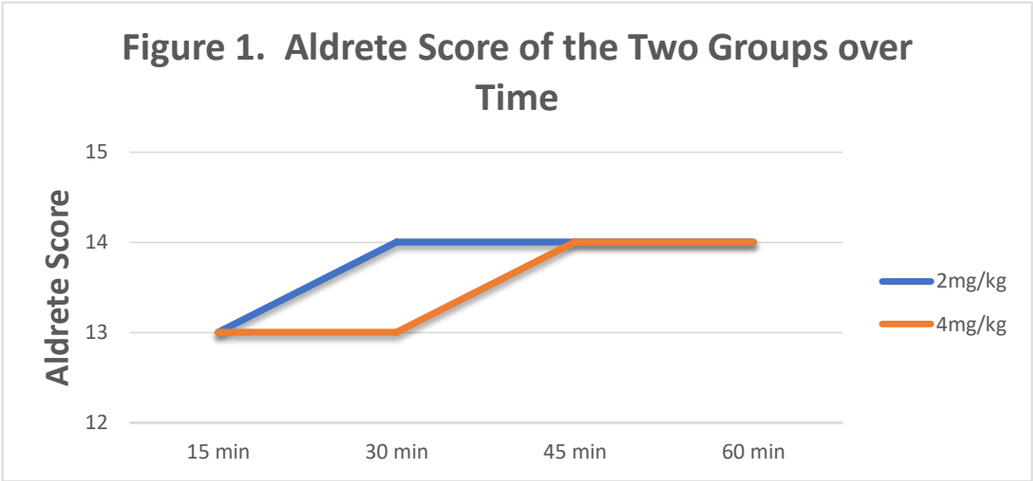
| Variable | Sugammadex 2 mg/kg N=40 | Sugammadex 4mg/kg N=40 | p-value |
|---|---|---|---|
| Age (yrs) | 6.46±3.08 | 6.26±3.08 | 0.7815 |
| Sex<br>Male<br>Female | <br>26 (65%)<br>14 (35%) | <br>27 (67.5%)<br>13 (32.5%) | 0.813 |
| ASA<br>1<br>2 | <br>23 (57.5%)<br>17 (42.5%) | <br>24 (60%)<br>16 (40%) | 0.820 |
| Weight (kg) | 21.21±8.38 | 21.90±11.11 | 0.7556 |
| Surgery time (min) | 120.18±67.16 | 121.68±79.0 | 0.9273 |
| Sugammadex Rescue<br>Yes<br>No | <br>38 (95%)<br>2 (5%) | <br>30 (75%)<br>10 (25%) | <br><br>0.012 |
| Recovery time to TOF 0.9 (sec) | 321.23±223.93 | 229.33±170.46 | 0.0422 |
| Modified Aldrete Score | 13.6±0.31 | 13.56±0.26 | 0.2766 |

The Recovery Time, from administration of Sugammadex to TOF ratio of 0.9, of 2 minutes or less was compared in terms of dosage, particularly 2 mg/kg and 4 mg/kg. The recovery time of 2 minutes or less occurred in about 5.0% of the patients given 2mg/kg while the same occurred in 25.0% of the patients given 4mg/kg. There were significantly more patients in the group given Sugammadex 4mg/kg that had a recovery time of ≤ 2min as compared to those given Sugammadex 2mg/kg (Table 2).

**Table 2.** Recovery Time (RT) from administration of Sugammadex to a TOF ratio of 0.9 in both groups (2mg/kg and 4mg/kg)

| Recovery Time to TOF 0.9 | Sugammadex 2mg/kg N=40 | Sugammadex 4mg/kg N=40 | p-value |
|---|---|---|---|
| ≤ 2min | 2 (5%) | 10 (25%) | 0.012 |
| >2 min | 38 (95%) | 30 (75%) | |

The median Modified Aldrete Score was also compared between the 2 groups. It is similar in the 2 groups. Aldrete score was recorded at 15-minute intervals in both groups. There was no significant difference in the Aldrete score between the two groups (p=0.2776).




The incidence of adverse effects in the PACU and during the 24-Hour Postoperative Period was compared between the 2 groups. The adverse effects experienced by the patients in the two doses of Sugammadex in the PACU and up to 24 hours postoperatively were not significantly different (Table 3).

**Table 3.** Adverse effects of Sugammadex in the Recovery Room and during the 24-Hour Postoperative Period in both groups

| Adverse events | Sugammadex 2mg/kg N=40 | Sugammadex 4mg/kg N=40 | p-value |
|---|---|---|---|
| PACU adverse events<br>Yes<br>No | <br>9 (22.5%)<br>31 (77.5%) | <br>6 (15%)<br>34 (85%) | 0.390 |
| Ward adverse events<br>Yes<br>No | <br>7 (17.5%)<br>33 (82.5%) | <br>5 (12.5%)<br>35 (87.5%) | 0.531 |

The most common adverse effects were vomiting, followed by low normal heart rate and hypertension. No significant difference was noted in the adverse effects between the two doses since a minimum sample size of 200 patients is needed to detect such a difference. No deaths occurred in the study (Table 4).

**Table 4**. Incidence of the Most Common Adverse Effects by Treatment Group in the PACU and During the 24-Hour Postop Period

| Adverse event | Sugammadex 2mg/kg N=40 | | Sugammadex 4mg/kg N=40 | |
|---|---|---|---|---|
| | PACU | Ward | PACU | Ward |
| Vomiting | 2 | 4 | 2 | 2 |
| Nausea | 1 | 0 | 0 | 1 |
| Low-normal Heart Rate | 3 | 1 | 2 | 2 |
| Hypertension | 1 | 1 | 1 | 0 |
| Bradycardia | 1 | 0 | 1 | 0 |
| Fever | 1 | 1 | 0 | 0 |
| Total | 9 | 7 | 6 | 5 |

## DISCUSSION

At the end of the procedure all 80 subjects reached a TOF ratio of 0.9. Between the 2 groups (Sugammadex 2mg/kg and 4mg/kg), there were more subjects (10 vs. 2) in the Sugammadex 4mg/kg group who achieved a Recovery Time to TOF ratio of 0.9 in less than 2 minutes. The observations in our study were consistent with the pharmacokinetics of Sugammadex showing a linear, dose-dependent relationship[15]. In a study by Plaud et al., when Sugammadex was administered at reappearance of T2 for the reversal of rocuronium-induced neuromuscular blockade in pediatric and adult surgical patients, a clear dose-response relationship was observed for children, adolescents, and adults with median times to a TOF ratio of 0.9 ranging from 4.6 to 0.6 min as the dose of Sugammadex increased from 0.5 mg/kg upwards[13]. Same findings were supported in two phase II studies[18,104] wherein a rapid and dose-dependent reduction in the mean time to recovery of the TOF ratio to 0.9 was shown from approximately 4.0 to 1.1 min with Sugammadex doses of 0.5–4.0 mg/kg, respectively, when administered at reappearance of T2 in adult patients with neuromuscular blockade induced by 0.6 mg/kg rocuronium[13,18,104]. Sugammadex dose of 4mg/kg was shown to be significantly more effective than Sugammadex 2mg/kg in children for the immediate rescue reversal of intense neuromuscular blockade. However, a clinical observation was made during the study. When the TOF ratio was ≥0.9 some patients still had abdominal breathing. A time lag of approximately 2-3 minutes was noted between the objective display of TOF ratio ≥0.9 and the clinical improvement from abdominal breathing to adequate chest rise and good tidal volume. Thus, a good clinical judgment as regards to the clinical parameters for extubation has to be exercised in correlation with the TOF reading especially in this population. A limitation of the study was that the time interval from the TOF reading of 0.9 to extubation was not recorded. This could have given us a deeper insight about the efficacy and safety profile of the particular study dose of Sugammadex.

Eventhough a time lag interval was observed between the TOF reading of ≥0.9 and improved clinical respiratory parameters for some patients in the higher dose group Sugammadex 4mg/kg, no patients developed residual neuromuscular blockade or recurarization in the PACU and within the 24-hour postoperative period in the two groups. All of the patients achieved a very satisfactory recovery discharge criteria based on Modified Aldrete Scale in the PACU. No adverse effects in the PACU and within the 24-hour postoperative period that led to discontinuation of a treated patient from the study. The median Modified Aldrete Scale was 14 in the two groups, out of a total score of 14. No scores below 1 were noted for the individual variables related to consciousness, activity, respiration, hemodynamics, oxygen saturation, pain, as well as nausea and vomiting. Minor or isolated adverse effects in the PACU include the following: 2 patients developed mild bradycardia[108](Appendix Table 8) which was responsive to single dose of atropine, 5 patients had low normal heart rate but were still given single dose atropine to achieve near-baseline value, 1 patient with nausea, 5 patients with vomiting (one of which was the same patient who developed low normal heart rate), 2 patients developed hypertension but asymptomatic, 1 patient had fever (37.9°C) which was controlled with tepid sponge bath. Similar side-effects were noted within the 24-hour postoperative period. The following were: mild vomiting (n=6, not likely to be related with Sugammadex alone), nausea (n=1), low normal heart rate (n=3, one was transient and no longer required atropine), hypertension (n=1, same patient who developed hypertension in PACU [130-140/60-70mmHg - 120/60mmHg] but continuously remained asymptomatic), and mild fever (n=1, 37.8°C). The most commonly occurring adverse effects in the two groups were vomiting and a low normal heart rate. The intensity of these adverse effects was described as mild to moderate. They were easily manageable and were not considered life-threatening. Furthermore, no serious adverse events suggestive of hypersensitivity and/or suspected anaphylaxis were observed during the study. The occurrence of adverse effects while in the PACU and during the 24-hour postoperative period did not differ significantly in the two groups. Sugammadex 4mg/kg was well-tolerated in children. The safety information collected in this study adds to the profile of Sugammadex established in previously published studies. In a study performed by

Plaud et al. and Sari et al.[13,92] across the different age groups (infants, children, and adolescents) the most common side effects were acute postoperative nausea / vomiting and pain related to the surgery. They could not directly correlate the side effects with Sugammadex[13,92]. An observation in our study was that the side effects of Sugammadex were usually noted within the first 15 to 30 minutes of administration. It is suggested that it should be diluted and given slowly especially in this population in order to minimize the occurrence of side effects.

## CONCLUSION

The dose of Sugammadex 4mg/kg was shown to be statistically effective in regaining spontaneous respiration in less than 2 minutes compared with Sugammadex 2mg/kg. All pediatric patients who received Sugammadex 2mg/kg and 4mg/kg, respectively, achieved Good Recovery Parameters in the PACU. Comparison of adverse effects both in PACU stay and during 24-hours postoperatively yielded no significant difference. The most frequently observed adverse effects were vomiting and low normal heart rate which was the same in both groups. The clinical severity of adverse effects was considered as mild to moderate. Sugammadex 4mg/kg compared with Sugammadex 2mg/kg can be considered safe and effective as an immediate reversal agent for rocuronium-induced intense neuromuscular blockade in children.

## RECOMMENDATION

While Sugammadex 4mg/kg showed statistically significant result compared with Sugammadex 2mg/kg in reversing an intense neuromuscular blockade in children, further study should be done to identify whether increasing the dose of Sugammadex from 4mg/kg to 6mg/kg, comparing the two groups, would translate to a shorter Recovery time to a TOF ratio of 0.9, thus, a faster return to spontaneous respiration. The time from arriving at a TOF ratio of 0.9 to the time of extubation should also be measured and compared between the two groups to increase the efficacy and safety assessment of Sugammadex in this age group. More documentation about the efficacy and safety profile of Sugammadex 6mg/kg in this special population should be performed and compared with the 4mg/kg. This effort could set the initial steps until the safe and appropriate dose for the immediate reversal of intense neuromuscular blockade in children will be established. This will indeed be lifesaving for many of our children who would be found in the real clinical scenario of an unanticipated difficult airway.

## REFERENCES


1. Jeff Harless, Ramesh Ramaiah, and Sanjay M Bhananker. Pediatric airway management. Int J Crit Illn Inj Sci. 2014 Jan-Mar; 4(1): 65–70.

2. Pennsylvania Patient Safety Authority. Management of Unanticipated Difficult Intubation ©2010 Vol. 7, No. 4 - December 2010.

3. J. Adam Law, MD,corresponding author Natasha Broemling, MD, Richard M. Cooper, MD, Pierre Drolet, MD, Laura V. Duggan, MD, Donald E. Griesdale, MD, Orlando R. Hung, MD, Philip M. Jones, MD, George Kovacs, MD, Simon Massey, MBBCh, Ian R. Morris, MD, Timothy Mullen, MD, Michael F. Murphy, MD, Roanne Preston, MD, Viren N. Naik, MD, Jeanette Scott, MBChB, Shean Stacey, MD, Timothy P. Turkstra, MD, David T. Wong, MD, and for the Canadian Airway Focus Group. The difficult airway with recommendations for management – Part 1 – Difficult tracheal intubation encountered in an unconscious/induced patient. Can J Anaesth. 2013; 60(11): 1089–1118.

4. Curtis R1, Lomax S, Patel B. Use of sugammadex in a 'can't intubate, can't ventilate' situation. Br J Anaesth. 2012 Apr;108(4):612-4. doi: 10.1093/bja/aer494. Epub 2012 Jan 26.

5. Bhananker SM, Ramamoorthy C, Geiduschek JM, Posner KL, Domino KB, Haberkern CM, Campos JS, Morray JP. Anesthesia-related cardiac arrest in children: update from the Pediatric Perioperative Cardiac Arrest Registry. Anesth Analg. 2007;105:344–350. [PubMed].

6. Murat I, Constant I, Maud'huy H. Perioperative anaesthetic morbidity in children: a database of 24,165 anaesthetics over a 30-month period. Paediatr Anaesth. 2004;14:158–166. [PubMed].

7. Heinrich S, Birkholz T, Ihmsen H, Irouschek A, Ackermann A, Schmidt J. Incidence and predictors of difficult laryngoscopy in 11,219 pediatric anesthesia procedures. Paediatr Anaesth. 2012;22:729–736. [PubMed].

8. Bom A. The Discovery and Preclinical Development of Sugammadex. MSD Research Institute, Newhouse, Scotland. 2010.

9. Tiberiu Ezri, Mona Boaz, Alexander Sherman, Marwan Armaly, Yitzhak Berlovitz. Sugammadex: An Update. The Journal of Critical Care Medicine 2016;2(1):16-21.

10. Mendonca C. Sugammadex to rescue a 'can't ventilate' scenario in an anticipated difficult intubation: is it the answer? Anaesthesia. 2013;68:795-9.

11. Miki Kasai, M.D., Chihiro Igarashi, M.D., Noriko Miyazawa, M.D., Ph.D., Shinichi Yamamoto, M.D., Ph.D., Takahiro Suzuki, M.D., Ph.D. Reversibility of rocuronium-induced profound neuromuscular blockade with sugammadex in neonates.

12. Meretoja MD, PhD. Neuromuscular block and current treatment strategies for its reversal in children. Pediatric Anesthesia 2010 20: 591–604. Department of Anaesthesiology, Hospital for Children and Adolescents, University of Helsinki, Finland.

13. Plaud B, Meretoja O, Hofmockel R, Raft J, Stoddart PA, van Kuijk JH, Hermens Y, Mirakhur RK. Reversal of Rocuronium-induced Neuromuscular Blockade with Sugammadex in Pediatric and Adult Surgical Patients. Anesthesiology 2009; 110:284–94.

14. Benigni A., Maffioletti M., Spotti A., Benigni A.M., Locatelli B.G., Sonzogni V. Efficacy and safety of a sugammadex dose of 4 mg/kg in early reversal of a deep neuromuscular block rocuronium-induced in infants and children: a case series.

15. Merck & Co Inc. BRIDION: EPAR – Product Information Annex I: Summary of product characteristics. European Medicines Agency; London, UK. 2013. Available from:http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Product_Information/human/000885/WC500052310.pdf. Accessed June 13, 2013.

16. Fuchs-Buder T. Neuromuscular Block Management by Current Reversal Agents and Sugammadex: An Update. University Department of Anaesthesia and Critical Care, Nancy / France. 2010.

17. Pühringer FK, Rex C, Sielenkämper AW, Claudius C, Larsen PB, Prins ME, Eikermann M, Khuenl-Brady KS. Reversal of profound, high-dose rocuronium-induced neuromuscular blockade by sugammadex at two different time points: an international, multicenter, randomized, dose-finding, safety assessor-blinded, phase II trial. Anesthesiology 2008; 109: 188–97.

18. Suy K, Morias K, Cammu G, Hans P, van Duijnhoven WG, Heeringa M, Demeyer. Effective reversal of moderate rocuronium- or vecuronium-induced neuromuscular block with sugammadex, a selective relaxant binding agent. Anesthesiology 2007; 106: 283–8.

19. Michael V Copp, Thomas F Barrett. Sugammadex: Role in current anaesthetic practice and its safety benefits for patients. World Journal of Anesthesiology. August 2015.

20. Caldwell J. Sugammadex: past, present and future. Adv Anaesth 2011; 29: 19-27 [DOI: 10.1016/j.aan.2011.07.007].

21. Steven Lobaz, Mark Clymer, Mario Sammut. Safety and Efficacy of Sugammadex for Neuromuscular Blockade Reversal. Clinical Medicine Insights:

Therapeutics 2014:6 1–14doi: 10.4137/CMt.s10241.

22. Osmer C, Vogele C, Zickman B, Hempelmann G: Comparative use of muscle relaxants and their reversal in three European countries: A survey in France, Germany and Great Britain. Eur J Anaesthesiol 1996; 13:389–99.

23. Naguib M. Pharmacology of muscle relaxant and their antagonist neuromuscular physiology and pharmacology. In: Miller RD,editor. Anaesthesia. 6th ed. Philadelphia: Churchill Livingston;2006. p. 481---572.

24. Barrow MEH, Johnson JK. A study of anticholinesterase and anticurare effects of some cholinesterase inhibitors. Br J Anaesth 1996; 38: 420–31.

25. Feldman A, Fauvel N. Recovery from a neuromuscular block. In: Pollard B, ed. Applied Neuromuscular Pharmacology. Oxford: Oxford University Press, 1994; 107–22.

26. van den Broek L, Proost JH, Wierda JM: Early and late reversibility of rocuronium bromide. Eur J Anaesthesiol Suppl 1994; 9:128–32.

27. Kirkegaard-Nielsen H, Helbo-Hansen HS, Lindholm P, Severinsen IK, Pedersen HS, Jansen EW: Optimum time for neostigmine reversal of atracurium induced neuromuscular blockade. Can J Anaesth 1996; 43:932–8.

28. Bevan JC, Collins L, Fowler C, Kahwaji R, Rosen HD, Smith MF, de Scheepers LD, Stephenson CA, Bevan DR. Early and late reversal of rocuronium and vecuronium with neostigmine in adults and children. Anesth Analg 1999; 89: 333–9.

29. Meretoja OA, Gebert R. Postoperative neuromuscular block following atracurium or alcuronium in children. Can J Anaesth 1990; 37: 743–746.

30. Bartkowski RR. Incomplete reversal of pancuronium neuromuscular blockade by neostigmine, pyridostigmine, and edrophonium. Anesth Analg 1987; 66: 594–598.

31. Beemer GH, Bjorksten AR, Dawson PJ, Dawson RJ, Heenan PJ, Robertson BA. Determinants of the reversal time of competitive neuromuscular block by anticholinesterases. Br J Anaesth 1991; 66: 469–475.

32. Cronnelly R, Morris RB, Miller RD. Edrophonium: duration of action and atropine requirement in humans during halothane anesthesia. Anesthesiology 1982; 57: 261–266.

33. Kirkegaard-Nielsen H, Helbo-Hansen HS, Lindholm P, Severinsen IK, Bülow K. Time to peak effect of neostigmine at antagonism of atracurium- or vecuronium-induced neuromuscular block. J Clin Anesth 1995; 7: 635–639.

34. Srivastava A, Hunter JM. Reversal of neuromuscular block. Br J Anaesth 2009; 103: 115–129.

35. Barber HE, Calvey TN, Muir KT. The relationship between the pharmacokinetics, cholinesterase inhibition and facilitation of twitch tension of the quaternary ammonium anticholinesterase drugs, neostigmine, pyridostigmine, edrophonium and 3-hydroxyphenyltrimethylammonium. Br J Pharmacol 1979; 66: 525–530.

36. Ansermino JM, Sanderson PM, Bevan JC et al. Acceleromyography improves detection of residual neuromuscular blockade in children. Can J Anaesth 1996; 43: 589–594.

37. Murphy GS, Szokol JW, Marymont JH, Greenberg SB, Avram MJ, Vender JS, Nisman M. Intraoperative acceleromyographic monitoring reduces the risk of residual neuromuscular blockade and adverse respiratory events in the postanesthesia care unit. Anesthesiology 2008; 109: 389–398.

38. Pino RM. Residual neuromuscular blockade: a persistent clinical problem. Int Anesthesiol Clin 2006; 44: 77–90.

39. Bevan DR, Smith CE, Donati F. Postoperative neuromuscular blockade: a comparison between atracurium, vecuronium, and pancuronium. Anesthesiology 1988; 69: 272–276.

40. Naguib M, Kopman AF, Ensor JE. Neuromuscular monitoring and postoperative residual curarisation: a meta-analysis. Br J Anaesth 2007; 98: 302–316.

41. Hunter JM. Is it always necessary to antagonize residual neuromuscular block? Do children differ from adults? Br J Anaesth 1996; 77: 707–709.

42. Stefan Josef Schaller, Heidrun Fink. Sugammadex as a reversal agent for neuromuscular block: an evidence-based review. Klinik für Anaesthesiologie, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; Department of Anesthesia, Massachusetts General Hospital, Boston, MA, USA Dove Press Journal: Core Evidence, 24 September 2013.

43. R. Kevin Jones, M.D., James E. Caldwell, M.B., Ch.B., Sorin J. Brull, M.D., Roy G. Soto, M.D. Reversal of Profound Rocuronium-induced Blockade with Sugammadex A Randomized Comparison with Neostigmine Anesthesiology 2008; 109:816–24.

44. Bom A, Bradley M, Cameron K, Clark JK, van Egmond J, Feilden H, MacLean EJ, Muir AW, Palin R, Rees DC, Zhang MQ: A novel concept of reversing neuromuscular block: Chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. Angew Chem 2002; 114:276–80.

45. Adam JM, Bennett DJ, Bom A, Clark JK, Feilden H, Hutchinson EJ, Palin R, Prosser A, Rees DC, Rosair GM, Stevenson D, Tarver GJ, Zhang MQ. Cyclodextrin-derived host molecules as reversal agents for the neuromuscular blocker rocuronium bromide: synthesis and structure-activity relationships. J Medicinal Chem 2002; 45: 1806–1816.

46. Bom A, Clark JK, Palin R. New approaches to reversal of neuromuscular block. Curr Opin Drug Discov Devel 2002; 5: 793–800.

47. Bom A, Hope F, Rutherford S, Thomson K. Preclinical pharmacology of sugammadex. J Crit Care 2009; 24: 29–35.

48. Hunter JM, Flockton EA. The doughnut and the hole: a new pharmacological concept for anaesthetists. Br J Anaesth 2006; 97: 123–126.

49. Abrishami A, Ho J, Wong J, Yin L, Chung F. Sugammadex, a selective reversal medication for preventing postoperative residual neuromuscular blockade. Cochrane Database Syst Rev 2009; 4: CD007362.

50. Ploeger BA, Smeets J, Strougo A, Drenth HJ, Ruigt G, Houwing N, Danhof M. Pharmacokinetic pharmacodynamic model for the reversal of neuromuscular blockade by sugammadex. Anesthesiology 2009; 110: 95–105.

51. Sparr HJ, Vermeyen KM, Beaufort AM, Rietbergen H, Proost JH, Saldien V, Velik-Salchner C, Wierda JM. Early reversal of profound rocuronium-induced neuromuscular blockade by sugammadex in a randomized multicenter study: efficacy, safety, and pharmacokinetics. Anesthesiology 2007; 106: 935–943.

52. Bom A, Hope F. Sugammadex restores spontaneous respiration after profound blockade with rocuronium (abstract). Anesthesiology 2008; 109: A356.

53. Akha AS, Rosa J, Jahr JS, Li A, Kiai K. Sugammadex: cyclodextrins, development of selective binding agents, pharmacology, clinical development, and future directions. Anesthesiol Clin. 2010;28(4):691–708.

54. Paton L1, Gupta S, Blacoe D. Successful use of sugammadex in a 'can't ventilate' scenario. Anaesthesia. 2013 Aug;68(8):861-4.

55. Bogumiła Wołoszczuk-Gębicka[1], Lidia Zawadzka-Głos[2], Jerzy Lenarczyk[1], Bożena Dorota Sitkowska[1], Iwona Rzewnicka[3].

[1]Department of Paediatric Anaesthesiology and Intensive Therapy, Medical University of Warsaw, Poland [2]Department of Paediatric Otolaryngology, Medical University of Warsaw, Poland [3]Department of Paediatric Surgery and Urology, Medical University of Warsaw, Poland. Two cases of the "cannot ventilate, cannot intubate" scenario in children in view of recent recommendations. Anaesthesiology Intensive Therapy 2014, vol. 46, no 2, 88–91.

56. Partownavid P, Romito BT, Ching W, Berry AA, Barkulis CT, Nguyen KP, Jahr JS. Sugammadex: A Comprehensive Review of the Published Human Science, Including Renal Studies. Am J Ther.2015;22:298-317.

57. Staikou C, Stamelos M, Stavroulakis E. Impact of anaesthetic drugs and adjuvants on ECG markers of torsadogenicity. Br J Anaesth. 2014;112:217-30.

58. de Kam PJ, van Kuijk J, Smeets J, Thomsen T, Peeters P. Sugammadex is not associated with QT/QTc prolongation: methodology aspects of an intravenous moxifloxacin-controlled thorough QT study. Int J Clin Pharmacol Ther. 2012;50(8):595–604.

59. Tsur A, Kalansky A. Hypersensitivity associated with sugammadex administration: a systematic review. Anaesthesia 2014; 69: 1251-1257 [PMID: 24848211 DOI: 10.1111/anae.12736].

60. Crisafi. Clinical Evaluation of Sugammadex: Efficacy and Safety. Anesthetic and Analgesic Drug Products AdvisoryCommittee (AADPAC) Meeting November 6, 2016.

61. D. Chambers, M. Paulden, F. Paton, M. Heirs, S. Duffy, J. M. Hunter, M. Sculpher, and N. Woolacott. Sugammadex for reversal of neuromuscular block after rapid sequence intubation: a systematic review and economic assessment. Br J Anaesth. 2010; 105(5):568–575.

62. eMC.Bridion100mg/ml.Sugammadex.http://www.medicines.org.uk/EMC/medicine/21299/SPC/Bridion+100+mg+ml+solution+for+injection/ 2013. Updated October 25, 2013.

63. Kam PJ, Heuvel MW, Grobara P, Zwiers A, Jadoul JL, Clerck Ed, Ramael S, Peeters PA. Flucloxacillin and diclofenac do not cause recurrence of neuromuscular blockade after reversal with sugammadex. Clin Drug Investig. 2012;32(3):203–12.

64. http://www.ema.europa.eu/humandocs/PDFs/EPAR/bridion/emea-combined-h885en.pdf Accessed 26 May, 2010.

65. Padmaja, Mantha. Monitoring of Neuromuscular Junction. Indian Journal of Anaesthesia, August 2002; 46 (4): 279-288.

66. Viby-Mogensen J, Jorgensen BC, Ording H. Residual curarization in the recovery room. Anesthesiology 1979;50:539-41.

67. J. E. Caldwell1 and R. D. Miller. Clinical implications of sugammadex. Anaesthesia, Journal of the Association of Anaesthetists of Great Britain and Ireland ,2009, 64 (Suppl. 1), pages 66–72.

68. Bragg P, Fisher DM, Shi J, Donati F, Meistelman C, Lau M, Sheiner LB. Comparison of twitch depression of the adductor pollicis and the respiratory muscles. Pharmacodynamic modeling without plasma concentrations. Anesthesiology 1994; 80: 310–9.

69. Donati F, Meistelman C, Plaud B. Vecuronium neuromuscular blockade at the diaphragm, the orbicularis oculi, and adductor pollicis muscles. Anesthesiology 1990; 73: 870–5.

70. Wright PM, Caldwell JE, Miller RD. Onset and duration of rocuronium and succinylcholine at the adductor pollicis and laryngeal adductor muscles in anesthetized humans. Anesthesiology 1994; 81: 1110–5.

71. Cantineau JP, Porte F, d'Honneur G, Duvaldestin P. Neuromuscular effects of rocuronium on the diaphragm and adductor pollicis muscles in anesthetized patients. Anesthesiology 1994; 81: 585–90.

72. Bonsu AK, Viby-Mogensen J, Fernando PU, Muchhal K, Tamilarasan A, Lambourne A. Relationship of post-tetanic count and train-of-four response during intense neuromuscular blockade caused by atracurium. British Journal of Anaesthesia 1987;59: 1089–92.

73. Engbaek J, Ostergaard D, Skovgaard LT, Viby-Mogensen J. Reversal of intense neuromuscular blockade following infusion of atracurium. Anesthesiology 1990;72: 803–6.

74. Eriksson LI, Lennmarken C, Staun P, Viby-Mogensen J. Use of post-tetanic count in assessment of a repetitive vecuronium-induced neuromuscular block. British Journal of Anaesthesia 1990; 65:487–93.

75. Ali HH, Utting JE, Gray C. Stimulus frequency in the detection of neuromuscular block in humans. Br J Anaesth 1970;42: 967-78.

76. McGrath C, Hunter J. Monitoring of Neuromuscular Block. Continuing Education in Anaesthesia, Critical Care & Pain 2006.Volume 6 Number 1 2006.

77. Eriksson LI, Sundman E, Olsson R, Nilsson L, Witt H, Ekberg O, Kuylenstierna R. Functional assessment of the pharynx at rest and during swallowing in partially paralyzed humans: simultaneous videomanometry and mechanomyography of awake human volunteers. Anesthesiology 1997;87: 1035-43.

78. Fuchs-Buder T, Meistelman C, Alla F, Grandjean A, Wuthrich Y, Donati F. Antagonism of Low Degrees of Atracurium-induced Neuromuscular Blockade Dose–Effect Relationship for Neostigmine. Anesthesiology 2010; 112:34–40.

79. Viby-Mogensen. Neuromuscular monitoring. In: Miller RD, ed. Anesthesia, 5th edition. New York: Churchill Livingstone, 2000; 1351–66.

80. Madsen MV, Scheppan S, Kissmeyer P, Mørk E, Rosenberg J, Gätke MR. Neuromuscular blockade for improvement of surgical conditions during laparotomy: protocol for a randomised study. Dan Med J 2015; 62(10): A5139.

81. Hemmerling TM, Donati F. Neuromuscular blockade at the larynx, the diaphragm and the corrugator supercilii muscle: a review. Can J Anaesth 2003; 50:779-94.

82. Kirov K, Motamed C, Ndoko SK, Dhonneur G. TOF count at corrugator supercilii reflects abdominal muscles relaxation better than at adductor pollicis. Br J Anaesth 2007; 98:611-4.

83. G. Dhonneur[1 2]*, K. Kirov[1], C. Motamed[1], R. Amathieu[1 2], W. Kamoun[1], V.Slavov[2] and S-K. Ndoko[1]. [1]Anaesthesia and Intensive Care Department, Jean Verdier University Hospital of Paris, 93143 Bondy Cedex,France.[2]Paris 13 University School of Medicine of Bobigny, 93000 Bobigny Cedex, France. Post-tetanic count at adductor pollicis is a better indicator of early diaphragmatic recovery than train-of four count at corrugator supercilii. Br J Anaesth 2007; 99: 376-9.

84. Fernando PU, Viby-Mogensen J, Bonsu AK et al. Relationship between posttetanic count and response to carinal stimulation during vecuronium-induced neuromuscular blockade. Acta Anaesthesiol Scand 1987;31: 593-6.

85. Marín PCE, Engelhardt T. Algorithm for difficult airway management in pediatrics. Rev Colomb Anestesiol. 2014;42:325–334.

86. Jimenez N, Posner KL, Cheney FW, Caplan RA, Lee LA, Domino KB: An update on pediatric anesthesia liability: a closed claims analysis. Anesth Analg 2007; 105: 147–153.

87. Cook TM, Woodal N, Frerk C; Fourth National Audit Project: Major complications of airway management in the UK: Results of the Fourth National Audit Project of the Royal College of Anaesthetists and the Difficult Airway Society. Part I: Anaesthesia. Br J Anaesth 2011: 106: 617–631.

88. Lee CM. Train-of-4 quantitation of competitive neuromuscular block.Anesth Analg 1975; 54: 649 - 53.

89. Expert Working Group (Efficacy) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). (August 25, 2007). "Guideline for Industry - Clinical safety data management: definitions and standards for expedited reporting." (PDF) FDA Center for Drug Evaluation and Research.

90. http://www.fda.gov/safety/medwatch/howtoreport/ucm053087.htm

91. Godai K, Hasegawa-Moriyama M, Kuniyoshi T, Kakoi T, Ikoma K, Isowaki S, Matsunaga A, Kanmura Y. Three cases of suspected sugammadex-induced hypersensitivity reactions.Br J Anaesth. 2012;109 (2):216–218.doi: 10.1093/bja/aes137.

92. Sinem Sarı[1], Banu Taşdemir[1], Sezen Sözkısacık[2], Feray Gürsoy[1]. [1]Adnan Menderes University, Department of Anesthesiology and Reanimation, Aydın, Turkey [2]Adnan Menderes University, Department of Pediatric Surgery, Aydın,Turkey. Side Effects of Sugammadex Use in Pediatric Patients. Journal of Clinical and Experimental Investigations 2013; 4 (3): 265-268.

93. https://www.asahq.org/resources/clinical-information/asa-physical-status classification-system

94. Loreto Godoy M.[a], M.D., Paola Pino A.[a], Registered Nurse, Gulliana Córdova L.[a],M.D., Juan Andrés Carrasco O.[a], M.D. and Andrés Castillo M.[a], M.D. [a.] Department of Pediatrics.Hospital Clínico. Pontificia Universidad Católica de Chile. Sedation and analgesia in children undergoing invasive procedures. Arch Argent Pediatr 2013; 111(1):22-28.

95. Pedersen T, Viby-Mogensen J, Bang U, OIsen NV, Jensen E, Engbaek J. Does perioperative tactile evaluation of the train-of-four response influence the frequency of postoperative residual neuromuscular blockade? Anesthesiology 1990;73: 835-9.

96. http://sketchymedicine.com/wp-content/uploads/2013/11/nmb1.png

97. http://www.scielo.br/img/revistas/rba/v62n3/en_a16chart01m.jpg

98. Abramson NS, Safar P. Randomized Clinical Study of Thiopental Loading in Comatose Survivors of Cardiac Arrest. Brain Resuscitation Clinical Trial I Study Group.N Engl J Med 1986; 314:397-403February 13, 1986.

99. Hussain N, Mendonca C (2012) Unanticipated Difficult Airway: Can Sugammadex Rescue can't Intubate can't Ventilate (CICV) Scenario? J Anesth Clin Res 3:e109. doi:10.4172/2155-6148.1000e109.

100. Miki Kasai, M.D.[1], Chihiro Igarashi,M.D.[1], Noriko Miyazawa, M.D., Ph.D.[1], Shinichi Yamamoto, M.D., Ph.D.[1], Takahiro Suzuki, M.D., Ph.D.[2] Tokyo Metropolitan Children's Medical Center, Department of Anesthesiology[1] Nihon University School of Medicine, Department of Anesthesiology[2]. Reversibility of Rocuronium-Induced Profound Neuromuscular Blockade with Sugammadex in Neonates.

101. American Society of Anesthesiologists: Practice Guidelines for Management of the Difficult Airway: An Updated Report. Anesthesiology 2003; 98: 1269-1277.

102. Resuscitation Council.Anaphylaxis Algorithm. March 2008. www.resus.org.uk

103. CPR & ECC Guidelines. Pediatric Bradycardia With a Pulse and Poor Perfusion Algorithm.American Heart Association ©2015.

104. Sorgenfrei IF, Norrild K, Larsen PB, Stensballe J, Østergaard D, Prins ME,Viby-Mogensen J: Reversal of rocuronium-induced neuromuscular block by the selective relaxant binding agent

sugammadex: A dose-finding and safety study. Anesthesiology 2006; 104:667–74.

105. Cope TM, Hunter JM. Selecting neuromuscular blocking drugs for elderly patients. Drugs Aging. 2003;20:125-40.

106. Kara T, Ozbagriacik O, Turk HS, Isil CT, Gokuc O, Unsal O, Seyhan E, Oba S. Sugammadex versus neostigmine in pediatric patients: a prospective randomized study.

107. Rev Bras Anestesiol. 2014 Nov-Dec; 64(6):400-5.doi: 10.1016/j.bjan.2014.03.001. Epub 2014 Aug 31.

108. François Donati, David R. Bevan. Neuromuscular Blocking Agents. *faculty.washington.edu/ramaiahr/BARASH.pdf*

109. Hartman ME, Cheifetz IM. Pediatric Emergencies and Resuscitation. In: Kliegman RM, Stanton ST BF, Geme III JW, Schor NF, Behrman RE, editors.Nelson Textbook of Pediatrics. 19th Ed. Philadelphia: Elsevier; 2011.p.280.

110. Zwer F (2016) Factors Affect Neuromuscular Transmission and Block. J Anesth Crit Care Open Access 6(1): 00216. DOI:10.15406/jaccoa.2016.06.00216

# PREVALENCE AND FACTORS ASSOCIATED WITH BULLYING IN PUBLIC GRADE 5 AND 6 ELEMENTARY PUPILS IN QUEZON CITY

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**ABSTRACT**

**BACKGROUND**: Bullying most often occurs in the school environment and can affect the social climate of the school as well as the surrounding community. Bullying interferes with learning and student development, and its long-term social and emotional ramifications are solely underestimated.

**OBJECTIVES**: To identify the prevalence and common risk factors for bullying among grade 5 and 6 students in a public elementary school in Quezon City.

**METHODS**: This is a prospective cross-sectional study where in prevalence rate of bullying and the associated risk factors of bullying were analyzed. The Illinois Bullying Scale (IBS) was used to measure the prevalence of bullying.

**RESULTS**: A total of 166 students between 10-12 years old of age participated in the study. All the respondents were old students. Majority of them were male (53.61%). Nine of ten students were Catholics. Majority of the fathers and half of the mothers were employed. The prevalence of bullying was at 15.66%. There was 18% prevalence of bullying on the victim subscale, 13.86% on the bully subscale, and 15.66% on the fight subscale.

**CONCLUSION**: Males have increased bully scores compared to female for both bully and fight subscales. Other factors such as being overweight or underweight, educational levels of parents, socioeconomic status, composition of the families play no significant association with bullying among students in public school. Schools and parents should become familiar with the school's definition of bullying, bullying prevention policies and the code of conduct.

**KEYWORDS**: Bullying, School Bullying, Victimization, Illinois Bullying Scale, Anti-Bullying Law.

## INTRODUCTION

Bullying is a widespread problem among adolescents. Recent surveys across different countries have shown that bullying can affect up to half of the youth. Bullying is defined as a repeated verbal, physical or psychological behavior that is harmful and involves the misuse of power by an individual or group towards one or more person (Public school education, NSW). Bullying is a serious problem among school-age youths, and intervention at every level is critical. The problem of bullying is not unusual to the Philippines. In fact, in the Asia-Pacific Regional Study on bullying, around 58% of the Filipino participants reported being made fun of by other kids, 45% being forced to do things, 36% being physically hurt, and 30% being left out of groups (A Measure of the Experience of Being Bullied: An Initial Validation in Philippine Schools by Lai, Ye, Chang, 2008). Bullying can have several adverse health effects if ignored.

Bullying most often occurs in the school environment (Ross, 1996) and can affect the social climate of the school, as well as the surrounding community. It not only affects the bully and the target of the bullying, but also the students who observe as bystanders, whether neutral, supportive, or opposed to the bullying behavior. Bullying interferes with learning and student development, and its long-term social and emotional ramifications are solely underestimated. Research suggests that comprehensive efforts that engage teachers and other school staff, students, parents, and community partners are more effective than

wholly classroom-based approaches (U.S Department of Education, 1998). Bullying is a serious problem among school-age youths, and intervention at every level is critical—from the janitorial and secretarial staff to school administrators, community leaders and other decision makers. Now more than ever, schools must re-evaluate the way they choose to respond to incidents of bullying and school violence. As schools struggle with new challenges in meeting the holistic needs of students, social workers can be "powerful tools" in influencing and implementing change. (Targeting Bullying, A Comprehensive Approach, NASW Practice Update)

In the Philippines, according to a recent report by the Department of Education (DepEd), approximately 31 bullying incidents are being reported daily in schools. A total of 6,363 cases of bullying in public as well as private elementary and high schools were recorded in the year 2014 versus 5,236 documented in 2013. As this figure was based on reported bullying incidences, it could actually be higher, seeing as many students are not able to report abuse due to fear of retaliation.

In categorizing various types of bullying among school-age youths, it is important to understand that the asymmetry of power that exists between the bully and the victim may be physical and or psychological (Nansel et, al., 2001). The actual aggressive, bullying behaviour(s) can manifest as physical, verbal, emotional or psychological, or sexual (sexual harassment). Physical bullying can range from poking, pinching, and biting to hitting, pulling hair, choking, kicking or beating. It involves any physical contact initiated by the bully towards the victim with the intent of causing physical discomfort or distress. Verbal bullying involves such things as teasing, name calling, threats, and spreading rumors.

Bullied children suffer pain, humiliation, loss of self-esteem, anxiety, insecurity, physical injury, shame, failure, days missed at school, a compromised learning environment, headaches, stomach pain and many attempt suicide. In the long run, bullied children are more prone to depression and poor self-esteem. Bullies have antisocial/delinquent behaviors, higher rates of being convicted of registered crimes, and may use drugs or alcohol and tobacco. Students feel less safe when bullying occurs in their school and are less satisfied with school life. *U.S DHHS (2001)

Bullying is a significant public health problem because it is prevalent and harmful. Between 20% and 56% of young people are involved in bullying annually (5-7). Thus, in a classroom of 30 students, between 6 and 17 students are involved in bullying as a victim, perpetrator, or both (bully-victim). The specific rate of bullying victimization and perpetration varies according to age, type of bullying, the time period over which bullying behaviors are assessed, and by subgroup. Younger (middle school-aged) children are more likely to be involved in bullying than high school-aged children. Verbal bullying is generally more prevalent than physical or cyber-bullying and bullying is more likely to occur over a longer time period—"ever" or over the "past year" as opposed to "the past few months". Specific subgroups are more likely to be victimized. For example, bullying victimization is more prevalent upon lesbian and gay youth—60% report victimization during the past 30 days prior to the survey compared with 28.8% of heterosexual youth. (Journal of Adolescent Health, Society of Adolescent Health and Medicine, 2017).

Local and international researches stress the need for a whole school approach to bullying which includes the adoption of an anti-bullying policy and anti-bullying initiatives (Fox et al, 2003; Gottfredson, Wilson & Skroban Najara, 2002; Olweus, 1993; Pepler & Craig, 2000; Rigby, 2002; Sampson, 2002; Scheckner et al, 2002; Shaw, 2001; Smith, 2000; Sudermann, Jaffe & Schieck, 1996) . The idea behind this approach, first proposed by Olweus (1993), is that the policy and the program reinforce each other and help communicate behavioural expectations for everyone involved in the daily activities of a school.

Until now, the whole school approach has not been widely implemented. While this approach addresses the immediate bullying incident and the primary individuals involved, it ignores the impact of environmental factors such as the school's culture, peer and bystander influence, and family dynamics that can have a huge effect on bullying incidents. Within the whole school approach, a component focusing

on individuals identified as at risk for being bullied or for bullying others is useful, but it should not be the exclusive focus of a policy or intervention at the school (Ma, Stewin & Mah, 2001).

## OBJECTIVES OF THE STUDY

### I. GENERAL OBJECTIVE:

To identify the prevalence and common risk factors for bullying among grade 5 and 6 students in a Public Elementary school in Quezon City

### II. SPECIFIC OBJECTIVES:

- To describe the demographic profiles of bullied grade 5 and 6 students of X Public School in Quezon City
- To identify the common risk factors of bullying among grade 5 and 6 students in X Public School in Quezon City
- To determine the prevalence rate of bullying among grade 5 and 6 students in X Public School in Quezon City
- To identifythe association between bullying and common risk factors of bullied grade 5 and 6 students in X Public School in Quezon City.

## METHODOLOGY

This is a prospective cross-sectional study where in prevalence rate of bullying and its associated risk factors of bullying was analyzed. This study was conducted during the month of October 2017 at Aurora A. Quezon Elementary School in Quezon City which was randomly selected through draw lots. The sections and even the students were randomly selected through draw lots also. The study population included all grade five and six students aged 10 to 12 years old who were randomly selected in Aurora A. Quezon Elementary School. It is located at the corner of the Cordillera and Luskot streets Galas, Quezon City. The school has 8 sections for both grade 5 and 6 levels. Each grade level has more than 400 students. In this study, students aged 10 to 12 years old were chosen as the study population since this age group is already in early adolescent stage when the students will have varied and rapid changes physically and think differently. Also, this study population belongs to the most bullied population in school as being reported in recent local statistics.

Descriptive statistics was used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables. Crude and adjusted odds ratios and the corresponding 95% confidence intervals from binary logistic regression were computed to determine the association between bullying and common risk factors. All valid data was included in the analysis. Missing variables was neither replaced nor estimated. Null hypotheses were rejected at 0.05α-level of significance. STATA 15.0 was used for data analysis.

A survey was conducted by administering a questionnaire to each grade 5 and 6 pupils in Aurora A. Quezon Elementary School in Quezon City. The questionnaire that was used is the Illinois Bully Scale (IBS). The questionnaire is appropriate for students starting in third grade. This questionnaire is an 18 item, self-report measure that contains three subscales for measuring the frequency of fighting, peer victimization, and bully behavior. Consisting of 9 items, the Bully subscale includes items that address how often a youth (8-18 years old) is engaged in bullying behavior, primarily in the form of social aggression. Consisting of 4 items, the victim subscale includes items that address both physical and verbal types of victimization by peers. The 5 items assess physical fighting.

The students who were able to give consent (by signing the informed consent and assent forms) were included in the study. These students were assigned to a room such as the school library where they would answer the tool. Once data collection is done, risk factors of those who were identified as victims were also gathered for analysis.

The validation process of this questionnaire underwent validity through content validity through the experts and reliability using Cronbach's alpha.

## ETHICAL CONSIDERATIONS

All data and personal information were kept confidential. Each student was given a folder with a questionnaire in it. The student was seated one seat apart from each other. The

primary investigator was available for any questions that may arise during the survey.

After answering the questionnaires, these were passed to the primary investigator. These will be kept confidential. Only the primary investigator would be able to go through the questionnaires. However, in cases that the student would react poorly to the questions, those who would be getting a score more than the cut off value, a referral to a school councilor or psychologist would be recommended to the school. This will also be reported to the parents and to the school heads to provide safety to the victims as this is included in the policy of Anti-Bullying Law of the Philippines.

Since the school has a psychologist, all students who performed poor in the survey would warrant a referral to the school guidance counselor.

**I. Legal Aspect**

This includes asking permission from school heads and administrators in conducting the survey and interview as part and parcel of the research or study. A letter addressed to the school division head office and head of a public elementary school were given. A courtesy call was done. A short presentation of the study was also conducted by the primary investigator to be the school head. Approval of the research was granted by the school division head office and the school head of the public elementary school.

**II. Parental consent**

The parents of the students were given a letter of invitation to attend a short presentation regarding the effects of bullying to students. The contact numbers or addresses of parents of the subjects were taken from the school registry. One weekend was allotted for a short lecture regarding bullying and the proposed research on bullying. After which, the informed consent was distributed to the parents. The informed consent was read and properly filled by the parents or guardians as well as the participants prior to the performance of the study.

## RESULTS

A total of 166 students were analyzed, with mean age of 10.93 ± 0.73 years, and 53% male. There were no transferees. Nine of ten students were Catholics. The median number of siblings was two, and 43% were firstborns. Majority of the fathers and half of the mothers were employed. Half of the parents were "married in church." Table 1 enumerates the socio-demographic profile of the respondents.

**Table 1.** Socio-demographic profile of 166 Grade 5 and 6 students

| | **Frequency (%); Mean ± SD; Median (Range)** |
|---|---|
| Age (years) | 10.93 ± 0.73 |
| Weight (kg) | 34.61 ± 7.85 |
| Height (cm) | 140.02 ± 9.78 |
| BMI ($kg/m^2$) | 17.62 ± 3.44 |
| Sex | |
| Male | 89 (53.61) |
| Female | 77 (46.39) |
| Transferee | |
| Old student | 166 (100) |
| New student | 0 |
| Religion | |
| Catholic | 148 (89.16) |
| Iglesia ni Christo | 4 (2.41) |
| Born Again | 8 (4.82) |
| Jehovah's witness | 5 (3.01) |
| Mormon | 1 (0.6) |
| Number of siblings | 2 (1—12) |
| Order in the family | |
| First child | 72 (43.37) |
| Second child | 31 (18.67) |
| Third child | 34 (20.48) |
| Fourth child | 10 (6.02) |

| | Frequency (%); Mean ± SD; Median (Range) |
|---|---|
| Fifth child | 12 (7.23) |
| Sixth child | 5 (3.01) |
| Seventh child | 1 (0.6) |
| 12th child | 1 (0.6) |
| Father's age (years) (n=159)* | 41.41 ± 8.43 |
| Father's educational attainment (n=159) | |
| Did not go to school | 0 |
| Elementary | 6 (3.77) |
| Elementary graduate | 0 |
| High school | 1 (0.63) |
| High school graduate | 59 (37.11) |
| College | 11 (6.92) |
| College graduate | 66 (41.51) |
| Vocational graduate | 1 (0.63) |
| Don't know | 15 (9.43) |
| Father's employment status (n=159) | |
| Employed | 148 (93.08) |
| Unemployed | 4 (2.52) |
| Don't know | 7 (4.4) |
| Mother's age (years) (n=164) | 38.52 ± 6.05 |
| Mother's educational attainment (n=164) | |
| Did not go to school | 1 (0.61) |
| Elementary | 8 (4.88) |
| Elementary graduate | 0 |
| High school | 4 (2.44) |
| High school graduate | 57 (34.79) |
| College | 9 (5.49) |
| College graduate | 71 (43.29) |
| Vocational graduate | 2 (1.22) |
| Don't know | 12 (7.32) |
| Mother's employment status (n=164) | |
| Employed | 81 (49.39) |
| Unemployed | 80 (48.78) |
| Don't know | 3 (1.83) |
| Parent's marriage status | |
| Married in church | 90 (54.22) |
| Not married | 26 (15.66) |
| Married in civil court | 45 (27.11) |
| Don't know | 5 (3.01) |
| Monthly income (PhP) | |
| 500- < 2,000 | 3 (1.81) |
| 2,000- <3,500 | 3 (1.81) |
| 3,500- 5,000 | 5 (3.01) |
| > 5,000 | 153 (92.17) |
| Don't know | 2 (1.2) |

**Seven students had deceased fathers. Two students had deceased mothers.*

In 15 of 18 items, more than half of the students had reported "never" to have experienced these bullying behaviors. There were three items were more than 50% of the students had experienced at least once, which were: *Other students made fun of me, Other students called me names,* and *I hit back when someone hit me first.* There were certain behaviors which were frequently experienced, 7 or more times, by some students, particularly: *I upset other students for the fun of it, Other students made fun of me, Other students called me names, I got hit and pushed by other students, I helped harass other students, I teased other students, I got in a physical fight, I threatened to hurt or hit another student,* and *I got into a physical fight because I was angry, I started conflicts, I encouraged people to fight,* and *I excluded students from my cliques.* (Table 2)

**Table 2**. Illinois Bully Scale results of 166 students

| | Never | 1 or 2 times | 3 or 4 times | 5 or 6 times | 7 or more times |
|---|---|---|---|---|---|
| | Frequency (%) | | | | |
| 1. I upset other students for the fun of it | **114 (68.67)** | 45 (27.11) | 6 (3.61) | 0 | **1 (0.60)** |
| 2. In a group I teased other students. | **136 (81.93)** | 22 (13.25) | 7 (4.22) | 1 (0.60) | 0 |
| 3. I fought students I could easily beat. | **137 (82.53)** | 27 (16.27) | 2 (1.20) | 0 | 0 |
| 4. Other students picked on me. | **107 (64.46)** | 45 (27.11) | 11 (6.63) | 2 (1.20) | 1 (0.60) |
| 5. Other students made fun of me | 77 (46.39) | **50 (30.12)** | **22 (13.25)** | **7 (4.22)** | **10 (6.02)** |
| 6. Other students called me names. | 77 (46.39) | **45 (27.11)** | **19 (11.45)** | **3 (1.81)** | **21 (12.65)** |
| 7. I got hit and pushed by other students. | **91 (54.82)** | 50 (30.12) | 17 (10.24) | 2 (1.20) | **6 (3.61)** |
| 8. I helped harass other students. | **116 (69.88)** | 27 (16.27) | 16 (9.64) | 1 (0.60) | **6 (3.61)** |
| 9. I teased other students. | **116 (69.88)** | 39 (23.49) | 6 (3.61) | 0 | **5 (3.01)** |
| 10. I got in a physical fight. | **118 (71.08)** | 39 (23.49) | 4 (2.41) | 1 (0.60) | **4 (2.41)** |
| 11. I threatened to hurt or hit another student. | **142 (85.54)** | 21 (12.65) | 1 (0.60) | 0 | **2 (1.20)** |
| 12. I got into a physical fight because I was angry. | **109 (65.66)** | 49 (29.52) | 6 (3.61) | 0 | **2 (1.20)** |
| 13. I hit back when someone hit me first. | 82 (49.40) | **65 (39.16)** | **14 (8.43)** | **4 (2.41)** | **1 (0.60)** |
| 14. I was mean to someone when I was angry. | **122 (73.49)** | 41 (24.70) | 0 | 3 (1.81) | 0 |
| 15. I spread rumors about other students. | **142 (85.54)** | 19 (11.45) | 2 (1.20) | 1 (0.60) | **2 (1.20)** |
| 16. I started (instigated) arguments or conflicts. | **144 (86.75)** | 20 (12.05) | 1 (0.60) | 0 | **1 (0.60)** |
| 17. I encouraged people to fight. | **145 (87.35)** | 16 (9.64) | 2 (1.20) | 2 (1.20) | **1 (0.60)** |
| 18. I excluded other students from my clique of friends. | **122 (73.49)** | 35 (21.08) | 2 (1.20) | 4 (2.41) | **3 (1.81)** |

Overall, the prevalence of bullying was at 15.66%. On various subscales, it showed 18% prevalence of bullying on the victim subscale, 13.86% on the bully subscale, and 15.66% on the fight subscale.

**Table 3.** Illinois Bully subscales scores (n=166)

| | Frequency (%); Median (Range) | Mean ± SD |
|---|---|---|
| Victim subscale* | 3 (0—12) | 3.14 ± 2.79 |
| Yes (score ≥ 5.93) | 30 (18.07) | |
| No (score < 5.93) | 136 (81.93) | |
| Bully subscale* | 2 (0—13) | 2.77 ± 2.61 |
| Yes (score ≥ 5.38) | 23 (13.86) | |
| No (score < 5.38) | 143 (86.14) | |
| Fight subscale* | 1 (0—11) | 1.84 ± 2.10 |
| Yes (score ≥ 3.94) | 26 (15.66) | |
| No (score < 3.94) | 140 (84.34) | |
| Total* | 7 (0—28) | 7.76 ± 5.69 |
| Yes (score ≥ 13.45) | 26 (15.66) | |
| No (score < 13.45) | 140 (84.34) | |

To determine predictors of bullying, binary logistic regression was performed. As an initial step, crude odds ratios for each of the sociodemographic characteristics were determined. For overall bullying scores, it was found that male sex and parents' marriage status to have increased odds for increased bullying scores. Males were 3.43 times more likely to have increased bullying scores compared to females (95% CI 1.3 – 9.05, p = 0.013).

**Table 4.1** Association of select characteristics with bullying based on total scores

| | **Bully total Yes (n=26)** | **Bully total No (n=140)** | **Crude OR (95% CI)** | **P-value** |
|---|---|---|---|---|
| | **Frequency (%); Mean ± SD; Median (Range)** | | | |
| Age (years) | 10.88 ± 0.71 | 10.93 ± .74 | 0.909 (0.51 – 1.61) | 0.744 |
| Sex (male) | 20 (76.92) | 69 (49.29) | 3.43 (1.3 – 9.05) | **0.013** |
| Height (m) | 1.41 ± 0.16 | 1.40 ± 0.83 | 4.19 (0.07 – 252. 98) | 0.492 |
| Weight (kg) | 32.34 ± 5.03 | 34.89 ± 8.45 | 0.959 (0.91 – 1.01) | 0.140 |
| BMI | 16.55 ± 3.45 | 17.75 ± 3.56 | 0.904 (0.79 – 1.03) | 0.198 |
| Religion | | | | |
| Catholic | 21 (80.77) | 127 (90.71) | (reference) | |
| Iglesia ni Cristo | 2 (7.69) | 2 (1.43) | 6.05 (0.81 – 45.3) | 0.080 |
| Born Again | 2 (7.69) | 6 (4.29) | 2.02 (0.38 – 10.66) | 0.409 |
| Jehovah's witness | 1 (3.85) | 4 (2.86) | 1.51 (0.16 – 14.19) | 0.718 |
| Mormon | 0 | 1 (1.71) | (omitted) | - |
| Number of siblings | 2 (1 – 5) | 2 (1 – 12) | 0.984 (0.77 – 1.26) | 0.897 |
| Order in family | | | | |
| First child | 12 (46.15) | 60 (42.86) | (reference) | |
| Second child | 3 (11.54) | 28 (20) | 0.536 (0.14 – 2.05) | 0.362 |
| Third child | 6 (23.08) | 28 (20) | 1.071 (0.36 – 3.15) | 0.900 |
| Fourth child | 1 (3.85) | 9 (6.43) | 0.556 (0.06 – 4.8) | 0.593 |
| Fifth child | 4 (15.38) | 8 (5.71) | 2.5 (0.64 – 9.65) | 0.184 |
| Sixth child | 0 | 5 (3.57) | (omitted) | - |
| Seventh child | 0 | 1 (0.71) | (omitted) | - |
| 12th child | 0 | 1 (0.71) | (omitted) | - |
| Income (n=164) | | | | |
| 500- < 2,000 | 0 | 3 (2.17) | (omitted) | - |
| 2,000- <3,500 | 1 (3.85) | 2 (1.45) | 2.826 (0.25 – 32.46) | 0.404 |
| 3,500- 5,000 | 2 (7.69) | 3 (2.17) | 3.768 (0.6 – 23.8) | 0.158 |
| > 5,000 | 23 (88.46) | 130 (94.20) | (reference) | - |
| Paternal employment status (n=152) | | | | |
| Employed | 24 (100) | 124 (96.88) | (reference) | - |
| Unemployed | 0 | 4 (3.13) | (omitted) | - |
| Maternal employment status (n=161) | | | | |
| Employed | 11 (45. 83) | 70 (51.09) | (reference) | - |
| Unemployed | 13 (54.17) | 67 (48.91) | 1.235 (0.52 – 2.95) | 0.635 |
| Parent's marriage status (n=161) | | | | |
| Married in church | | | | |
| Not married | 18 (78.26) | 72 (52.17) | (reference) | - |
| Married in civil court | 3 (13.04) | 23 (16.67) | 0.522 (0.14 – 1.93) | 0.330 |
| | 2 (8.70) | 43 (31.16) | 0.186 (0.04 – 0.84) | **0.029** |

On multivariate analysis, males were nearly three times as likely to have increased bully scores compared to females. Also, respondents whose marriage status was that of civil marriage were 80% less likely to have increased bully scores.

**Table 4.2** Adjusted Odds Ratio

| | Adjusted Odds Ratio | 95% Confidence Interval | P-value |
|---|---|---|---|
| Age (years) | 0.765 | 0.41 – 1.44 | 0.408 |
| Sex (male) | 2.929 | 1.06 – 8.10 | **0.034** |
| Parent's marriage status | | | |
| Married in church | (reference) | - | - |
| Not married | 0.449 | 0.12 – 1.72 | 0.243 |
| Married in civil court | 0.192 | 0.04 – 0.88 | **0.034** |

*$R^2$ = 9.21%; p-value = 0.016*

As an initial step, crude odds ratios for each of the socio-demographic characteristics for victim scores were determined. However, the study showed insufficient evidence to demonstrate an association between these characteristics and increased victim scores.

**Table 5.1** Association of select characteristics with bullying based on victim subscale scores

| | Victim score Yes (n=30) | Victim score No (n=136) | Crude OR (95% CI) | P-value |
|---|---|---|---|---|
| | Frequency (%); Mean ± SD; Median (Range) | | | |
| Age (years) | 10. 97 ± 0.72 | 10.91 ± 0.74 | 1.09 (0.64 – 1.87) | 0.748 |
| Sex (male) | 18 (60) | 71 (52.21) | 1.37 (0.61 – 3.06) | 0.440 |
| Height (m) | 1.42 ± 0.15 | 1.39 ± 0.08 | 10.8 (0.23 – 504) | 0.225 |
| Weight (kg) | 33.23 ± 7.57 | 34.77 ± 8.16 | 0.97 (0.93 – 1.03) | 0.344 |
| BMI | 16.65 ± 3.80 | 17.76 ± 3.49 | 0.91 (0.81 – 1.03) | 0.125 |
| Religion | | | | |
| Catholic | 26 (86.67) | 122 (89.71) | (reference) | - |
| Iglesia ni Christo | 1 (3.33) | 3 (2.21) | 1.56 (0.16 – 15.64) | 0.703 |
| Born Again | 1 (3.33) | 7 (5.15) | 0.67 (0.08 – 5.68) | 0.714 |
| Jehovah's witness | 1 (3.33) | 4 (2.94) | 1.17 (0.13 – 10.93) | 0.889 |
| Mormon | 1 (3.33) | 0 | (omitted) | - |
| Number of siblings | 2 (1 – 6) | 2 (1 – 12) | 0.94 (0.73 – 1.2) | 0.608 |
| Order in family | | | | |
| First child | 14 (46.47) | 58 (42.65) | (reference) | - |
| Second child | 3 (10) | 28 (20.59) | 0.44 (0.12 – 1.67) | 0.230 |
| Third child | 8 (26.67) | 26 (19.12) | 1.27 (0.48 – 3.4) | 0.629 |
| Fourth child | 1 (3.33) | 9 (6.62) | 0.46 (0.05 – 3.9) | 0.479 |
| Fifth child | 3 (10) | 9 (6.62) | 1.38 (0.33 – 5.78) | 0.658 |
| Sixth child | 1 (3.33) | 4 (2.94) | 1.04 (0.11 – 10) | 0.976 |
| Seventh child | 0 | 1 (0.74) | (omitted) | - |
| 12th child | 0 | 1 (0.74) | (omitted) | - |
| Income (n=164) | | | | |
| 500- < 2,000 | 0 | 3 (2.22) | (omitted) | - |
| 2,000- <3,500 | 2 (6.90) | 1 (0.74) | 10.24 (0.89 – 117.3) | 0.062 |
| 3,500- 5,000 | 2 (6.90) | 3 (2.22) | 3.41 (0.54 – 21.49) | 0.191 |
| > 5,000 | 25 (86.21) | 128 (94.81) | (reference) | - |
| Paternal employment status (n=152) | | | | |
| Employed | 28 (100) | 120 (96.77) | (reference) | - |
| Unemployed | 0 | 4 (3.23) | (omitted) | - |
| Maternal employment status (n=161) | | | | |
| Employed | 12 (42.86) | 69 (51.88) | (reference) | - |
| Unemployed | 16 (57.14) | 64 (48.12) | 1.438 (0.63 – 3.27) | 0.387 |
| Parent's marriage status (n=161) | | | | |
| Married in church | | | | |
| Not married | 16 (59.26) | 74 (55.22) | (reference) | - |
| Married in civil court | 5 (18.52) | 21 (15.67) | 1.1 (0.36 – 3.36) | 0.865 |
| | 6 (22.22) | 39 (29.10) | 0.71 (0.26 – 1.96) | 0.511 |

For the bully subscale scores, males were 2.794 times more likely to have increased bully scores compared to females (95% CI 1,04 to 7.49, p = 0.041).

**Table 6.1** Association of select characteristics with bullying based on bully subscale scores

| | Yes (n=23) | No (n=143) | Crude OR (95% CI) | P-value |
|---|---|---|---|---|
| | Frequency (%); Mean ± SD; Median (Range) | | | |
| Age (years) | 11.04 ± 0.71 | 10.91 ± 0.74 | 1.284 (0.7 – 2.34) | 0.416 |
| Sex (male) | 17 (73.91) | 72 (50.35) | 2.794 (1.04 – 7.49) | **0.041** |
| Height (m) | 1.38 ± 0.08 | 1.404 ± 0.1 | 0.042 (0.001 – 6.25) | 0.215 |
| Weight (kg) | 32.61 ± 5.75 | 34.8 ± 8.34 | 0.965 (0.91 – 1.02) | 0.227 |
| BMI | 17.14 ± 2.3 | 17.63 ± 3.73 | 0.96 (0.85 – 1.09) | 0.542 |
| Religion | | | | |
| Catholic | 21 (91.3) | 127 (88.81) | (reference) | - |
| Iglesia ni Cristo | 1 (4.35) | 3 (2.1) | 2.02 (0.2 – 20.3) | 0.552 |
| Born Again | 1 (4.35) | 7 (4.9) | 0.86 (0.1 – 7.38) | 0.894 |
| Jehovah's witness | 0 | 5 (3.5) | (omitted) | - |
| Mormon | 0 | 1 (0.7_ | (omitted) | - |
| Number of siblings | 3 (1—6) | 2 (1—12) | 1.127 (0.89 – 1.42) | 0.316 |
| Order in family | | | | |
| First child | 10 (43.48) | 62 (43.36) | (reference) | - |
| Second child | 2 (8.7) | 29 (20.28) | 0.427 (0.09 – 2.08) | 0.292 |
| Third child | 4 (17.4) | 30 (20.98) | 0.827 (0.24 – 2.85) | 0.763 |
| Fourth child | 1 (4.35) | 9 (6.29) | 0.689 (0.08 – 6.04) | 0.737 |
| Fifth child | 4 (17.39) | 8 (5.59) | 3.1 (0.78 – 12.24) | 0.106 |
| Sixth child | 2 (8.7) | 3 (2.1) | 4.13 (0.61 – 27.91) | 0.145 |
| Seventh child | 0 | 1 (0.7) | (omitted) | - |
| 12th child | 0 | 1 (0.7) | (omitted) | - |
| Income (n=164) | | | | |
| 500- < 2,000 | 1 (4.35) | 2 (1.42) | 3.325 (0.29 – 38.38) | 0.336 |
| 2,000- <3,500 | 1 (4.35) | 2 (1.42) | 3.325 (0.29 – 38.38) | 0.336 |
| 3,500- 5,000 | 1 (4.35) | 4 (2.84) | 1.663 (0.176 – 15.63) | 0.657 |
| > 5,000 | 20 (86.96) | 133 (94.33) | (Reference) | - |
| Paternal employment status (n=152) | | | | |
| Employed | 20 (95.24) | 128 (97.71) | (reference) | - |
| Unemployed | 1 (4.76) | 3 (2.29) | 2.133 (0.21 – 21.53) | 0.521 |
| Maternal employment status (n=161) | | | | |
| Employed | 12 (54.55) | 69 (49.64) | (reference) | - |
| Unemployed | 19 (45.45) | 70 (50.36) | 0.82 (0.33 – 2.03) | 0.669 |
| Parent's marriage status (n=161) | | | | |
| Married in church | 12 (57.14) | 78 (55.71) | (reference) | - |
| Not married | 4 (19.05) | 22 (15.71) | 1.18 (0.35 – 4.03) | 0.790 |
| Married in civil court | 5 (23.81) | 40 (28.57) | 0.813 (0.27 – 2.47) | 0.714 |

Adjusting for age, male sex remained to have increased odds for increased bully subscale scores. Specifically, males had 2.727 more likely to have increased bully subscale scores. This model explained 3.76% of the variation in bully subscale scores but was not statistically significant (p = 0.0814).

**Table 6.2** Predictors of bully subscale scores

| | Adjusted Odds Ratio | 95% Confidence Interval | P-value |
|---|---|---|---|
| Age (years) | 1.214 | 0.66 – 2.22 | 0.529 |
| Sex (male) | 2.727 | 1.01 – 7.34 | **0.047** |

*$R^2$ = 3.76%; p-value = 0.0814*

For the fight subscale scores, males were six times more likely to have increased bully scores compared to females (95% CI 1.96 to 18.29, p = 0.002).

**Table 7.1** Association of select characteristics with bullying based on fight subscale scores

| | **Yes (n=26)** | **No (n=140)** | **Crude OR (95% CI)** | **P-value** |
|---|---|---|---|---|
| | **Frequency (%); Mean ± SD; Median (Range)** | | | |
| Age (years) | 11.07 ± 0.69 | 10.9 ± 0.74 | 1.391 (0.78 – 2.4) | 0.261 |
| Sex (male) | 22 (84.62) | 67 (47.86) | 5.99 (1.96 – 18.29) | **0.002** |
| Height (m) | 1.39 ± 0.08 | 1.40 ± 0.10 | 0.487 (0.006 – 39.84) | 0.749 |
| Weight (kg) | 35.35 ± 6.33 | 34.33 ± 8.34 | 1.02 (0.96 – 1.07) | 0.556 |
| BMI | 18.19 ± 2.95 | 17.44 ± 3.65 | 1.06 (0.94 – 1.19) | 0.329 |
| Religion | | | | |
| Catholic | 22 (84.62) | 126 (90) | (reference) | - |
| Iglesia ni Cristo | 3 (11.54) | 1 (0.71) | 17.18 (1.7 – 172.7) | **0.016** |
| Born Again | 1 (3.85) | 7 (5) | 0.82 (0.09 – 6.98) | 0.854 |
| Jehovah's witness | 0 | 5 (3.57) | (omitted) | - |
| Mormon | 0 | 1 (0.71) | (omitted) | - |
| Number of siblings | 2 (1 – 5) | 2 (1 – 12) | 0.935 (0.72 – 1.2) | 0.614 |
| Order in family | | | | |
| First child | 14 (53.85) | 58 (41.43) | (reference) | - |
| Second child | 4 (15.38) | 27 (19.29) | 0.614 (0.18 – 2.04) | 0.426 |
| Third child | 3 (11.54) | 31 (22.14) | 0.40 (0.11 – 1.5) | 0.175 |
| Fourth child | 1 (3.85) | 9 (6.43) | 0.46 (0.05 – 3.94) | 0.479 |
| Fifth child | 4 (15.38) | 8 (5.71) | 2.07 (0.5 – 7.87) | 0.285 |
| Sixth child | 0 | 5 (3.57) | (omitted) | - |
| Seventh child | 0 | 1 (0.71) | (omitted) | - |
| 12th child | 0 | 1 (0.71) | (omitted) | - |
| Income (n=164) | | | | |
| 500- < 2,000 | 0 | 3 (2.17) | (omitted) | - |
| 2,000- <3,500 | 1 (3.85) | 2 (1.45) | 2.826 (0.25 – 32.46) | 0.404 |
| 3,500- 5,000 | 2 (7.69) | 3 (2.17) | 3.77 (0.6 – 23.8) | 0.158 |
| > 5,000 | 23 (88.46) | 130 (94.20) | (reference) | - |
| Paternal employment status (n=152) | | | | |
| Employed | 23 (100) | 125 (96.90) | (reference) | - |
| Unemployed | 0 | 4 (3.10) | (omitted) | - |
| Maternal employment status (n=161) | | | | |
| Employed | 11 (44) | 70 (51.47) | (reference) | - |
| Unemployed | 14 (56) | 66 (48.53) | 1.35 (0.57 – 3.18) | 0.493 |
| Parent's marriage status (n=161) | | | | |
| Married in church | | | | |
| Not married | 18 (78.26) | 72 (52.17) | (reference) | - |
| Married in civil court | 2 (8.70) | 24 (17.39) | 0.33 (0.07 – 1.54) | 0.160 |
| | 3 (13.04) | 42 (30.43) | 0.28 (0.08 – 1.03) | 0.055 |

Adjusting for age, the table below showed that male sex remained to have increased odds for increased bully subscale scores. Specifically, males were 5.794 times more likely to have increased fight subscale scores.

Compared to Catholic students, respondents who were Iglesia ni Cristo (INC) religion were 27.93 times more likely to have increased fight subscale scores.

This model explained 14.17% of the variation in fight subscale scores (p = 0.0005).

**Table 7.2** Adjusted Odds Ratio

| | Adjusted Odds Ratio | 95% Confidence Interval | P-value |
|---|---|---|---|
| Age (years) | 1.291 | 0.70 – 2.37 | 0.410 |
| Sex (male) | 5.794 | 1.83 – 18.39 | **0.003** |
| Religion | | | |
| Catholic | (reference) | - | - |
| Iglesia ni Cristo | 17.93 | 1.37 – 233.93 | **0.028** |
| Born Again | 0.701 | 0.08 – 6.33 | 0.751 |
| Jehovah's witness | (omitted) | - | - |
| Mormon | (omitted) | - | - |

*$R^2$ = 14.17%; p-value = 0.0005*

## DISCUSSION

Bullying in school is an issue that continue to receive attention from researchers, educators, parents and students. This study focused on the prevalence of bullying in a public elementary school in Quezon City. Based from the data that was gathered, school bullying is still prevalent. The overall prevalence of bullying was at 15.66%.

According to independent study, it was found that parents whose students attend public schools reported higher bullying rates (29 percent) than parents whose children attend private boarding schools (22 percent). Minority students were also found to be more at risk of bullying in public schools than private ones.

Using the Illinois Bullying Scale, the prevalence of bullying was 18% on victim subscale, 13.86% on bully subscale and 15.66% on fight subscale. Hence, it can be concluded that, among the 3 subscales, there are more students who are being bullied.

There were three items in the IBS were more than 50% of the students had experienced at least once, which were: *Other students made fun of me, Other students called me names,* and *I hit back when someone hit me first (Table 2).*This can be further deduce that verbal and physical abuses were observed more frequently among the students in a public elementary school. Based on the recent statistics on bullying, verbal bullying is the most common type of bullying which is about 77% of all students being bullied.

No single factor puts a child at risk of being bullied or bullying others. Bullying can happen anywhere – workplace, home and even in school. In some studies, children who are bullied have one or more of the following risk factors such as overweight of underweight, perceived to be weak, depressed and have low self-esteem, and also those who are unpopular. On the other hand, children who are more likely to bully others are those who are aggressive, violent, have less parental involvement, have more friends and popular.

Among the socio-demographic data listed in the study, male gender revealed to be more likely to have increased risk for bullying for both bully and fight subscales. This can be further explained since for decades, in many researches, boys were found out to be inherently more aggressive than girls, and playground scuffles usually ended with a boy in detention. In the 1990s, though, Finnish researcher Kaj Bjorkqvist began interviewing adolescent girls about their interactions. He found was that girls are no less aggressive than boys; they are just aggressive in different ways[14]. This further strengthens the fact that gender is a significant factor of bullying.

The study also showed that compared to Catholic students, those respondents who were of Iglesia Ni Cristo (INC) religion were 17.93 times more likely to have increased fight subscale scores. However, no recent data or studies that would support that INC religion are prone to be involved in fighting. This factor was statistically significant in the data, but this could be even an artifactual association meaning association by chance but not a real association.

Respondents whose parents' marriage status was that of civil marriage were 80% less likely to have increased bully scores. However, in a recent study performed in Turkey on school bullying by Turkmen et al, marital status of the parents were observed to have no association

being a victim of violence[17].In contrast to this, some studies revealed that significantly higher proportion of children with divorced or widowed parents were reported being involved in the form of bullying than children with regular married parents, especially as bullies[18]. This may be attributed to the stressful environment in which children from broken families endure. But since this study showed, in general, a large proportion of children having married parents either civil or in church, then less likely that these students would have increased bully scores as being shown in table 4.2

On the other hand, this study showed that the Body Mass Index (BMI), educational levels of parents, socioeconomic status, composition of the families of the students were reported to be not associated with bullying.

## CONCLUSION

This study concludes that, even with Anti-Bullying Law being implemented, bullying is still prevalent in schools, especially in public schools. The study showed that the prevalence of bullying was at 15.66%. Majority of the students who were involved were victims (18%).

Males have increased bully scores compared to female for both bully and fight subscales. Other factors such as being overweight or underweight, educational levels of parents, socioeconomic status, composition of the families play no significant association with bullying among students in public school. Bullying is a psychological and pedagogical problem connected with public health. It must be solved by various professionals immediately. Adolescents should be guided by adults and the family should play a vital role in protecting the welfare of their children. Since bullying is still prevalent, early identification of children at risk for bullying should be done to prevent further consequences. Through early intervention, young children will be less likely to succumb to the accumulating risks and negative effects of bullying.

Teachers and even parents should become familiar with the school's definition of bullying, bullying prevention policies and the code of conduct. This is to ensure that the same policy is being enforced throughout the school. it would also be helpful if teachers and other faculty staff will attend a bullying training prevention programs or in-service in order to learn more about bullying and their obligation as a teacher related to this issue.

## REFERENCES


1. American Association of University Women Educational Foundation. (2001). Hostile hallways; Bullying, teasing, and sexual harassment in school. Washington DC: Author

2. Askew, S. (1989). Aggressive behaviour in boys; To what extent is it institutionalized? In D. P. Tattum & D. A Lane (Eds), Bullying in Schools (pp. 59-71). Hanley, Stoke-on-Trent, UK: Trentham Books.

3. Nansel, T. R, Overpeck, M. Pilla, R. S, Ruan, WJ Simons-Morton, B, & Scheidt, P (2001). Bullying Behavior among U. S youth: Prevalence and association with psychosocial adjustment. JAMA, 285, 2094-2100.

4. Olweus, D. (1993). Bullying in Schools: What we know and what we can do. Cambridge, MA: Blackwells.

5. Ross, D. M (1996). Childhood bullying and teasing: What school personnel, other professionals, and parents can do. Alexandria, VA: American Counseling Association.

6. Smith, P.K & Thompson, D. (1991). Dealing with bully/Victim problems in the U.K In P.K Smith & D. Thompson (Eds) Practical approaches to bullying (pp. 1-12). London: FultonStephenson,

7. Arseneault L., W. E. Bullying Victimization Uniquley Contributes to Adjustment Problems in Young Children: A Nationally Representative Cohort Study. Pediatrics. 2006 Jul;118(1):130-8

8. Juvonen J, G. Bullying Among Young Adolescents: The Strong, the Weak, and the Troubles . Pediatrics. 2003; 112; 1231

9. Gini G. Pozzoli T. Association Between Bullying and Psychosomatic Problems: A Meta-Analysis. Pediatrics. 2009; 123; 1059

10. http://www.performwell.org

11. Hamburger M.E., Basile, K.C., Vivolo, A.M. (2011). Measuring Bullying Victimization, perpetration, and Bystander Experiences: A Compendium of Assessment Tools

12. National Center for Education Statistics: nces.ed.gov

13. Ouano, J. B. A Measure of the Experience of Being Bullied: An Initial Validation in Philippine Schools. Philippine Journal of Counseling Psychology. 2013. 15: 14-27

14. Edmonds, Molly. Are there difference between male and female bullies. 2017

15. Juvonen J, G. Bullying Among Young Adolescents: The Strong, the Weak, and the Troubles . Pediatrics. 2003; 112; 1231

16. Magklara K, Shapinakas P, et al. Bullying behavior in schools, socioeconomic position and psychiatric morbidity: a cross-sectional study in late adolescents in Greece. Child and Adolescent Psychiatry and Mental Health. 2012. 6:8

17. Turkmen D, Dokgoz M, Polat H, et al. Bullying among High School Sutdents. Amaltea Medical, 2010.

18. Abdulsalam, A, Daihani A, Francis K, et al. Prevalence and Associated Factors of Peer Victimization (Bullying) among grade 7 and 8 Middle School Students in Kuwait. International Journal of Pediatrics, 2016.

# RISK FACTORS FOR AMPHOTERICIN B NEPHROTOXICITY AMONG CHILDREN SIX MONTHS TO EIGHTEEN YEARS OLD ADMITTED AT THE PHILIPPINE CHILDREN'S MEDICAL CENTER

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**ABSTRACT**

**BACKGROUND**: Amphotericin B is used in pediatrics for severe fungal infections despite its known nephrotoxic side effects. Tubular injury and renal vasoconstriction range from 15-58% with exact risk factors that predispose children to developing these complications still undefined.

**OBJECTIVES**: To determine the risk factors for nephrotoxicity with deoxycholate Amphotericin B treatment among children 6 months-18 years old at the Philippine Children's Medical Center from 2006-2017

**METHODS**: This is a retrospective case-control study of 150 patients. Cases had decrease in eGFR by at least 25% and/or developed hypokalemia after at least one dose of Amphotericin. Those who did not develop nephrotoxicity were considered controls. Risk factors evaluated were age, sex, nutritional status, underlying medical condition, cumulative dose, concomitant use of nephrotoxic drugs used, treatment with diuretics and intravenous hydration. Results were analyzed using univariate and multivariate regression models.

**RESULTS**: Using logistic regression, underlying malignancy had the highest odds ratio of 33.1 and nutritional status of z score=0 showed the lowest at 0.158. Duration of treatment >14 days had 1.75 times chance of developing nephrotoxicity while total cumulative dose >7.1 mg/kg had 1.5 times more chance of developing nephrotoxicity. Subjects given diuretics had 5.5 times more odds, while those not given concomitant nephrotoxic medications were 5.33% less likely to develop renal toxicity.

**CONCLUSION:** Risk factors for nephrotoxicity were malignancy as an underlying medical condition, duration of amphotericin treatment of >14 days, cumulative dose >7.1 mg/kg and diuretic use. Normal nutritional status and no other concomitant nephrotoxic medication use had lesser odds of developing nephrotoxicity.

**RECOMMENDATION:** Clinicians should consider these risk factors, institute measures to monitor occurrence of nephrotoxicity and the need for alternative fungal therapy in these children. With identification of the population at risk, prospective research on determining the specific onset of renal effects and possible intervention is recommended.

**KEYWORDS**: Amphotericin B, Nephrotoxicity, Risk factors

## INTRODUCTION

Amphotericin is widely used as an antifungal agent and has been increasingly prescribed in the pediatric age group because of the increasing number of immunocompromised patients on oncologic treatments and antibiotic use (1,2). Although numerous antifungal agents have been approved for use, deoxycholate amphotericin B has been considered the "standard" therapy for most invasive fungal infections and the standard comparison for new antifungal drugs. However, deoxycholate amphotericin B has several disadvantages because of infusion-related events and most importantly, nephrotoxicity (1).

There are two main effects of Amphotericin B on the kidney: reduction in glomerular filtration rate and tubular dysfunction (2,3). A decreased glomerular filtration rate is evidenced by increased serum urea and

creatinine. Tubulopathy, especially in the distal portion, is characterized by hypokalemia due to potassium wasting, metabolic acidosis, hypomagnesemia and loss of urine concentrating ability (1). Renal effects of amphotericin may occur in up to 30-80% of adult and young patients, with very few researches in children (2,4).

Currently, there are two forms of amphotericin available in our institution, the deoxycholate amphotericin and lipid complex amphotericin, with the latter shown to have similar efficacy and nephrotoxicity lower than that of conventional amphotericin B. The primary disadvantage to its use, however, is the financial cost for daily treatment compared to the conventional amphotericin treatment (1,5). Also, its use has not been thoroughly evaluated whether their use in "low-risk" patients would be of significant benefit.

Amphotericin B induced nephrotoxicity, although often reversible, has been associated with increased morbidity and mortality along with increased hospital days and higher cost (1). In addition to affecting the kidney directly, the changes in the glomerular filtration rate and tubular secretion may affect the character of other nephrotoxins, which may increase their systemic exposure and risk for severe renal damage (2). Hence, identification and possible attenuation of nephrotoxicity is critical.

This study will determine the risk factors for acute kidney injury and hypokalemia among children 6mo-18yo who underwent deoxycholate Amphotericin B treatment at the Philippine Children's Medical Center from January 2006-January 2017.

Due to lack of studies in the pediatric age group, the time to onset and other factors contributing to renal dysfunction varies considerably among patients hence the need for close monitoring of patients on treatment. Some studies recommend monitoring renal toxicity by obtaining urea and creatinine among other parameters every other day throughout therapy (2). This is on the premise that there are currently no guidelines in monitoring and identification of patients that need close observation.

Determination of the incidence of renal aberrations associated with Amphotericin B use in our setting will promote awareness on the matter and encourage rational use of antifungal therapy. Knowing the risk factors for amphotericin B nephrotoxicity in children will help in the early identification of the patients in need for close monitoring, allow prevention of nephrotoxicity if the risks are modifiable and possibly recommend use of the lipid form to decrease its renal effects.

## METHODOLOGY

This is a retrospective case control study of patients aged 6 months old to 18 years old treated with deoxycholate Amphotericin B from January 2006 to January 2017 at the Philippine Children's Medical Center. Patients who were given at least one dose of deoxycholate Amphotericin B and had a drug related nephrotoxicity defined as decrease in GFR by at least 25% and/or developed hypokalemia were regarded as cases while those who were given the said treatment and did not develop nephrotoxicity were considered as controls.

List of patients were obtained from the Pharmacy Division. Individual charts were reviewed to obtain demographic profile and other information pertaining to the potential risk factors for nephrotoxicity, such as: nutritional state, cumulative deoxycholate Amphotericin B dose, underlying medical condition, recent nephrotoxic drug use, diuretic use and intravenous hydration use. Renal effects of Amphotericin B were based on their decline in renal function as per pRIFLE criteria and occurrence of hypokalemia among these patients.

Only those patients who had baseline creatinine and serum potassium levels prior to the initiation of Amphotericin B were included in the study. The subjects included in the study also had estimated glomerular function (eGFR) by modified Schwartz technique of more than 15 ml/min/1.73m$^2$ and baseline serum potassium levels equal to or greater than 3.5mmol/L prior to the initiation of Amphotericin B.

Excluded patients were those less than six months old and those using the lipid formulation of Amphotericin B. Patients with incomplete data on their baseline creatinine and serum potassium levels were not included. Subjects with baseline estimated glomerular function of less than or equal to 15

ml/min/1.73m$^2$ and whose serum potassium were less than 3.5 were also excluded.

Other toxic effects from Amphotericin use and other renal events that may have caused loss or end stage renal disease were not included the study. Because of the nature of a retrospective study, the accuracy of the data collected relied on the adequate documentation from the patient's records.

The list of patients on deoxycholate Amphotericin B treatment from January 2006 to January 2017 were obtained from the hospital pharmacy. Information regarding each patient were reviewed and logged from medical charts at the Medical Records Section. Factors evaluated were: age (6 months-35months old, 36 months-9 years old and 10-18 years old), sex (male or female), nutritional status (based on z scores for weight for height/length) , primary underlying medical condition (malignancy, neurological illness, surgical or postoperative and others),cumulative dose of amphotericin given, nephrotoxic drug use during and within 30 days before the initiation of Amphotericin treatment (aminoglycosides, vancomycin, acyclovir, NSAIDS, radiocontrast agents, chemotherapy such as platinum based compounds, ifosfamide and methotrexate, calcineurin inhibitors, allopurinol) , diuretics (furosemide) use and use of intravenous hydration (hydration at above maintenance, maintenance or without use of intravenous hydration).

Occurrence of an acute kidney injury was based on creatinine levels taken prior to the initiation of amphotericin treatment and compared with repeat creatinine levels taken after at least 1 dose of Amphotericin B. The estimated glomerular filtration rate that showed at least a 25% increase from baseline is considered an acute kidney injury event. Patients who at the end of treatment did not have a decrease in estimated glomerular function of at least 25% were considered as with no acute kidney injury.

Tubular effects of Amphotericin were assessed through derangements in potassium levels. Baseline serum potassium levels prior to the initiation of Amphotericin B treatment were compared to subsequent levels throughout the treatment. Patients who had serum potassium less than 3.5mmol/L after at least 1 dose of Amphotericin were considered as having an episode of hypokalemia.

Data collected were described using means, standard deviations, frequency and percentages. Association were tested using the t-test for normally distributed variables and Mann Whitney Test for non-normal distributions. Chi-square or Fischer's exact test were used for categorical variables.

Those variables which showed significance on univariate analysis were entered to the logistic regression model. For all tests, a 95% confidence level was considered significant.

**RESULTS**

A total of 318 patient charts were reviewed with only 150 (75 cases and 75 controls) patients who met the inclusion criteria of the study. The most common reason for exclusion was absent baseline or follow-up monitoring of creatinine and serum potassium during treatment. Of those who developed nephrotoxicity, 73 subjects presented with hypokalemia (with 3 patients both with hypokalemia and acute kidney injury) while 2 subjects developed acute kidney injury. There were no patients who underwent renal replacement therapy for nephrotoxicity.

Although there was no significance in the distribution between age groups, majority of the patients given Amphotericin B were between 6 months old to 9 years old. There was also no gender predilection as to the occurrence of nephrotoxicity between males and females. Majority of the patients enrolled had z scores for weight for length/height equal to zero and were significantly different among cases and controls. Distributions of patients with z scores falling above or below zero were not significant between the two groups (Table 1).

Table 1. **Demographic Characteristics and Nutritional Status of Subjects given Deoxycholate Amphotericin B**

| Variables | With Toxicity (Cases) n=75 | Without Toxicity (Controls) n=75 | P value |
|---|---|---|---|
| Age in months/yrs | | | 0.363 |
| 6-35 months | 26 (34.7%) | 34 (45.3%) | |
| 36 months to 9 yrs | 34 (45.3%) | 31 (41.3%) | |
| 10 yrs+ | 15 (20%) | 10 (13.4%) | |
| Gender | | | 0.999 |
| Male | 45 (60%) | 44 (58.7%) | |
| Female | 30 (40%) | 31 (41.3%) | |
| BMI, mean +/SD | 15.97 ±2.94 | 15.78 ±3.62 | 0.734 |
| Weight for Length/Height | | | 0.136 |
| z= 0 | 20 (26.7%) | 34 (45.2%) | 0.0265* |
| z -1 | 18 (24%) | 11 (14.7%) | 0.214 |
| z-2 | 14 (18.6%) | 8 (10.7%) | 0.248 |
| z-3 | 11 (14.7%) | 11 (14.7%) | 1.00 |
| z+1-+3 | 12 (16%) | 11 (14.7%) | 1.00 |

*p value significant at ≤ 0.05

Majority of patients who received Amphotericin B had underlying malignancies (blood or solid tumors). Cancer patients and those who underwent recent surgical procedures were significantly associated with Amphotericin B nephrotoxicity based on univariate analysis. Other patients who were given Amphotericin B had underlying neurologic illnesses, pneumonia, cardiac conditions and primary immunodeficiencies (Table 2)

**Table 2**. Underlying Medical Condition of Patients given Deoxycholate Amphotericin B

| Variables | With Toxicity (Cases) n=75 | Without Toxicity (Controls) n=75 | P value |
|---|---|---|---|
| Underlying medical condition | | | 0.0007* |
| malignancy | 39 (52%) | 17 (22.7%) | 0.0003* |
| neurologic illness | 9 (12%) | 15 (20%) | 0.265 |
| Pneumonia | 15 (20%) | 18 (24%) | 0.694 |
| Surgical | 1 (1.4%) | 11 (14.7%) | 0.005* |
| Others | 11 (14.7) | 14 (18.6%) | 0.662 |

*p value significant at ≤ 0.05

The most common indications for initiating Amphotericin treatment for both groups were blood and urine growth of *Candida* species. Total cumulative dose (mean ±SD) of Amphotericin for the controls were at 7.104 ± 4.48 mg/kg while a cumulative dose of 8.065 ± 7.58 mg/kg was documented for those who developed nephrotoxicity. Indications for treatment and cumulative dose were not significantly associated with hypokalemia or increased creatinine. The average length of treatment before cases developed nephrotoxicity were at 14.33 ±9.47 days while those who did not develop nephrotoxicity were treated for as long as 8.65 ±7.86 days. Treatment duration was a significant determinant for nephrotoxicity (p <0.0001) (Table 3).

**Table 3.** Indications for Use, Treatment Duration and Cumulative Dose of Patients given Deoxycholate Amphotericin B

| Variables | With Toxicity (Cases) n=75 | Without Toxicity (Controls) n=75 | P value |
|---|---|---|---|
| Indication for Amphotericin treatment | | | 0.105 |
| Candidemia | 31 (41.3%) | 23 (30.7%) | 0.234 |
| Candiduria | 17 (22.7%) | 31 (41.3%) | 0.022 |
| Persistent fever on prolonged antibiotics | 18 (24%) | 15 (20%) | 0.693 |
| Progression of other symptoms despite antibiotics | 9 (12%) | 6 (8%) | 0.587 |
| Duration of Amphotericin Treatment, in days, mean +/SD | 14.33 ±9.47 | 8.65 ±7.86 | <0.0001* |
| Total cumulative Amphotericin dose, (mg/kg), mean +/-SD | 7.104 ±4.48 | 8.065 ±7.58 | 0.182 |

*p value significant at ≤ 0.05

Controls had a baseline creatinine of 31.35 ± 18.54 umol/L (eGFR 116.13 ± 39.65 ml/min/1.73m$^2$) and baseline serum potassium levels of 4.02 ± 0.477 mmol/L. On the other hand, cases had baseline creatinine of 34.44 +/ 22.56 umol/L (eGFR 121.89 ± 38.72 ml/min/1.73m$^2$) with baseline serum potassium levels were at 3.98 ± 0.314 mmol/L. Majority of patients from both groups were maintained on maintenance hydration during treatment. Baseline creatinine, eGFR and serum potassium levels and the presence of hydration during treatment were not significant determinants for renal toxicity (Table 4).

**Table 4.** Baseline Creatinine, estimated Glomerular Filtration Rates (eGFR), Potassium levels and Hydration administered to patients given Deoxycholate Amphotericin B

| Variables | With Toxicity (Cases) n=75 | Without Toxicity (Controls) n=75 | P value |
|---|---|---|---|
| Baseline Creatinine, mean (umol/L) +/-SD | 34.44 ±22.56 | 31.35 ±18.54 | 0.309 |
| Baseline eGFR (ml/min/1.73m$^{2)}$, mean +/-SD | 121.89 ±38.72 | 116.13 ±39.65 | 0.369 |
| Baseline Potassium (mmol/L), mean +/-SD | 3.98 ±0.314 | 4.02 ± 0.477 | 0.491 |
| Hydration rate | | | 0.147 |
| none | 11 (14.7%) | 5 (6.7%) | 0.185 |
| maintenance | 50 (66.7%) | 60 (80%) | 0.096 |
| above maintenance | 14 (18.6%) | 10 (13.3%) | 0.505 |

*p value significant at ≤ 0.05

Sixty percent of patients who developed nephrotoxicity were given diuretics during their course of therapy. Vancomycin and aminoglycosides, alone or in combination, were among the common nephrotoxic drugs that were given together with Amphotericin B. Other drugs used in some patients were acyclovir, ifosfamide, carboplatin, cisplatin and ibuprofen. The use of diuretics and concomitant nephrotoxic medications during Amphotericin B treatment were significantly associated with the occurrence of renal toxicity (Table 5).

Table 5. Concomitant Use of Diuretics other Medications among Patients given Deoxycholate Amphotericin B

| Variables | With Toxicity (Cases) n=75 | Without Toxicity (Controls) n=75 | P value |
|---|---|---|---|
| Use of Diuretics N (%) | 45 (60%) | 14 (18.7%) | <0.0001* |
| Concomitant medications | | | 0.0003* |
| None | 2 (2.7%) | 21 (28%) | <0.0001* |
| Vancomycin alone | 11 (14.7%) | 10 (13.3%) | 1.00 |
| Vancomycin + aminoglycoside | 16 (21.3%) | 7 (9.3%) | 0.068 |
| Vancomycin + aminoglycoside + others | 3 (4%) | 0 | 0.244 |
| Vancomycin + others | 5 (6.7%) | 3 (4%) | 0.719 |
| Aminoglycoside only | 28 (37.3%) | 27 (36%) | 1.00 |
| Aminoglycosides + others | 6 (8%) | 2 (2.7%) | 0.276 |
| Others | 4 (5.3%) | 5 (6.7%) | 0.999 |

*p value significant at ≤ 0.05

Based on univariate analysis ($p \leq 0.05$) the following were the significant variables associated with nephrotoxicity: patient's underlying condition ( $p = 0.0007$), underlying malignancy and recent surgical procedure ($p= 0.0003$ and 0.005 respectively) , duration of amphotericin treatment ($p <0.0001$), use of diuretics ($p <0.0001$) and use of other nephrotoxic drugs during Amphotericin treatment (0.0003).

Logistic regression was done to determine the independent factors of nephrotoxicity. Results showed 6 variables significantly associated with outcome of nephrotoxicity: a weight for height/length z score of 0, malignancy as underlying medical condition, duration of treatment, cumulative dose of Amphotericin B, diuretic use and use of concomitant nephrotoxic medications (Table 6).

**Table 6.** Logistic Regression Analaysis using the ENTER method for the different variables from the Univariate analysis

| Variable | Odds Ratio | p value | Confidence interval |
|---|---|---|---|
| Z score (weight for length/height)= 0 | 0.1585 | 0.0098* | 0.0392- 0.6412 |
| Underlying malignancy | 33.1210 | 0.0004* | 4.7267- 232.0854 |
| Underlying surgical condition | 0.1201 | 0.1625 | 0.0061-2.3506 |
| Candiduria as indication for treatment | 1.5045 | 0.5453 | 0.4005-5.6522 |
| Duration of amphotericin treatment (> 14 days) | 1.7554 | 0.0000* | 1.3633-2.2602 |
| Total Cummulative amphotericin dose (<7.1mg/kg) | 0.6659 | 0.0004* | 0.5312-0.8347 |
| No hydration given during treatment | 3.5197 | 0.3327 | 0.2759-44.8996 |
| At least maintenance hydration during treatment | 1.4052 | 0.7221 | 0.2155-9.1608 |
| Diuretic use during treatment | 5.4823 | 0.0082* | 1.5514-19.3730 |
| No concomitant nephrotoxic medications given | 0.0533 | 0.0115* | 0.0055-0.5182 |
| Given Vancomycin + aminoglycoside during treatment | 2.9390 | 0.2118 | 0.5410-15.9655 |

*p value significant at ≤0.05

A z score of 0 showed an OR of 0.158, or 15.8% less chance of nephrotoxicity among those with normal nutritional status compared to other z scores. Patients who had cancer as the primary disease had the highest odds of based on the model, indicating that those who had malignancy had 33.1 times chance of developing nephrotoxicity compared to other medical conditions.

A total duration of more than 14 days of treatment was associated with a 1.75 times chance of developing nephrotoxicity while patients who were given more than a cumulative Amphotericin B dose of 7.1 mg/kg had 1.5 times more of developing hypokalemia or acute renal failure.

Patients who were given diuretics during their course of treatment had 5.5 times odds of nephrotoxicity. Those who were not given other concomitant nephrotoxic medications (such as vancomycin, aminoglycosides, cyclosporine and others) were 5.33% less likely to develop nephrotoxicity as compared to those who were given at least one added drug.

**DISCUSSION**

As seen is other studies, hypokalemia was the more prominent manifestation of renal injury in patients given Amphotericin B (11). Although nephrotoxicity is a well-known complication of deoxycholate Amphotericin B treatment, the exact risk factors that predispose patients to developing this complication has not been fully identified. Differences on reports also are partially due to different definitions of nephrotoxicity. Based on our study, the independent determinants of the occurrence of nephrotoxic effects of Amphotericin are nutritional status, the underlying medical condition, cumulative dose and duration of treatment and concomitant use of diuretics and nephrotoxic drugs.

Several investigators have found inconsistent results on the effects of nutritional status. And since these are mostly done in the adult population, only the weight was the basis for analysis with increased risk in the heavier or obese patients (9,11) with no correlates done yet on children. In our study, nutritional status was based on weight for corresponding height or length and not on weight alone. Children with normal nutritional status (z=0) corresponded to 15.8% less odds of nephrotoxicity compared to those who belong above or below the score.

Among adults, known underlying medical conditions that may be confounding factors for Amphotericin B nephrotoxicity is diabetes mellitus, ischemic heart disease and patients who had previous chemotherapy (3). Based on a study of Karimzadeh et al on cancer adult patients, nearly one-third developed nephrotoxicity a week after initiating Amphotericin treatment. Currently, there is no study on the nephrotoxic side effects of antifungal treatment directed to a special or high-risk population in children. From our results, it is shown that children who have malignancies were at highest odds of developing the unwanted renal effects of Amphotericin B. This is perhaps due to their previous chemotherapy received or their immunosuppressed state that have implications on their baseline renal status.

As seen in a retrospective study of adult patients, total dose and duration of therapy was a determinant of nephrotoxicity as attributed to the degree of exposure of the kidneys to the drug (9). Harbath et al noted that with longer duration of treatment, the greater the cumulative risk of nephrotoxicity. Results from the study of Fischer and colleagues also noted that each 0.10mg/kg/day increment in Amphotericin B dose was associated with a 1.8 fold increase in the risk of nephrotoxicity. In our study, a duration of more than 14 days of treatment and doses of amphotericin above 7.1mg/kg was already associated with occurrence of hypokalemia or acute kidney injury. These findings can assist the clinicians is assessing the risk-benefit ratio of high dose or prolonged Amphotericin B treatment.

Diuretics administered during the course of treatment were associated with a 12.5 increase in the risk of nephrotoxicity as noted from Fisher et al. This is similar to our results, showing that concomitant diuretic use conferred 5.5 times odds of nephrotoxicity compared to those who were not given any. This is presumably due to the direct effect of diuretics on the kidneys and a relative hypovolemia that may be experienced during diuretic treatment (9). This is also consistent with reports saying that saline loading can reverse nephrotoxicity in patients who may

be intravascularly volume depleted during treatment. In our study, however, there was no noted association with additional fluid supplementation with the occurrence of nephrotoxicity. Nevertheless, with the above results, if diuretic therapy is warranted, meticulous attention must be given to possible shifts in intravascular volume status and re-assessment of renal function.

Although not significant in our study, it is observed in several reports that the use of nephrotoxic drugs such as aminoglycosides, vancomycin and cyclosporine are confounding factors for the occurrence of nephrotoxicity (8,9,12). Some reports though, show contrasting results (10,11). This combination of nephrotoxic medications with amphotericin B were associated with higher renal toxicity possibly because patients who were given more concomitant drugs were also sicker hence a decreased glomerular filtration rate and not solely on the direct nephrotoxic effect of these drugs to the kidneys (13). Our study shows that those who were not given concomitant nephrotoxic drugs had lesser odds of having nephrotoxicity secondary to Amphotericin B. Perhaps not only because of less exposure to nephrotoxins but because these patients were relatively less sick compared to others who required more medications.

## CONCLUSION AND RECOMMENDATIONS

Patients who were given deoxycholate Amphotericin B were mostly between 6 months old to 9 years old and had z scores for weight for length/height equal to zero. There was no gender predilection as to the occurrence of nephrotoxicity between males and females. Majority of patients who received Amphotericin B had underlying malignancies and were given the drug due to blood and urine growth of *Candida* species. Vancomycin and aminoglycosides, alone or in combination, were among the common nephrotoxic drugs that were given together with Amphotericin B. Baseline creatinine, eGFR and serum potassium levels and the presence of hydration during treatment were not significant determinants for occurrence of hypokalemia and/or acute renal failure.

From this study, the associated risk factors with nephrotoxicity due to deoxycholate Amphotericin B therapy were malignancy as an underlying medical condition, duration of Amphotericin treatment of more than 14 days, cumulative Amphotericin dose of more than 7.1 mg/kg and diuretic use during treatment. Patients who had normal nutritional status (weight for height/length z score of 0) and with no other administered concomitant nephrotoxic medications had lesser odds of developing hypokalemia or acute kidney injury during Amphotericin treatment.

Because the development of acute kidney injury and electrolyte imbalances are associated with a longer hospital stay, higher mortality and additional medical costs, clinicians should consider the above risk factors among patients for Amphotericin treatment. Alternative fungal therapy and measures to decrease Amphotericin nephrotoxicity can be considered to address children who require treatment.

The retrospective nature of this study places limitations on accuracy, particularly in the recordkeeping. With the identification of children with malignancy as a high-risk population, further research on the effects of Amphotericin B on these children can be explored as to determine specific timing of monitoring for renal effects and timely intervention for complications. Determination of the specific onset and dose of deoxycholate Amphotericin B where manifestations of nephrotoxicity will begin can also be a future area for investigation.

## REFERENCES


1. Bes D, M Rosanova, N Sberna, Pharm D, Arrizurieta E. Deoxycholate amphotericin B and nephrotoxicity in the pediatric setting.Pediatr Infec Dis.2014 (33): 198-206.

2. Goldman R, Koren G. Amphotericin B nephrotoxicity in children.J Pediatr Hematol Oncol.2004; 26(7):421-6.

3. Berdichevski R, Luis LB, Crestana L, Manfro RC. Amphoericin B-related nephrotoxicity in low-risk patients. Braz J Infect Dis. 2006;10(2):94-9

4. Luber A, Maa L, Lam M, Gugliemo BJ. Risk factors for amphotericin B-induced

nephrotoxicity. J Antimicrob Chemother.1999.43, 267-71

5. Horwitz E, Shavit O, Shouval R, Hoffman A, Shapiro M, Moses A. Evaluating real-life clinical and economical burden of amphotericin-B deoxycholate adverse reactions. Int j Clin Pharm. 2012; (34):611

6. Laborin RL, Vargas MN. Amphotericin B:side effects and toxicity. Rev Iberoam Micol 2009;26(4):223-7.

7. Bagnis C, Deray G. Amphotericin B nephrotoxicity. Saudi J Kidney Dis Transplant. 2002; 13(4):481-91.

8. Dutta A, Palazzi D. Risk factors of amphotericin B toxicity in the non-neonatal pediatric population. Pediatr Infec Dis J. 2012; 31(9):910-4

9. Fisher MA, Talbot GH, Maislin G et al.Risk factors for amphotericin B-associated nephrotoxicity. Am J Med. 1989. 87:547-52

10. Soler Y, Plaza N, Prieto M. pRIFLE (pediatric risk, injury,failure,loss,end stage renal disease) score identifies acute kidney injury and predicts mortality in critically ill children: a prospective study.Pediatr Crit Care Med.2013; 14(4): 189-95.

11. Bates D, Su L, Yu D et al. Correlates of acute renal failure in patients receiving parenteral amphotericin B. Kidney international.2001; 60: 1452-1459

12. Harbath S, Pestotnik S, Lloyd J et al. The epidemiology of nephrotoxicity associated with conventional amphotericin b therapy. American Journal of Medicine. 2001.11:529-534

13. Goldman R, Ong M, Wolpin J et al.Pharmacologic risk factors for amphotericin b nephrotoxicity in children. J Clin Pharmacol. 2007; 47:1049-1054

# PREVALENCE AND FACTORS ASSOCIATED WITH SEROPROTECTION AFTER PRIMARY SERIES OF HEPATITIS B VACCINATION AMONG CHILDREN SEEN IN THE OUTPATIENT SETTING OF PHILIPPINE CHILDREN'S MEDICAL CENTER

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**ABSTRACT**

**BACKGROUND:** Hepatitis B is a vaccine-preventable condition that could develop into cirrhosis and hepatocellular carcinoma. Identifying patients at risk for Hepatitis B infection despite the universal implementation of Hepatitis B vaccination will help improve the immunization program.

**OBJECTIVE:** To determine the prevalence and factors associated with seroprotection among children 3 months to 18 years old primary Hepatitis B vaccination series.

**METHODOLOGY:** This is a cross-sectional study among children 3 months to 18years old with complete Hepatitis B immunization. Demographic, social and clinical data were correlated with response to HBsAg, Anti-HBs and Total Anti-HBc tests.

**RESULTS:** Among 110 subjects from different age groups, 52% had seroprotective anti-HBs levels ($\geq$10 mIU/ml). Seventy four percent seroprotection was seen in subjects with <5 years interval from vaccination, 26% in cases after 5-10 years, and 38% at 10 years after vaccination with significant difference. Other factors such as gender, geographic area, type of vaccine, schedule, age at first dose and place of vaccine were not associated with seroprotection.

**CONCLUSION:** Fifty two percent seroprotection from Hepatitis B infection among different age groups was demonstrated in our study. Interval year after vaccination was the only factor established to have significant association with seroprotection, with < 50% decline of anti-HBs level at 5 years or more after vaccination.

**RECOMMENDATIONS:** Community studies with larger population are needed. Anti-HBs detection 5 years or more post-vaccination may be considered to identify patients at risk for breakthrough infection. Repeat serologic testing among non-seroprotected subjects is recommended.

**KEYWORDS**: Seroprotection, booster

## INTRODUCTION

Information on the prevalence of antibody response among children who completed the vaccine in the country is still limited, if not unavailable. Despite the success of the universal infant hepatitis B vaccination program in some parts of the world, chronic HBV infection with consequent cirrhosis and hepatocellular carcinoma remains to be common in certain hyperendemic areas like the Philippines. In earlier studies, factors affecting such response to Hepatitis B vaccine have shown varying results (1). Furthermore, among children who initially responded to a primary three-dose vaccination series, around 15–50% demonstrate low or undetectable anti-HBs levels 5–10 years after primary vaccination (2). Data on these aspects can help identify certain areas that need improvement in our national immunization program to fully combat the scourge of Hepatitis B infection.

The burden of Hepatitis B infection cannot be underestimated. The disease accounts for 30% of cirrhosis and 53% of hepatocellular carcinoma (HCC) cases worldwide and as such, responsible for a staggering half a million deaths every year. In young children, most Hepatitis B infections are asymptomatic and unrecognized until complications (chronic liver disease, cirrhosis, hepatoma) develop after decades, typically during mid-adulthood. Since the

infection and its sequelae are not essentially an issue for childhood survival, Hepatitis B immunization primarily targeting young children remains to be underestimated (3). In contrast, this should be the period to emphasize prevention of the said disease and studies on younger population should be prioritized.

This paper aims to review the prevalence of seropositivity to Anti-HBs among children given primary series of Hepatitis B vaccine, and probe on the factors that may affect response. After overcoming all the funding obstacles on vaccine availability, an evaluation of the outcome of the immunization program and Hepatitis B status among our patients may help guide future policies and priorities in the continuity of the battle against this infection.

Unfortunately, the progress in Hepatitis B control in the Philippines has still been slow, erratic and to date, inadequate (3). This study can give a glimpse of the Hepatitis B status including the response to the widely implemented universal immunization program, and provide insights on possible areas of improvement and reinforcement.

**OBJECTIVES OF THE STUDY**
**GENERAL OBJECTIVES:**

To determine the prevalence and factors associated with seroprotection among children 3 months to 18 years old with primary Hepatitis B vaccination series

**SPECIFIC OBJECTIVES:**

1. To describe the clinicodemographic profile of children ages 3 months-18 years old with primary series of Hepatitis B vaccine
    a. age
    b. gender
    c. geographical area
    d. socioeconomic status
    e. educational attainment of parents
    f. nutritional status

2. To determine the HBsAg, Anti HBs and Anti HBc positivity rate among children ages 3 months-18 years old with primary Hepatitis B vaccine series

3. To determine if the following factors are significantly associated with seroprotection among children ages 3 months-18 years old with primary Hepatitis B vaccine series
    a. gender
    b. geographic area
    c. site of vaccine injection
    d. place where vaccine was given
    e. age at first dose of vaccine
    f. type of hepatitis B vaccine
    g. vaccine schedule
    h. interval years from the last dose of vaccine

4. To determine the seropositivity to HBsAg among Anti HBs-negative children ages 3 months-18 years old given primary Hepatitis B vaccineseries.

5. To determine if the following factors are significantly associated with seropositivity to HBsAg among children ages 3 months-18 years old with primary Hepatitis B vaccine series
    a. age
    b. gender
    c. educational attainment of parents
    d. socioeconomic status
    e. maternal HBsAg status
    f. history of blood transfusion
    g. mode of birth delivery
    h. history of sexual contact, for children 10-18 years old
    i. history of IV illicit drug use, for children 10-18 years old

**METHODOLOGY**

This was a prospective cross-sectional study. The study was done among children ages 3 months to 18 years old seen at PCMC outpatient department with records showing complete immunization of primary series of Hepatitis B vaccine. Children without any record of immunization history, children in immunocompromised state (on immunosuppressive drugs, malnourished, diagnosed cases of tuberculosis, diagnosed cases of chronic liver disease - except for symptomatic or asymptomatic cases of acute or chronic hepatitis B, immunologic, hematologic-oncologic, renal conditions), those who received the last dose of vaccine within four weeks from the time of conduct of the study, and those who received booster doses of hepatitis B vaccine were excluded.

Based on statistics that children aged 1 to 5 years old with presenting symptoms of HBV infection would have probability of presenting with acute hepatitis at a rate of 5% to 15%, then, using the 5% rate of disease to develop with +/-5% margin of error estimated at CI 95%, the sample size needed in this study was 100 cases as representative of patients in the general and adolescent pediatrics who have immunization records showing the status of hepatitis B vaccine during their infancy.

Subject recruitment of children ages 3 months to 18 years old among patients at the pay and charity outpatient department with records of immunization of primary series of Hepatitis B vaccine was done where the principal investigator screened for potential subjects who agreed to join the study. Upon agreement and signing of Informed Consent/Assent, history and physical examination were done. Laboratory requests were given to the parents for the child's serologic examination where approximately 5 ml of blood was obtained. HBsAg, Anti-HBs and Total Anti-HBc determination using enzyme-linked immunosorbent assay (ELISA) method was done. The Anti-HBs assay was used to detect antibodies to the surface antigen of HBV in the serum samples with a sensitivity of 99.2% and specificity of 99.4%. The Anti-HBc assay determined the presence of total antibodies to the core antigen of HBV with a sensitivity of 99.53% and specificity of 99.5%. The HBsAg assay detected the presence of surface antigen of the virus with a sensitivity of 100% and specificity of 99.94%. All the immunologic assays made use of Bio-Rad Detection Kit.

Complications like pain, hematoma, minimal bleeding and inflammation following blood extraction were explained in detail. Measures like applying adequate pressure over the puncture site to control the bleeding and avoid hematoma formation were advised. Proper infection control policies were followed. Subjects were notified in person or via phone communication of the results of serologic tests.

The following data were gathered from the subjects:

a. age
b. gender
c. weight, length/height, weight-for-length/weight-for-height/BMI to determine nutritional status
d. presence of jaundice, icteric sclera, organomegaly and other physical findings that may pertain to liver diseaseparental educational attainment either elementary, high school, college, others.
e. parents' occupation
f. vital status of each parent, whether alive or deceased. If deceased, identify the cause of death
g. socioeconomic status based on the income bracket followed by the social service of the institution
h. geographical area or the patient's place of residence based on the regions of the country where the child spent more than half of his lifetime
i. objective evidence of maternal HBsAg status before the time patient received vaccine, whether reactive, nonreactive, or unknown
j. history of transfusion of any blood product at least one week prior to the conduct of the study
k. mode of delivery either vaginal or caesarean section
l. history of sexual contact, for subjects 10-18 years old
m. history of illicit IV drug use, for subjects 10-18 years old

Patients' immunization records were also evaluated to determine the following:

a. site of vaccine injection (buttocks, thigh, deltoid, others)
b. age at the first dose of vaccine (within 24 hours, more than 24 hours- 7 days, or more than 7 days)
c. age at succeeding doses to identify the schedule that was followed (0-1-2, 0-1-6, 0-6-10-14, others)
d. interval in years from the last dose of vaccine to the conduct of the study (0-4 years, 5-10 years, more than 10 years)
e. place of vaccine administration either through private clinic, local health center or both
f. type of vaccine given whether monovalent or combination with or without monovalent dose

Seropositivity to Anti-HBs, Total Anti-HBc and HbsAg, were the primary outcomes of interest in this study. Determination of patients'

demographic profile and analysis of factors affecting seropositivity were the secondary outcome measures. The association between the above variables and the serologic results were statistically analyzed.

Patients' categorical profiles such as demographics, Anti-HBs, Total Anti-HBc positivity results and HbsAg seropositivity were expressed in frequency and percentages. In testing associations among patients' profiles and Anti-HBs, Anti-HBc or HBsAg seropositivity results, Chi square test of independence with 2x2 Fischer Exact test adjustment were performed. Analysis in predicting seroprotection were done using Multi-logistic regression modelling as well as estimating the Odds ratio CI 95%. Any associated p-value less than 0.05alpha were considered significant. STRATA ver 14 was used as statistical software in processing the data.

## RESULTS

Among the 202 children screened with immunization records, only 163 subjects had complete primary series of Hepatitis B immunization (Figure 1). Upon exclusion of other criteria, 111 parents agreed to sign the informed consent, however, one refused blood re-extraction for validation of result, hence was considered a drop-out. A total of 110 subjects were left as study participants where fifty subjects (45%) were male and sixty subjects (55%) were female. The male to female ratio was 1:1.2. All subjects completed their vaccination within 1 year of age. Majority belonged to the age group 3-9 years old (42%) followed by infants 3 months to 2 years of age (38%) with a mean age of 5.23 +/- 4.34 SD (Table 1). Eighty eight study participants (80%) came from the National Capital Region (NCR) belonging to the middle income socioeconomic status (49%) but most parents were able to attain tertiary level of education (61%). Eighty five subjects (79%) had normal nutritional status while 13 patients were overweight (12%), and 10 patients were obese (9%).

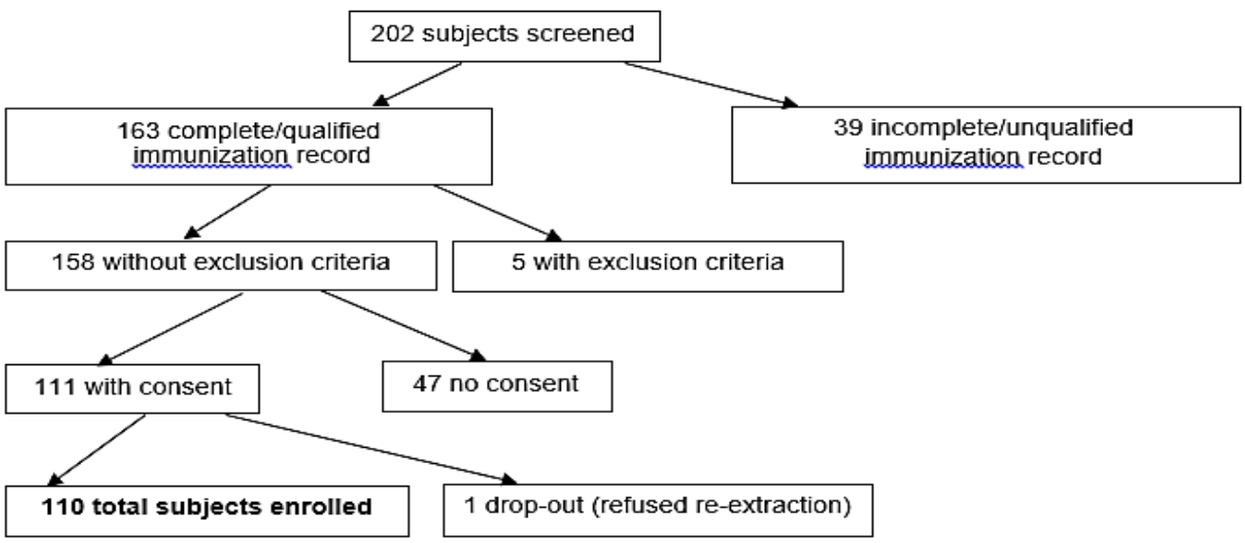


**Figure 1**: Recruitment of study participants

**Table 1:** Distribution of Anti-HBs Levels According to demographic Factors

| SOCIODEMOGRAPHIC VARIABLES | TOTAL (n/%) |
|---|---|
| Age | |
| 3 months-2 years old | 41 (38%) |
| 3-9 years old | 46 (42%) |
| 10-18 years old | 23 (20%) |

| | |
|---|---|
| Mean +/- SD | 5.23 +/- 4.34 |
| Gender | |
| Male | 50 (45%) |
| Female | 60 (55%) |
| Geographic area | |
| Region I-V | 22 (20%) |
| NCR | 88 (80%) |
| Region VI-VIII | 0 (0%) |
| Region IX-XIII | 0 (0%) |
| Socioeconomic status | |
| C1 | 13 (12%) |
| C2 | 54 (49%) |
| C3 | 23 (21%) |
| Indigent | 0 (0%) |
| Pay | 20 (18%) |
| Parents' Educational attainment | |
| Elementary | 3 (3%) |
| High School | 37 (33%) |
| College | 67 (61%) |
| Others - Postgraduate | 3 (3%) |
| Nutritional status | |
| Normal | 87 (79%) |
| Overweight | 13 (12%) |
| Obese | 10 (9%) |

**Seropositivity to Anti-HBs, Anti-HBc, HBsAg**

As depicted in Figure 2, reactivity to anti-HBs (≥10mIU/ml) was noted in 54% (59/110) of the study population. Among them, 52% (57/110) were due to protection from Hepatitis B immunization having concomitant nonreactive results to anti-HBc (Table 2) and nonreactive result to HBsAg. Three out of 110 participants (3%) were found to be reactive to anti-HBc, while 1 subject (1%) had reactive results to HBsAg.

**Figure 2:** Seropositivity to Anti-HBs, Total Anti-HBc And HBsAg

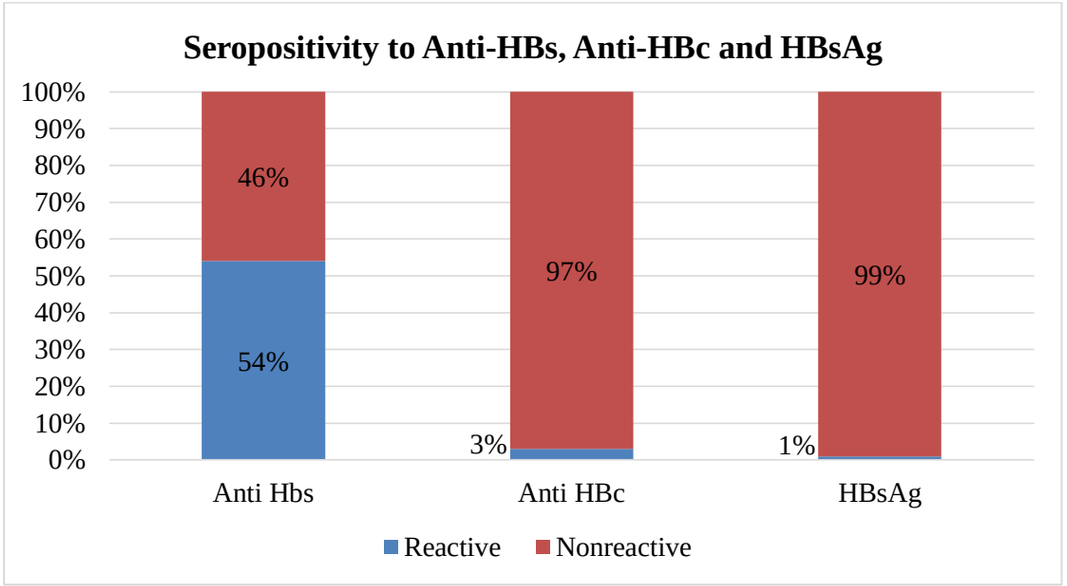

**Table 2:** Anti-HBs And Total Anti-HBc Positivity Results

| | Anti-HBs | |
|---|---|---|
| | **Reactive (≥ 10 mIU/ml)** | **Nonreactive (< 10 mIU/ml)** |
| **Total Anti HBc** | | |
| **Reactive** | 2 (2%) | 1 (1%) |
| **Nonreactive** | 57 (52%) | 50 (45%) |

**Factors affecting response after primary series of Hepatitis B Vaccine**

Of 57 seroprotected patients from hepatitis B vaccine, the highest seroprotection rate was noted at 82% (32/39) among the youngest age group of 3 months to 2 years, followed by 41% (19/46) among the 3 years to 9 years old group, and only 26% (6/23) from the adolescents 10-18 years old (Figure 3, Table 3).

Fourteen percent of the nonseroprotected subjects (7/51) wereas young as 1-2 years old who all received vaccination from the local health center within NCR using combination with or without monovalent dose. Forty three percent (3/7) of them followed 0-6-10-14 schedule, another 43% (3/7) followed nonspecific schedule, and 14% (1/7) followed 0-1-2 schedule for their vaccine.

**Figure 3:** Seropositivity to Anti-HBs among Age groups

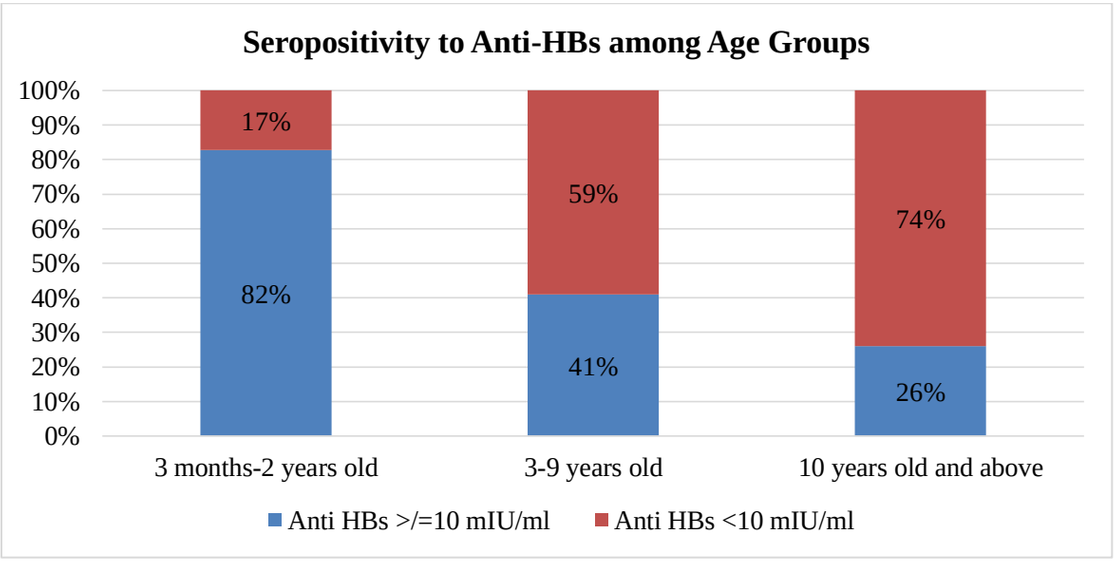


**Table 3: Seroprotective Anti-HBs Level by Age Group**

| | **SEROPROTECTED (N/%) N= 57** | **NON-SEROPROTECTED (N/%) N=51** | **TOTAL N=108** |
|---|---|---|---|
| **Age** | | | |
| 3 months-2years old | 32 (82%) | 7 (18%) | 39 (36%) |
| 3-9 years old | 19 (41%) | 27 (59%) | 46 (43%) |
| 10-18 years old | 6 (26%) | 17 (74%) | 23 (21%) |

Both male and female groups had almost equal seroprotected patients, although, majority of the seroprotected group at 56% (32/57) were female. Gender was not found to be significantly associated with seroprotection. Seventy seven percent (44/57) from the seroprotected group came from NCR (National Capital Region). However, only 52% of patients from NCR were seroprotected and 48% were nonseroprotected (Table 4). Considering the place of vaccine, the number of seroprotected and nonseroprotected subjects were almost equally distributed among those who received vaccine from the private clinic and from the local health center (Table 4). Although 72% (41/57) of the seroprotected group received their vaccines from local health center, this was not statistically significant, like geographic area when analyzed with seroprotection. All subject participants received their vaccine injections in the thigh.

**Table 4:** Factors Affecting Response to Primary Series of Hepatitis B Vaccine

| VARIABLES | SEROPROTECTED (N/%) N= 57 | NON-SEROPROTECTED (N/%) N=51 | TOTAL N=108 | P VALUE |
|---|---|---|---|---|
| **Gender** | | | | |
| Male | 25 (51%) | 24 (49%) | 49 (45%) | 0.74 |
| Female | 32 (54%) | 27 (46%) | 59 (55%) | |
| **Geographic Area** | | | | |
| Region I | 1 (100%) | 0 (0%) | 1 (1%) | |
| Region II | 0 (0%) | 1 (100%) | 1 (1%) | 0.15 |
| Region III | 2 (29%) | 5 (71%) | 7 (6%) | |
| Region IV-A | 10 (77%) | 3 (23%) | 13 (12%) | |
| NCR | 44 (52%) | 42 (48%) | 86 (80%) | |
| **Place of vaccine** | | | | |
| Private | 8 (53%) | 7 (47%) | 15 (14%) | |
| Local health center | 41 (50%) | 41 (50%) | 82 (76%) | 0.37 |
| Combination | 8 (73%) | 3 (27%) | 11 (10%) | |
| **Site of Administration** | | | | |
| Buttocks | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| Thigh | 57 (53%) | 51 (47%) | 108 (100%) | |
| Deltoid | 0 (0%) | 0 (0%) | 0 (0%) | |
| Others | 0 (0%) | 0 (0%) | 0 (0%) | |
| **Age at first dose of vaccine** | | | | |
| Within 24 hrs old | 29 (55%) | 24 (45%) | 53 (49%) | 0.79 |
| >24 hours–7 days old | 8 (57%) | 6 (43%) | 14 (13%) | |
| >7 days | 20 (49%) | 21 (51%) | 41 (38%) | |
| **Schedule of vaccine** | | | | |
| 0-1-2 | | | | |
| 0-1-6 | 9 (32%) | 19 (68%) | 28 (26%) | |
| 0-6w-10w-14w | 3 (100%) | 0 (0%) | 3 (3%) | 0.05 |
| Others | 15 (68%) | 7 (32%) | 22 (20%) | |
| | 30 (55%) | 25 (45%) | 55 (51%) | |
| **Type of vaccine** | | | | |
| Monovalent alone | 22 (41%) | 32 (59%) | 54 (50%) | |
| Combination with or without monovalent | 35 (65%) | 19 (35%) | 54 (50%) | **0.01** |
| **Interval years from the last dose of vaccine** | | | | |
| 0-4 years | | | | |
| 5-10years | 42 (74%) | 15 (26%) | 57 (53%) | **0.0000** |
| ≥11 years | 10 (26%) | 28 (74%) | 38 (35%) | |
| | 5 (38%) | 8 (62%) | 13 (12%) | |

Data gathered from this study showed that only 49% (53/110) of the population received their initial dose within 24 hours as recommended. Fifty one percent (29/57) of the seroprotected group received their first dose within 24 hours of birth, while 35% (20/57)

were given beyond 1 week of age. Findings also revealed that 53% of the seroprotected group (30/57) followed nonspecific schedules with minimum of one month interval between doses. Meanwhile, 63% (19/28) of those who followed 0-1-2 schedule were not seroprotected. On the other hand, all who used the 0-1-6 schedule were seroprotected (3/3), and about 68% (15/22) who followed the 0-6-10-14 schedulewere seroprotected. After analysis, none of the parameters (age at first dose and schedule) showed significant difference in association with seroprotection, having p values of 0.79 and 0.05 respectively.

The type of vaccine used either monovalent or combination type showed a significant difference when analyzed against seroprotection rate. Thirty five patients (61%) from the seroprotected group were given combination vaccine, with a significant p value of 0.01. There were 59% (32/54) who were nonseroprotected among those who received monovalent type in all vaccine doses. Meanwhile, majority of those given combination vaccine were seroprotected at 65% (35/54). Protective anti-HBs levels were maintained in 74% (42/57) of subjects less than 5 years after vaccination, 26% (10/38) in cases after 5-10 years, and only 38% in cases after 10 years from the last vaccine. This interval year from the last vaccination was analyzed and was noted to be significant.

Multilogistic regression analysis done on statistically significant variables (type of vaccine and interval years from last vaccination) revealed that only the time intervalafter vaccination was significantly associated with seroprotection (Table 5). Children are 7 (1/0.13) times less likely seroprotected 5-10 years after the last vaccination and childrenare 4 (1/0.22) times less likely seroprotected more than 10 years after vaccination compared to children less than 5 years after vaccination.

**Table 5:** Multilogistic Regression Analysis of Interval Years as a factor affecting Seroprotection

| | **Odds Ratio** | **P>\|z\|** | **[95% Conf. Interval]** |
|---|---|---|---|
| **Interval years from last vaccine dose** | | | |
| 0-4 years | | | |
| 5-10 years | 0.1275510 | 0.000 | 0.0502177 0.3239747 |
| >10 years | 0.2232143 | 0.020 | 0.0631043 0.7895593 |

Out of 110 subjects, the only patient found to be reactive to HBsAg had concomitant reactive result to Total anti-HBc and nonreactive result to anti-HBs (Table 2). The subject is a 6 year old female, born to an HBsAg positive mother via normal spontaneous delivery, with no history of blood transfusion, and who received a birth dose of Hepatitis B vaccine together with an immunoglobulin within 12 hours of birth from a private hospital (Table 6). There were no symptoms of jaundice, fever, abdominal pain nor hepatomegaly at the time of serological survey.

**Table 6:** Profile of 3 Study Participants Positive for Anti-HBc

| **Child No.** | **Age** | **Gender** | **Age at first dose of HB vaccine** | **Maternal HBsAg Status** | **HBsAg** | **Total Anti-HBc** | **Anti-HBs** |
|---|---|---|---|---|---|---|---|
| 18 | 4 months | M | 7 days old | Unknown | NR | R | R 263.935 |
| 56 | 6 years & 4 months | F | 1 day old (HBIg within 12 hours) | R | R | R | NR <2 |
| 96 | 4 years & 7 months | F | 1 month old | Unknown | NR | R | R-27.677 |

## DISCUSSION

Hepatitis B immunization remains to be the mainstay in the prevention of Hepatitis B infection due to unavailability of specific treatment for the said virus. A complete series of Hepatitis B vaccine induces protective antibody levels in more than 95% of infants, children and young adults (10).

In our country, the prevalence rate of the anti-HBs antibody among the pediatric Hepatitis B vaccinees after 5-11 years since primary immunization was determined by Mendoza et al in 2007, and was found to be 71.5% (211/295) (7). In an unpublished study in Cagayan de Oro in 2014, about 54% of children had Anti-HBs seroprotective levels ($\geq$10mIU/ml) 5-6 years after three doses of Hepatitis B vaccine (17), while a separate unpublished study in Cebu City in 2015 found a seroprotection rate of 48% among children 3-6 years old (18). In our study done among different age groups, 52% of the overall population were found to have seroprotective Anti-HBs levels after a complete Hepatitis B immunization. However when grouped according to age, 82% were seroprotected among ages 3 months to 2 years old, 41% among 3-9 years old, and only 26% among 10-18 years old (Figure 3, Table 3). Due to higher availability of immunization records among younger infants and children, majority of the study participants came from the said age groups and less from the adolescent group, which contributed to the non-homogenous distribution of subjects by age.

Previous studies demonstrated that anti-HBs titers decline over time (19, 20). Though according to a meta-analysis, protection provided by three or four doses of monovalent HB vaccine persists for at least two decades in the great majority of immunocompetent individuals (21), in some studies, antibodies have been demonstrated to become negative in 15-50% of the vaccine responders within 5-10 years (2, 22). In this study, seroprotective levels were detected in 74% of children less than 5 years after the vaccination, in 26% of cases 5-10 years after vaccination, and 38% in cases 11 years onwards after vaccination. A study in 2014 found that 88% seroprotection was seen in less than 5 years after vaccination, however, with less significant decrease to 78% between 5-10 years after vaccination, and 74% 10 years after vaccination (23). In contrast, we found 26% and 38% seroprotection rate among cases 5-10 years and more than 10 years after vaccination, respectively. This is quite alarming since more than 50% of subjects are already at risk for breakthrough Hepatitis B infection 5 years after their vaccination. Although a decreasing trend was observed, the higher seroprotection rate 11 years after vaccination compared with 5-10 years after vaccine may be due to recruitment bias that affected the distribution of subjects by age. Although in general, Hepatitis B booster dose is not advised among immunocompetent persons due to vaccine-induced immune memory that is said to persist for more than 20 years following immunization (10, 24), a booster series is still recommended (25). Nonseroprotected subjects in this study were advised to receive three-dose booster of Hepatitis B vaccine. According to Su et al in 2013, 95% maintained protective Anti-HBs level after a three-dose booster (26). Unfortunately, repeat post vaccination testing was beyond the scope of our study.

Male gender is said to be associated with nonresponse to Hepatitis B vaccine, owing to the effect of the sex hormone testosterone which damages the production of the immunoglobulins (27). Moreover, numerous immunological genes are also found in the X chromosome while only few ones are mapped in the Y chromosome (28). Similar to other findings (19, 20, 24), no gender difference was observed in this study.

Philippine Children's Medical Center is a referral center in an urban setting. Most subjects who came from National Capital Region had almost equal seroprotection and nonseroprotection rate. The youngest among the nonseroprotected group aged 1-2 years old came from NCR, specifically Quezon City. According to He et al in 2015, the possibility of a low level of or even a negative anti-HBs for children at or under age 3 should be a concern (25). Circumstances surrounding administration of the vaccine should be investigated especially vaccine handling and storage. The effectiveness of vaccine depends on the source of procurement and proper maintenance of cold chain, which is largely affected by the place where the vaccine was given. In a developing tropical country like ours, adherence to the recommended vaccine storage of refrigerator temperature between 2-8C

remains a challenge among local health centers. In a study by Mohammad Afzal in 2011, 100% seroprotection was observed in children who received vaccine from a private source (29). This is different from the findings in this study showing an equal seroprotection and nonseroprotection rate among patients from both private and local health center. Although majority of the seroprotected group were patients who received vaccine from local health centers, this did not show any significant difference.

The WHO recommends that the first dose of vaccine be administered within 24 hours of birth to prevent mother to child transmission of infection (30). Our data shows that only 48% (53/110) of the study population received their initial dose of vaccine within 24 hours as recommended with majority already covered by the Mandatory Infants and Children Health Immunization Act of 2011 that should provide infants with a birth dose of Hepatitis B vaccine within 24 hours of birth (30).Some authors reported lower proportions of individuals with anti-HBs ≥ 10 mIU/mL if the first vaccine dose had been given directly after birth (31, 32). Findings in this study are in accordance with the observations by Mele, et al and Schonberger, et al wherein a first dose within 24 hours of birth does not appear to be related to lower proportions of individuals with anti-HBs ≥ 10 mIU/mL, supporting the use of vaccination schedules starting at birth in order to attain timely vaccination and higher vaccination rates (33, 34).

Dosing schedule is another important factor in the development of antibody response and titer level. There should be a minimum gap of 8 weeks between the 2nd and 3rd doses, and at least 16 weeks between the 1st and 3rd doses of Hepatitis B vaccination (35). However, to minimize frequency of healthcare visits, the dosing schedule has been negotiated in the EPI at 6,10,14. The WHO has recommended 0-1-2 schedule for highly endemic countries like the Philippines however, in this study, 68% of those who followed this schedule were seronegative which is similar to the findings of Mapandi of 43% seroprotection rate (14). On the other hand, Yao et al demonstrated a lower seropositive anti-HBs level with other schedules compared to 0-1-6 schedule (36), while our findings in this study showed 100% seroprotection among the 0-1-6 group. Although some studies have shown the 6, 10, 14 schedule to be effective, Afzal, et al demonstrated a low seroprotection rate of 68% with lower geometric mean titer levels achieved compared to the 0-1-6 schedule (29). A 0, 6,10,14 schedule is being followed by our government and implemented at our local health centers. Our data showed 68% seroprotection among those who followed this schedule, although overall, the schedule of vaccine had no significant association with seroprotection.

Studies have compared combined and monovalent vaccines and have shown little difference between the two regimens in terms of immunogenicity after a dose of HBV at birth (37). *C*ombination vaccine is reported to have shown good immunogenicity and good long term anti-HBs persistence which could advantageously replace separate monovalent vaccines in areas of high Hepatitis B endemicity in terms of clinical, economic and strategic benefits (38). In our study, majority from the seroprotected group were given combination vaccine although this did not show significant difference on multilogistic regression. A higher seroprotection of 65% was also seen among those who were given combination vaccine with or without monovalent dose. Interestingly, 59% of those who only received monovalent vaccine were nonseroprotected. The variation in vaccine brands used may contribute to the difference in immunogenicity of the monovalent vaccine and could be a possible explanation for this finding.

In an HBV endemic country like Taiwan, the prevalence of HBsAg has decreased from 9.8% to 0.7% 15 years after the implementation of universal Hepatitis B vaccination. In China, HBsAg seroprevalence has decreased markedly from over 8.5% in 1992 to <1% in 2006 since routine immunization was recommended by the CDC (39). This study revealed that 1% among the subject participants was seropositive to HBsAg which was a case of vertical transmission. This is closely similar to two separate local and unpublished studies in 2014 and 2015 reporting an HBsAg seropositivity rate of 0.3% and 0.6% among preschool and school aged children in Cebu City and Cagayan de Oro, respectively (17, 18). According to Wong, the Philippines is still highly endemic for Hepatitis B with a prevalence rate of 16.7% (4). Since this is a single-center study among children with limited

sample size, the finding of 1% Hepatitis B infection rate is not reflective of the true national HBsAg prevalence and cannot be used to conclude the effectiveness of our country's universal immunization program.

## CONCLUSION AND RECOMMENDATION

In this study, 52% of patients among different age groups were found to have seroprotective anti-HBs levels. A decrease in seroprotection rate against Hepatitis B infection was demonstrated at< 50% after 5 years and more post-vaccination with statistical significance. Gender, geographic area, age at first dose, place of vaccine, schedule and type of vaccine were among the other factors analyzed which, in this study, cannot conclusively affect seroprotection rate due to the declining protective immunity seen with increasing time.

There is a definite need to review the implementation of Hepatitis B immunization program in our country. Further studies in the community, or among different hospital institutions should be carried out for larger study population to determine seroprotection rate and factors affecting it 1-2 months after vaccination. Detection of anti-HBs levels 5 years or more post vaccination may be considered to determine the need for booster dose, and successfully prevent breakthrough Hepatitis B infection. Follow-up studies on the subjects who had no seroprotection should be done, with repeat anti-HBs testing post completion of booster doses, to identify the true nonresponders.

## REFERENCES


1. **Chang, YC, Wang JH, et al.** Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study. *BMC Public Health* 2014, 14: 991

2. **Huang,LM, et al.** Long-term response to hepatitis B vaccinationand response to booster in children born to mothers with Hepatitis B e antigen. Hepatology 1999; 29:954-9

3. **Shepard, CW et al.** Hepatitis B Virus Infection: Epidemiology and Vaccination*Epidemiol Rev* 2006;28:112–125

4. **Wong,S, Ong,J et al.**Hepatitis B Infection among adults in the Philippines: A National Seroprevalence Study. World J Hepatol 2013 April 27; 5(4):214-219

5. **Lingao AL, et al.**Seroepidemiology of hepatitis B virus in the Philippines.*Am J Epidemio* 1986 Mar;123(3):473-80

6. Implementing Guidelines on Hepatitis B Immunization for Infants, Administrative Order No. 2006-0015. Republic of the Philippines. Department of Health, June 2006

7. **Mendoza,B, Chan,V, et al.**Detection of Anti-HBs, Anti-HBc and HBsAg among Pediatric Vaccinees Years after Primary Hepatitis B Immunization. Philippine Journal of Science 136 (1): 57-63, June 2007

8. **Van Herck,K, Van Damme,P,** Benefits of Early Hepatitis B Immunization Programs for Newborns and Infants. *Pediatric Infectious Disease Journal* 2008; 27: 861-869

9. **Filippelli, M, Lionetti, E,** Hepatitis B vaccine by intradermal route in non-responder patients: An update. *World J Gastroenterol* 2014 August 14; 20(30): 10383-10394

10. **Hamborsky J, Kroger A, Wolfe S.** Centers for Disease Control and Prevention. Epidemiology and Prevention of Vaccine-Preventable Diseases., eds. 13th ed. Washington D.C. Public Health Foundation, 2015

11. **Lu, JJY, et al.**Hepatitis B immunity in adolescents and necessity for boost vaccination: 23 years after nationwide hepatitis B virus vaccination program in Taiwan. *Vaccine*(2009) 6613–6618

12. **Gitlin, N**. Hepatitis B: Diagnosis, prevention, and Treatment. Clinical Chemistry, 1997, 43:1500-1506

13. Recommended Immunization Schedule for Persons Aged 0 Through 6 Years – United States 2010. www.aap.org

14. **Mapandi, Isnihaya**, Serologic Status Of Neonates Born To Hepatitis B Positive

Mothers And Given Hepatitis B Vaccine At Birth In A Tertiary Government Hospital From January 2007 To June 2008: A Pilot Study. *PIDSP Journal 2010* Vol 11 No.2, 32-39

15. **Coppola, N. et al**, The Long-term Immunogenicity of recombinant hepatitis B Virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC Infectious Diseases* (2015) 15:149

16. World Health Organization Hepatitis B, www.who.int

17. **Demegillo, M et al.**Persistence of Protective Hepatitis B Antibody Levels and Response to Booster Immunization of Elementary Students of West City Central School, 5 to 6 Years After Primary Immunization, 2014, unpublished

18. **Balangue-Tundag, ME et al**. HBsAg Carriage among Filipino Preschool Children in Lapu-Lapu City, Cebu, 2015, unpublished

19. **Dassah, S, et al.** Seroconversion of Hepatitis B Vaccine in Young children in the Kassena Nankana District of Ghana: A Cross-Sectional Study, *PLOS ONE* 2015; 10 (12): 1-10.

20. **El-Sayed, B, et al,** Long-term protection of Hepatitis B vaccinationamong Egyptian children. *Egypt J Pediatr Allergy Immunol* 2011; 9 (1): 35-40.

21. **Poorolajal, et al**. Long-term protection provided by hepatitis B vaccine and need for booster dose: A Meta-analysis. *Vaccine*2010; 28: 623-31.

*22.* **Wu JS, et al**. Hepatitis Bvaccination in high-risk infants: 10 year follow-up. *J Infect Dis* 1999;179:1319–25.

23. **Rezaei, M, et al**, Seroprotection after Hepatitis B vaccination in children aged 1 to 15 years in central province of Iran, Semnan, *J Prev Med Hyg 2014;* 55:1-3

24. **Van Herck et al.** Benefits of Early Hepatitis B immunization programs for newborns and infants. *Pediatr Infect Dis J* 2008; 27: 861-9

25. **He, F et al.** The Serum Anti-HBs Level among Children who received Hepatitis B Vaccination during Infancy in Mianyang City, China: A Cross-Sectional Study. *Viral Immunology* 2015; 29,(1): 1-9.

26. **Su, FH et al,** Efficacy of Hepatitis B vaccine boosters among neonatally vaccinated university freshmen in Taiwan. *J Hepatol* 2013; 58:684-689

27. **Kanda, et al.** Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clinical and experimental immunology* 1996; 106: 410-415.

28. **Yang, S, et al.** Factors influencing immunologic response to Hepatitis B vaccine in adults. *Scientific Reports* 2016*;* 6:27251

29. **Afzal, M, et al.** Seroprotection after Hepatitis B Vaccinationin Expanded Programme on Immunization, *BJMP* 2011; 4 (4): a445

30. WHO Western Pacific Region Hepatitis B Vaccination in the Philippines. www.upto.who.int

31. **Hammitt LL, et al**. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. *Vaccine*. 2007;25:6958–6964.

32. **Petersen KM, et al.** Duration of hepatitis B immunity in low risk children receiving hepatitis B vaccinations from birth. *Pediatr Infect Dis J*. 2004;23:650–655.

33. **Mele, A et al.** Effectiveness of Hepatitis B Vaccination in Babies born to Hepatitis B surface antigen-positive mothers in Italy. *J Infect Dis* 2001; 184:905-908

34. **Schonberger, K, et al.** Determinants of Long-termProtection after Hepatitis B Vaccination in Infancy: A Meta-analysis. *Pediatr Infect Dis J* 2013; 32 (4): 307-313

35. **Mast, EE, et al**. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. Recommendations of

Advisory Committee on Immunization Practices, Center, 2005, www.cdc.gov

36. **Yao, J, et al.** The Response of Hepatitis B vaccination on seronegative adults with different vaccination schedules. *Human Vaccines & Immunotherapeutics* 2015; 11 (5): 1102-1107.

37. **Bravo, et al.**The New DTPw-HBV-Hib Combination vaccine can be used at the WHO schedule with a monovalent dose of hepatitis B vaccine at birth. *SEA Trop Med Public Health* 1998; 29 (4): 772-778

38. **Poovorawan, et al.** Long term antibody persistence after booster vaccination with combined tetravalent diphtheria, tetanus, whole cell Bordetella pertussis and hepatitis B vaccine in healthy infants. *Ann Trop Pedia* 1997; 17: 301-308

39. **Luo, et al.** Impact of the Implementation of a vaccination strategy on Hepatitis B virus infections in China over a 20-year period. *Int J Infectious Dis* 2012; 16 (2): e82-88.

# ACCURACY OF TRANSCUTANEOUS BILIRUBIN DETERMINATION IN NEONATAL HYPERBILIRUBINEMIA: A META-ANALYSIS

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**ABSTRACT**

**BACKGROUND:** Timely initiation of therapy for neonatal hyperbilirubinemia is routinely made based on total serum bilirubin levels. However, serial samplings by invasive needle pricks are needed for laboratory analyses. Studies comparing the correlation between serum bilirubin and transcutaneous bilirubin have yielded diverse results. A meta- analysis was done to find out the relationship between transcutaneous bilirubin measurements and serum bilirubin values.

**OBJECTIVE:** This study aims to analyze scientific articles regarding the accuracy of transcutaneous bilirubin measurements among healthy neonates as an alternative screening for hyperbilirubinemia.

**STUDY DESIGN:** Diagnostic Accuracy meta- analysis

**METHODS:** Studies on the accuracy of transcutaneous bilirubin measurements were identified through intensive literature search. Local studies were confirmed thru personal communication.

**RESULTS:** Three hundred eighteen studies were identified through literature search. Ten studies met the eligibility criteria. Eight of the ten studies reported results as correlation coefficients. The pooled estimates of correlation coefficients is high at r = 0.85 (95% CI = 0.84 to 0.857). Five studies reported results with data for diagnostic accuracy. The pooled analysis for sensitivity and specificity are high at 0.84 (95% CI 0.8-0.88) and 0.79 (95% CI 0.77-0.81) respectively. The pooled likelihood ratio has a significant difference with a pooled positive LR of 4.19 (95% CI 2.98-5.9, P<0.01) while the negative likelihood ratio is 0.23 (95% CI: 0.17 to 0.29). The AUC for transcutaneous bilirubinometry is 0.89.

**CONCLUSIONS:** Transcutaneous bilirubin measurement can be an alternative in monitoring the risk of healthy neonates for hyperbilirubinemia based on the pooled analysis of correlation coefficient and diagnostic accuracy.

**KEYWORDS:** Transcutaneous bilirubin, Term and late preterm, serum bilirubin, hyperbilirubinemia

## INTRODUCTION

Jaundice is one of the most common conditions needing medical care during the first two weeks of life. It is a relatively prevalent disease that affects approximately 2.4-15% of newborns. Sixty percent of term and 80% of preterm neonates develop jaundice in their first week of life. Ten percent of breastfed neonates are persistently jaundiced requiring readmissions

Neonatal jaundice is categorized into physiologic and pathologic hyperbilirubinemia. Physiologic hyperbilirubinemia conotes to an immaturity of neonates to metabolize increased bilirubin production. It usually appears after 24 hours of age and bilirubin level peaks up to 12-15mg/dl and resolves spontaneously. Pathologic hyperbilirubinemia is jaundice in the first 24 hours of life with increasing bilirubin concentration for more than 5mg/dl in 24 hours. It also requires intervention for its resolution.

Considering the overall socioeconomic burden associated with hyperbilirubinemia, the American Academy of Pediatrics in 2004 issued guidelines for the management of hyperbilirubinemia in term or near-term newborn. It recommends serum bilirubin measurement in all jaundiced infants in the first 24 hours of life and for infants appearing with disproportionate jaundice for the infant's age.

Serum samples are obtained by an invasive capillary puncture or venipuncture giving neonates and parents an additional burden. Another method was generated using a device that measures the intensity of specific wavelength when directed into the skin may alleviate apprehension of most parents from an invasive procedure.

The purpose of this meta-analysis is to determine the impact of non-invasive transcutaneous bilirubin determination by its sensitivity and nearness to serum bilirubin determination. The usefulness of transcutaneous bilirubinometry to decrease the need for blood sampling compared to serum total bilirubin in the management of jaundiced healthy neonates. Thus the research question is: "How accurate is transcutaneous bilirubin determination as screening tool to serum bilirubin in determining level of bilirubin in neonates?"

## METHODOLOGY

Electronic search was done through a systematic review of all English articles using MEDLINE (Ovid, PubMed), WHO search portal, Herdin and the Cochrane Controlled Trials Register from January 1990 to October 2017 and EMBASE from January 1990 to October 2017. Additional studies were identified thru personal communication with specialist, researcher, institution and organization. Manual search of reference lists and abstracts were searched from different specialty society conferences scientific program. Manual search for relevant studies was conducted for researches of residents or consultants from different hospitals and institution.

Medical subject heading terms used were cross-sectional studies, neonates, term and late preterm neonates, newborn, transcutaneous bilirubinometer, transcutaneous bilirubin determination, transcutaneous bilinometry, hyperbilirubinemia and serum bilirubin determination.

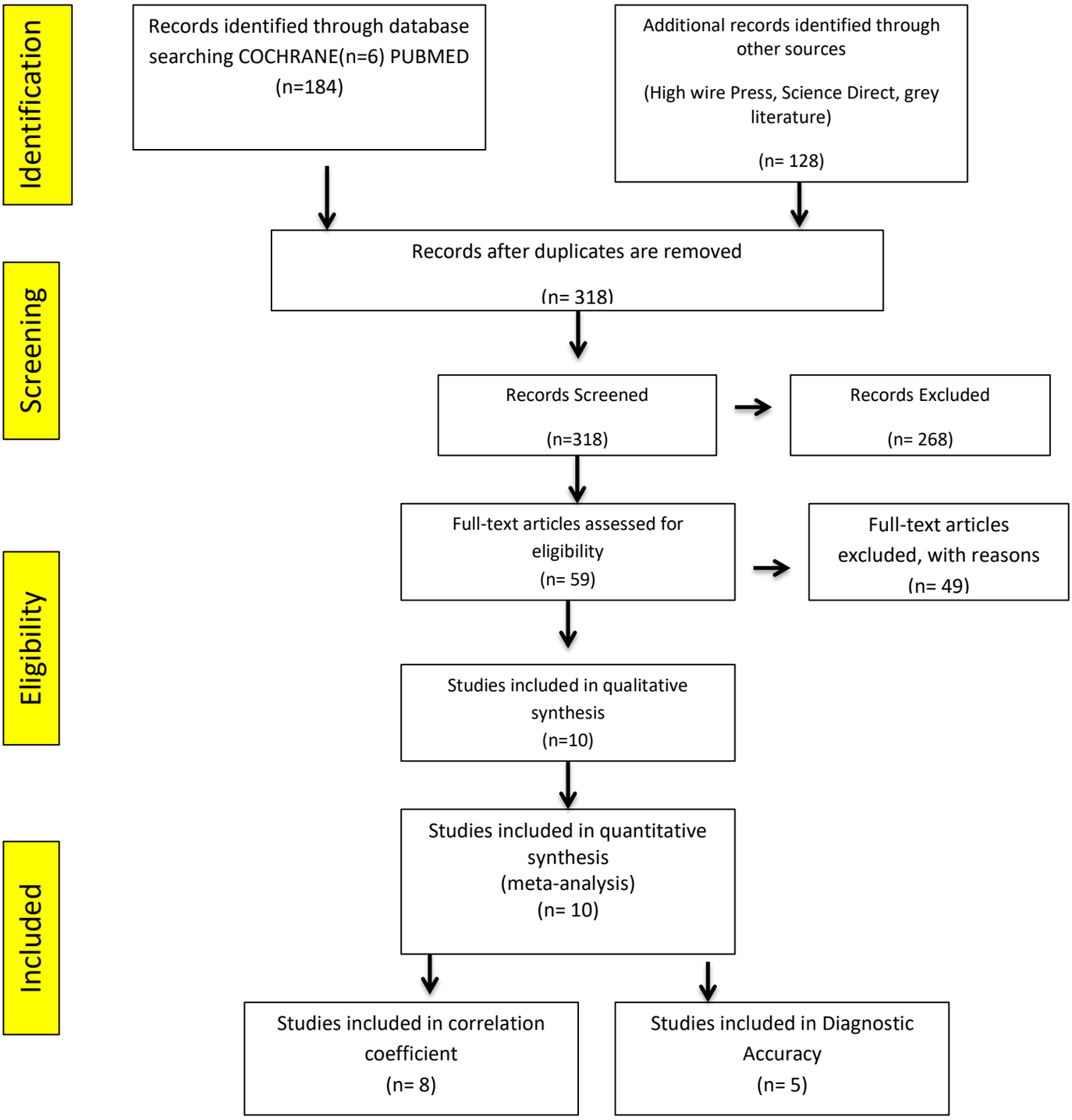
Diagnostic accuracy studies comparing transcutaneous bilirubin determination and serum bilirubin determination for neonatal hyperbilirubinemia were searched and appraised for validity. This meta-analysis included all cross-sectional studies evaluating the use of transcutaneous bilirubin and subsequently with serum bilirubin levels. The participants of the included studies are neonates, term or late preterm neonates age 35 to 37 weeks, which require bilirubin measurement as a universal screening test or for monitoring the risk for hyperbilirubinemia.

Two independent researchers conducted a literature review in accordance to PRISMA standards for selecting qualified studies for meta-analysis. Full texts of identified studies were reviewed for its significance separately by the investigators. Issues and discrepancies in the selection of studies were discussed and resolved. A third party was recruited as mediator if there are any unresolvable issues encountered in the article selections.

We included researches that studies any type of hyperbilirubinemia determined by transcutaneous bilirubin compared to serum bilirubin determination. We required that the authors had state in their methodology section the sensitivity and specificity for both transcutaneus and serum bilirubin levels among neonates. Researches that studied accuracy of transcutaneous bilirubinometry in the diagnosis of hyperbilirubinemia having correlation coefficients as there data were also included to further optimize the quality of the research. The studies not meeting the eligibility criteria and abstracts for which complete results were not available were not examined in the analysis.

We excluded basic research and animal studies. We also excluded from our primary analysis studies that focused on patients with unstable health states, such as those following acute hospitalization and needing interventions such as phototherapy or exchange transfusions.

**Fig. 1** Data Extraction And Evaluation Of Studies




A meta-analysis was performed on the available data from the correlation coefficients between measurements of TcB and TSB. All correlations were first converted to Fisher z scores before being pooled. The resulting pooled Fisher z scores were then transformed back into standard correlation coefficients for ease of interpretation. Data was analyzed using StatsDirect 3.1.11 to generate forest plot for the primary outcome

Data on the sensitivity, specificity, and positive and negative predictive values were pooled together and presented in a forrest plot. Summary Receiver Operating Characteritics, (SROC) with analysis of the area under the curve (AUC) were also obtained. Data was analyzed using Meta-Disc v.1.4.

## RESULTS

The literature search generated a total of 318 studies with potential relevant citations. A total of 268 articles were excluded for non-fulfillment of eligibility criteria. Fifty- nine studies were reviewed and assessed for eligibility. Forty-nine studies were excluded. Reasons vary, but mostly, it did not state in their methodology section the sensitivity and specificity for both transcutaneus and serum bilirubin levels among neonates.

A trial of ten cross- sectional studies appraised was considered eligible for further analysis. These full articles were reviewed. However, the lack of information from four (4) of the published manuscripts, most prominently the lack of values of true positives, true negatives, false positive and false negatives needed to construct the aforementioned 2x2 table. In order to optimize the significance of the study, an additional study was pooled for the correlation coefficient and was analyzed. A total of 5 prospective cross- sectional studies were included in the final meta-analysis of diagnostic accuracy, while eight cross-sectional studies were included in the meta-analysis of correlation coefficient.

As shown in table 1, the characteristics of the included studies where identified providing 5,948-paired measurements of TcB and TSB in 5, 310 patients who fulfilled the inclusion criteria. All have comparable baseline characteristics. However, included studies varied according to gestational age of the participants being either term or late preterm; site of transcutaneous bilirubin measurement (forehead: 8 studies; sternum: 4 studies); transcutaneous bilirubin device used (BiliCheck: 10 studies; JM-103: 2 studies; VanHou: 1 study); and agreement statistic used for comparison (correlation co- efficient: 1 study; and sensitivity and specificity: 5 studies. There are 7 studies reporting results by both correlation coefficient and Bland- Altman method. Studies conducted transcutaneous and serum bilirubin estimations within a short interval of time ranging either simultaneously to an hour.

Table 2 showed the results for risk of bias assessments on the I ncluded studies. Using the QUADAS tool, the majority of the included studies were assessed as low risk for bias with respect to patient selection, index test, reference standard, and flow and timing.

**Table 1**. Characteristics of the ten Included Studies in the Meta-analysis

| Author, Year | Population, Characteristics, Ethnicity | Measurements/ Sample | TcB Site | TcB Device | Comparison Method | TsB Method | Maximum Interval Between tests, min | Comments |
|---|---|---|---|---|---|---|---|---|
| **Alsaedi 2006** | Healthy term neonates | 631/631 | Forehead | BiliCheck® | r, BA Sn, Sp | Heel prick | 10 mins | jaundiced gestational age of 37-42 weeks |
| **Bhutani 2000** | Well-baby nurseries of pennsylvania | 1788/490 | Skin | BiliCheck® | r, BA | Heel prick | 10 mins | Some measurements were done earlier due to the staff's discretion for clinical jaundice |
| **Olusanya 2016** | Healthy late and term preterm | 1553/2107 | Sternum | BiliCheck® or JM-103 | r, BA | Heel prick | 1 hour | Gestational age ≥35 weeks or birth weight ≥2.2 kg |

| Author, Year | Population, Characteristics, Ethnicity | Measurements/ Sample | TcB Site | TcB Device | Comparison Method | TsB Method | Maximum Interval Between tests, min | Comments |
|---|---|---|---|---|---|---|---|---|
| **Boo 2007** | Healthy Malaysian Term with hyperbilirubinemia | 345/ 288 | Forehead and Sternum | BiliCheck® | r, BA | Venous puncture | 30 mins | Indian and chinese Jaundiced neonates |
| **Holland 2009** | Term neonates | 70/343 | Forehead and Sternum | BiliCheck® | r | Heel prick | 10 mins | More than 3 weeks between 1-5 dyas old |
| **Kolman 2007** | Hispanic healthy infants | 192/198 | Forehead | BiliCheck® | r, BA Sn, Sp | Venous puncture | 30 mins | Has not had TSB level before |
| **Mohamed 2014** | Healthy neonates | 347/141 | Forehead or sternum | BiliCheck® | Sn, Sp | Venous puncture | simultaneous | >/= 35 to 37 weeks; weigs more than 2000g |
| **Romagnoli 2013** | Healthy term and late preterm neonates | 298/298 | Forehead | BiliCheck® or JM-103 | Sn, Sp | Heel prick | Soon after TcB determination | > 35 weeks visually jaundiced &or / prior to discharged |
| **Srinivas 2015** | Healthy term neonates | 552/ 512 | Forehead | BiliCheck® | r, BA Sn, Sp | Heel prick | Obtained serum samples when TcB is greater than the 95$^{th}$ percentile only | Retrospective study |
| **Zhan 2016** | Healthy term neonates | 172/302 | Forehead | Bilicheck ® and VanHou | r, BA | Radial artery puncture | With serum sample for bilirubin in 24 hours | Must have to have bilirubin sample prior to inclusion to studies |

**Table 2.** Risk of Bias Assessments of the Included Studies

| Author, Year | Risk of Bias | | | | Applicability Criteria | | |
|---|---|---|---|---|---|---|---|
| | Patient Selection | Index Test | Reference Standard | Flow and Timing | Patient Selection | Index Test | Reference Standard |
| Alsaedi 2016 | ✓ | ✓ | ✓ | ✓ | ? | ✓ | ✓ |
| Bhutani 2000 | ✓ | ✓ | ✓ | ? | ✓ | ✓ | ? |
| Olusanya 2016 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Boo 2007 | ✓ | ✓ | ✓ | ✓ | ? | ✓ | ✓ |
| Holland 2009 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Kolman 2007 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Mohamed 2014 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Romagnoli 2013 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Svinas 2015 | ✓ | ✓ | ✓ | ? | ✓ | ✓ | ✓ |
| Zhan 2016 | ✓ | ✓ | ✓ | ? | ✓ | ✓ | ✓ |

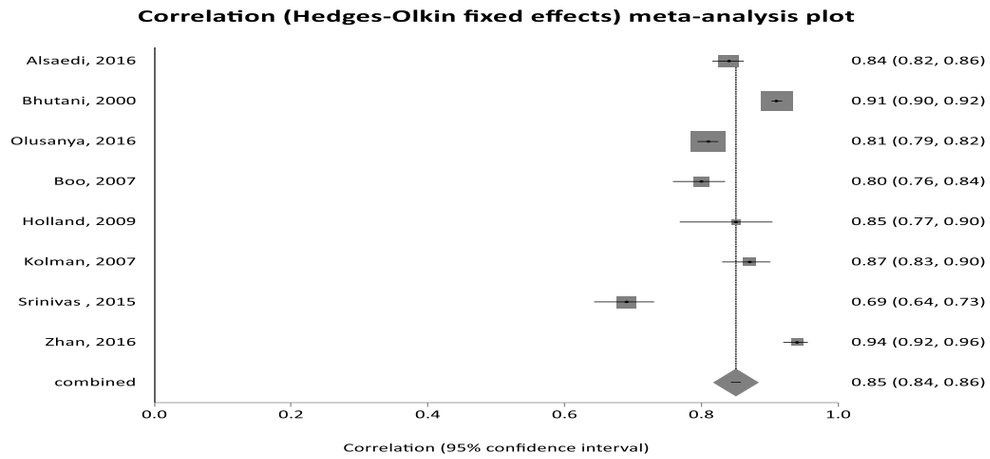
**✓, low risk; X, high risk; ? unclea**

**Pooled Analysis of Correlation Coefficients**

Eight studies reported results as correlation coefficients. Figure 2 illustrates eight studies, which provided results for correlation coefficients. The pooled estimates of correlation coefficient r = 0.85 (95% CI = 0.84 to 0.857). All results of TcB regardless of the site of measurement have a positive correlation with the true value of serum bilirubin.

However, the classical measure of heterogeneity, Cochran's Q at 307.62 is high indicating significant heterogeneity among the pooled studies. $I^2$ statistic is the percentage of variation across studies that is due to heterogeneity rather than chance. $I^2$ was 97.7%. The high level of heterogeneity may be explained by the differences among hospitals where the studies were conducted in terms of their method, device and site of determination.

**Figure 2.** Pooled estimates of correlation coefficients according to the site of TcB measurement




**Pooled Tests for Diagnostic Accuracy**

Figure 3 and 4 demonstrates the analysis of the pooled data for the sensitivity and specificity of the studies. Five studies were included in the study, and results of the studies measuring the sensitivity of TcB do not significantly differ. Overall sensitivity of TcB based on the pooled analysis is high at 0.84 (95% CI 0.8-0.88, P=0.22). This further demonstrates that TcB determination generally has a high sensitivity thereby strengthening its utility for neonates. As illustrated in figure 4, the results of these studies measuring the specificity of TcB significantly differ. Specificity holds the fraction of those with the disease correctly identified as negative by the test. Overall specificity of TcB based on the pooled analysis is high at 0.79 (95% CI 0.77-0.81, P<0.01).

**Figure 3.** Forest plot evaluating Sensitivity

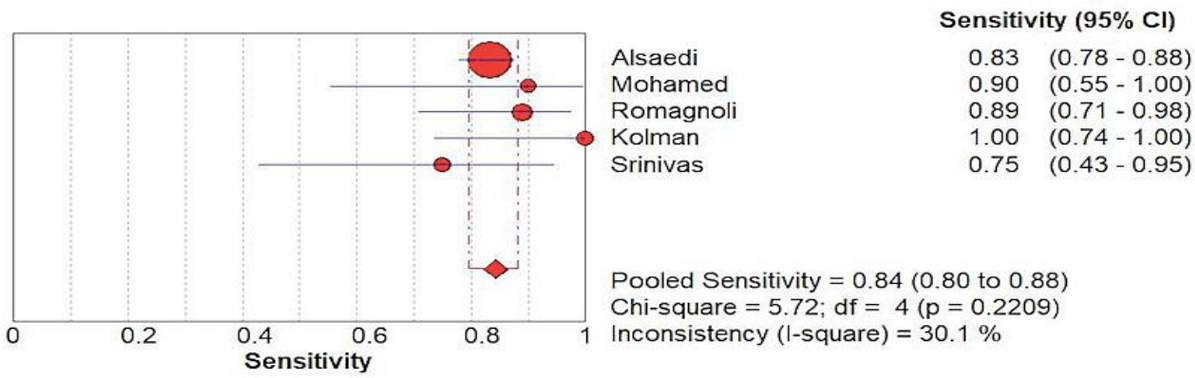
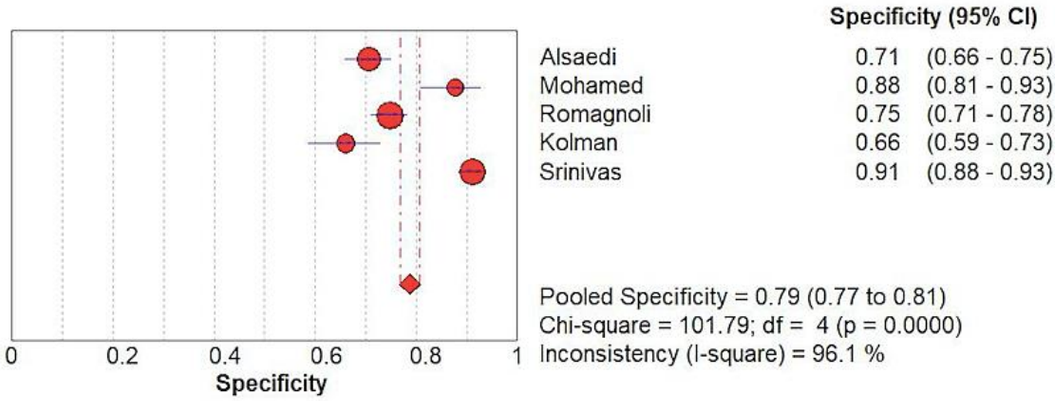


**Figure 4.** Forest plot evaluating Specificity




The pooled positive likelihood ratio tells us the increase in the probability of disease if the test is positive. This shows a significant difference with a pooled positive LR at 4.19 (95% CI 2.98-5.9, P<0.01) while the negative likelihood ratio is 0.23 (95% CI: 0.17 to 0.29). These represent that if the result of transcutaneous bilirubin measurement is positive, the probability of having the disease is high, but having a negative result would not mean that the patient has no disease. Clinical correlation is still warranted.

**Figure 5**. Forest plot evaluating Positive Likelihood Ratio

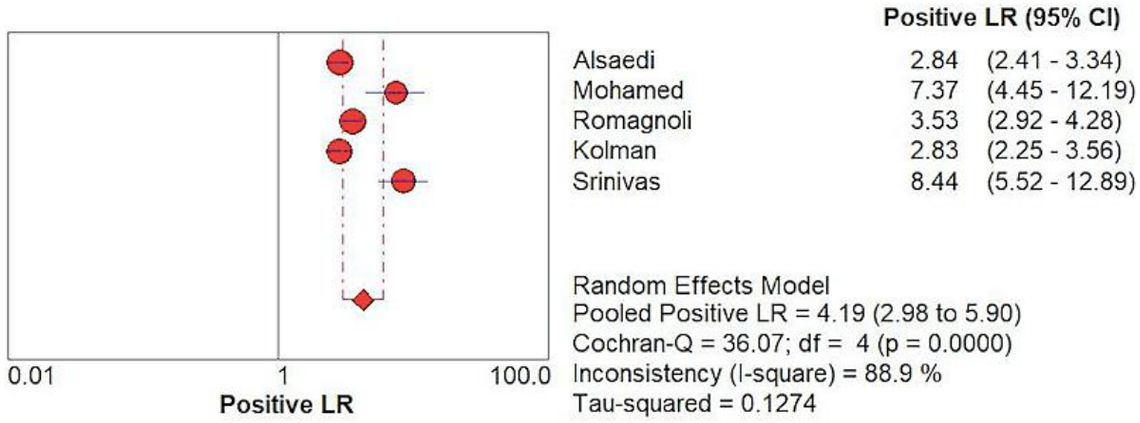
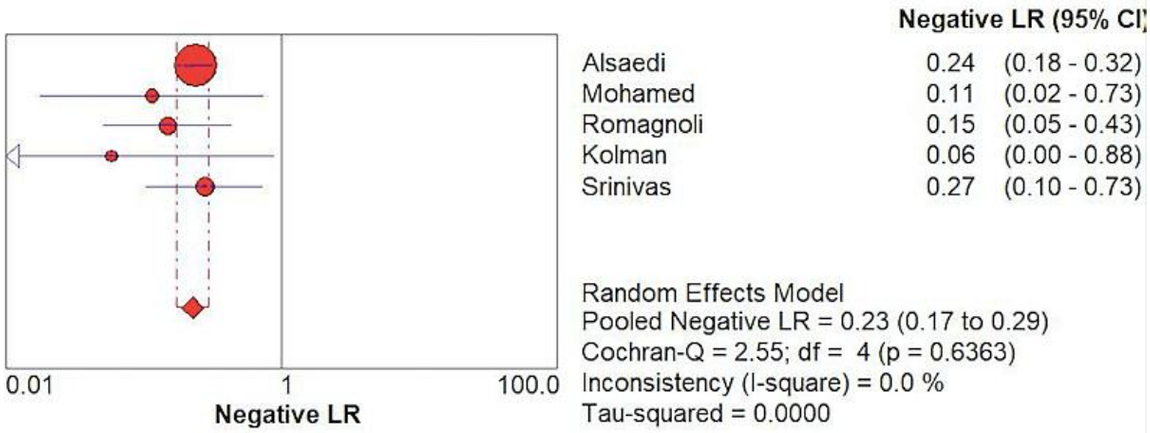
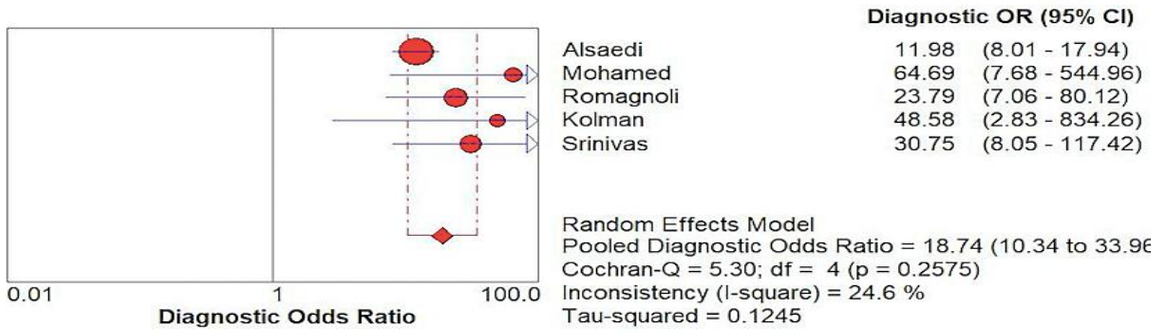



**Figure 6**. Forest plot evaluating Negative Likelihood Ratio



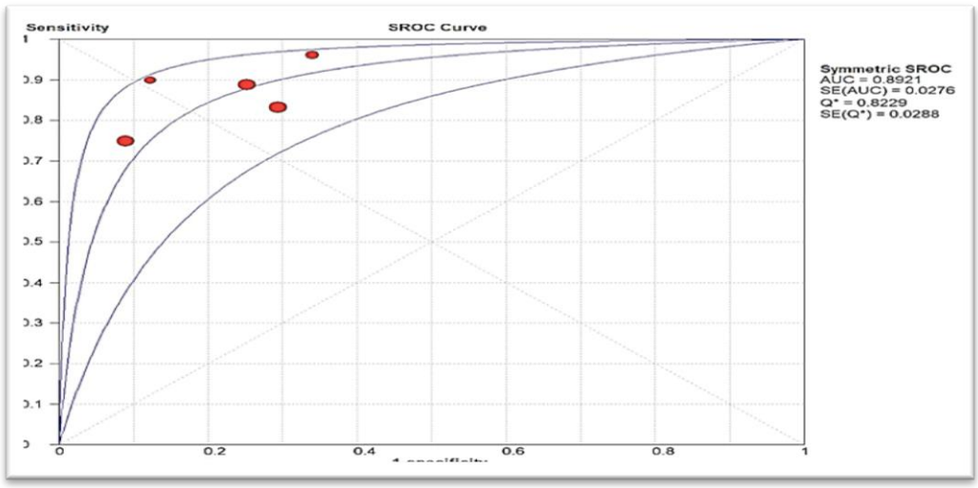
The diagnostic odds ratio (DOR) is a measure of the effectiveness of the diagnostic test: the ratio of the odds of a positive test result among the diseased to the odds of a positive test result among the non-diseased. It is used to discriminate subjects with the disease from subjects without the disease. The diagnostic odds ratio ranges from zero to infinity, with higher DOR indicative of better test performance. In this study, the pooled diagnostic odds ratio is 18.74 (95% CI: 10.34 to 33.96).

**Figure 7.** Forest plot evaluating Diagnostic Odds Ratio




The summary receiver operating characteristics curve (SROC) was analyzed in order to overcome data pooling difficulties. The AUC for transcutaneous bilirubinometry is 0.89, which is a reasonably good value compared to a perfect AUC value of one (1.0).

**Figure 8.** ROC Curve




## DISCUSSION

Universal screening for neonatal hyperbilirubinemia is controversial, although neonatal jaundice is common and benign in term and near preterm neonates. However, bilirubin encephalopathy or kernicterus may occur and may cause potential long-term impairments and poor outcomes from the initially well infant. Hence, the American Academy of Pediatrics (AAP) recommends universal screening with bilirubin levels or targeted screening based on risk factors. However, the U.S. Preventive Services Task Force and the American Academy of Family Physicians found insufficient evidence that screening improves outcomes.[i] Moreover, universal screening may increase the use of phototherapy, sometimes inappropriately. Several studies supporting the use of transcutaneous bilirubin determination as a near

alternative to serum bilirubin determination, its use for the detection and screening of hyperbilirubinemia in neonates is now accepted worldwide. This review supports the guidelines of AAP[11] that transcutaneous bilirubin determination is suitable to monitor probability of hyperbilirubinemia in neonates, within certain limits.

This study pooled all available data and those who met the criteria to confirm the accuracy of transcutaneous bilirubinometry to further guide practitioners in implementing its use. The identified studies evaluating the diagnostic accuracy of various transcutaneous bilirubin devices in a healthy term and near preterm population found that transcutaneous bilirubin measurements correlate reasonably well with the serum bilirubin estimation in healthy neonates, principally for the two commonly used transcutaneous bilirubin devices in practice, BiliCheck® and JM-103. The accuracy of these two devices was similar for the measurement at the forehead site but noted to be more accurate over the sternum in some studies as illustrated by Mohamed, Holland and Boo. However, according to the study done by Olusanya, increased pigmentation of the skin can affect the bilirubin result done transcutaneously as compared with serum. This can be explained by how transcutaneous bilirubin devices measures bilirubin using reflectance of light in the multi-wavelength spectral reflectance technique, which allows the device to determine the optical densities attributed to bilirubin, dermal thickness, heme and melanin in the epidermal and dermal layers in the infant skin.

The analysis of absolute transcutaneous – serum bilirubin measurement difference plots revealed minimal to no bias in measurements, irrespective of the site and type of device. Looking at the diagnostic accuracy of transcutaneous bilirubin devices in term and near preterm population, we presented a pooled data for bias and precision estimates along with the more commonly used measure of the correlation coefficient. The latter typically describes the strength of a relation between 2 variables rather than agreement between them. Thus, the clinical utility of correlation coefficient data is limited because they intuitively do not provide information regarding expected differences between the measurements conducted on a given patient by 2 separate tests.

The results of this study showed that transcutaneous bilirubin determination has a sensitivity of 84%, showing that 84% of patients with elevated serum bilirubin will also have an elevated bilirubin result transcutaneously. A specificity of 79% will mean that the patient without hyperbilirubinemia will have low levels of bilirubin transcutaneously.

An area under the curve showed an 89% accuracy for transcutaneous bilirubin indicating a good alternative to serum bilirubin as supported by all studies included in the meta analysis. While the odds ratio of 18.74 indicates that transcutaneous bilirubin determination will 18.74 times more likely to reflect the actual serum bilirubin level.

The likelihood ratio indicates that there is 4 times more likely that the patient is positive for the disease, a negative result would not mean that the patient has no disease due to a low negative likelihood ratio of 0.23. Hence, Clinical correlation is still warranted. Regardless of the site of measurement, there was a significant heterogeneity noted in the pooled estimates from the different studies with a correlation coefficient of 0.85. Hence, determination of bilirubin via transcutaneous bilirubin determination will not differ regardless of the site of determination.

According to Nagar, the pooled estimates of bias are comparable in the use of these devices as a result in a marked decrease in blood sampling for assessment of neonatal jaundice. It may yield the nearest estimate of the tests accuracy, and may still provide clinicians with helpful information on the utility of transcutaneous bilirubinometry[ii]. However, there is a lower threshold for the initiation of phototherapy for near preterm infant, with certain guidelines providing specific cutoffs for each gestational week according to the postnatal age. Thus, the information from this meta-analysis should be incorporated in clinical practice, taking into consideration the thresholds for phototherapy in high-risk infants. Similarly, a transcutaneous reading above the phototherapy threshold may be sufficient grounds to initiate phototherapy without the invasive test in most situations. The latter recommendation is made

despite knowing that some of these infants may be classified as below the phototherapy threshold based on serum bilirubin results because those infants are still likely to be reasonably close to the threshold.

Moreover, our analysis has several limitations. First, as mentioned above, there is presence of heterogeneity among the study categories and variables- both clinical and statistical, that were used for establishing hyperbilirubinemia. Some factors that may not be comparable in the trial might have affected the clinical outcomes derived. The high level of heterogeneity seen from the comparison on the incidence of transcutaneousmay be attributed to differences among the local hospital set-up, their practices, and the way the bilirubin levels are obtained. These differences may explain the statistical heterogeneity in some of the secondary outcomes investigated. Second, although we have pooled similar data across all trials, the number of participants per trial may be not sufficient to exclude significant clinical benefit. Thirdly, the setting of most trials was done only in single hospital centers and may have inherent bias related to their local practice habits. Finally, a possibility of publication bias based on funnel plot may discount our extensive search for relevant studies using multiple search items and removing language restriction.

Finally, although initial results seem to be promising for the use of transcutaneous bilirubin determination to early detect hyperbilirubinemia, there is still insufficient evidence to conclude to pediatricians that its use is comparable with the accuracy of the serum due to the pooled studies high heterogeneity. In addition, studies that investigate transcutaneous bilirubin determination device which has more benefit including superiority in terms of ease of use, durability, accuracy and route, are still lacking. These are areas that are yet to be ventured when it comes to evaluation of hyperbilirubinemia in large-scale randomized controlled trials or cross sectional studies for hyperbilirubinemia.

## CONCLUSION

The use of transcutaneous bilirubin determination was associated with statistically significant reduction in the incidence of hyperbilirubinemia in high-risk infants. This further strengthened the use of transcutaneous bilirubin devices, particularly JM-103 and BiliCheck®, measure serum bilirubin values in term healthy neonates with rational accuracy. Incorporating the use of transcutaneous bilirubin devices in clinical practice could help reduce the need for blood sampling for the management of high-risk infants or those at risk for hyperbilirubinemia.

The investigators suggest that an analysis of subgroups be done in order to add valuable evidence in the analysis of the diagnostic accuracy of transcutaneous bilirubinometry. A larger and well designed, randomized control trial are needed to determine whether the gestational age, post-natal age, body weight, race, and site of TcB measurement have any influence on the accuracy of transcutaneous bilirubin measurement for hyperbilirubinemia.

**References:**


1. Alsaedi, Saad. Transcutaneous bilirubin measurement in healthy Saudi term newborns. Saudi Medical Journal. 2016 Feb 37 (2) 142-146

2. BhutaniVK, GourleyGR, AdlerS, KreamerB, Dalin C, Johnson LH. Noninvasive measure- ment of total serum bilirubin in a multira- cial predischarge newborn population to assess the risk of severe hyperbiliru- binemia. *Pediatrics.* 2000; 106(2).

3. Olusanya BO, Imosemi DO, Emokpae AA. Differences between transcutaneous and serum bilirubin measurements in Black African neonates. *Pediatrics* 2016; 138(3).

4. Boo NY, Ishak S. Prediction of severe hyperbilirubinaemia using the Bilicheck transcutaneous bilirubinometer. J Paediatr Child Health. 2007; 43:297–302.

5. Holland, Lorne MD, MS, Implementing and validating transcutaneous bilinometry for neonates. American Journal of Clinical Pathology, Vol 132, Issue 4, October 2009, pages 555-561

6. Kolman KB Mathieson KM Frias C. A comparison of transcutaneous and total serum bilirubin in newborn Hispanic infants

at 35 or more weeks of gestation. *J Am Board FAM Med*. 2007; 20:266–271.

7. Mohamed I, Blanchard AC, Delvin E, Cousineau J, Carceller A. Plotting transcutaneous bilirubin measurements on specific transcutaneous nomogram results in better prediction of significant hyperbilirubinemia in healthy term and near-term newborns: a pilot study. Neonatology. 2014; 105:306–311

8. Romagnoli C, Catenazzi P, Barone G, Giordano L, Riccardi R, Zuppa AA, et al. BiliCheck vs JM-103 in identifying neonates not at risk of hyperbilirubinaemia. Ital J Pediatr. 2013; 39:46

9. Srinivas GL, Cuff CD, Ebeling MD, Mcelligott JT. Transcutaneous bilirubinometry is a reliably conservative method of assessing neonatal jaundice. J Matern Fetal Neonatal Med. 2015:1–5

10. Zhan, Canyang, and et al. Evaluation of the bilicheck transcutaneous bilirubinometer in the Chinese newborns. Biomedical research 2016; 27 (4): 1390- 1394.

11. Muchowski, Karen et al. Evaluation and treatment of neonatal hyperbilirubinemia. AM Fam Physician.2014 Jun 1;98(11): 873-878

12. Nagar, Gaurav MD. Reliability of transcutaneous bilirubin devices in preterm infants: infants: a systematic review. Pediatrics 2013; 132:871-881a systematic review. Pediatrics 2013; 132:871-881

# EFFECTS OF PROBIOTIC PROPHYLAXIS ON THE INCIDENCE OF VENTILATOR - ASSOCIATED PNEUMONIA AMONG CRITICALLY ILL PEDIATRIC PATIENTS: A META-ANALYSIS

CAROLYN GRACE C. TONGSON, MD, MARIA EVA I. JOPSON, MD

**ABSTRACT**

**BACKGROUND**: Among critically ill pediatric patients, a common complication experienced is nosocomial pneumonia. One field that garnered special interests as an alternative and promising way of preventing infection is the utilization of Probiotics. But whether it can prevent occurrence of ventilator-associated pneumonia (VAP) among critically ill pediatric patients remains unclear

**OBJECTIVES**: To determine whether probiotic supplementation will prevent the incidence of ventilator-associated pneumonia among critically ill pediatric patients.

**METHODS**: Literature search was conducted in PubMed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews to identify all relevant randomized controlled trials (RCTs) published between 1980 and 2016. The reviewers extracted data and reviewed the quality of the studies independently.

**RESULTS**: Three randomized controlled studies with a total of 327 pediatric patients admitted at the PICU were analyzed. Pooled analysis showed a statistically significant reduction in nosocomial pneumonia rates (odd ratio [OR] = 0.31, 95% CI 0.18 to 0.55, $P< 0.0001$, $I^2 = 53\%$) and statistically significant difference was found regarding overall mortality (OR =0.51 , 95% CI 0.30 to 0.88, $P = 0.01$, $I^2 = 0\%$) due to probiotics. However, no statistically significant difference was found between groups regarding duration of stay in the PICU (Mean Difference [MD] in days = 2.93, 95% CI 1.84 to 4.01, $P < 0.00001$, $I^2 = 97\%$ ), and duration of stay in the hospital (MD = 4.33 days, 95% CI 2.85 to 5.81, $P <$ .00001, $I^2 = 97\%$).

**CONCLUSION AND RECOMMENDATIONS:** The use of probiotics was associated with statistically significant reduction in the incidence of VAP in critically ill children. However, larger and well-designed, multi-center, RCTs are needed to further establish the effects of probiotic in the pediatric population of critically ill children who are at risk of developing nosocomial infection.

**KEYWORDS**: Probiotics, Ventilator Associated Pneumonia, Pediatric Intensive Care Unit

## INTRODUCTION

Despite progress in public health and hospital care, nosocomial infection is a major public health concern in both developing and developed countries. An increased risk in morbidity and mortality has been seen among in-patients inflicted with nosocomial infection. They are caused by a wide range of pathogens, and common sites of infection are said to be the bloodstream, respiratory tract, and urinary tract. The World Health Organization (WHO) showed that, at any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospitals. The highest frequencies were reported to have a prevalence of 11.8% and 10.0% respectively in the Eastern Mediterranean and South-East Asian Regions.

Among critically ill patients, a common complication experienced is nosocomial pneumonia, specifically in patients who are intubated for more than 48 hours. This renders nosocomial pneumonia to be responsible for significant in-hospital morbidity and mortality. When mechanically ventilated patients develop nosocomial pneumonia, it is then termed ventilator associated pneumonia (VAP). Given the condition of these patients, multiple risk factors were identified and thought to increase

bacterial colonization of the aero-digestive tract and facilitate the entry of pathogenic bacteria into the lower respiratory tract.

Recent findings suggest that frequent use of antibiotics may lead to the emergence of multidrug-resistant organisms, or may lead to the depletion of good microorganisms, and thereby just putting children more at risk to infection. In effect, it is this endemicity and persistence in resistant strains from widespread use of antimicrobials that facilitate bacterial infection spread in the pediatric health care setting. Many strains that were once susceptible to antimicrobials are now rendered resistant to treatment. And this is a problem particularly in developing countries such as the Philippines where more expensive second-line antibiotics may not be easily available or affordable for the Filipino families.

This recent trend on resistance to antimicrobials pushed for the creation of improved surveillance and implementation of more effective preventive interventions. One field that garnered special interests as an alternative and promising way of preventing infection is the utilization of Probiotics. Lately, the use of harmless bacteria through Probiotics to displace pathogenic strains has gained much attention in addressing different infections, including hospital-acquired ones. However, whether probiotics can prevent occurrence of nosocomial pneumonia among admitted critically-ill pediatric patients is still unclear. Therefore, there is a need to determine if children admitted at the pediatric intensive care unit and supplemented with probiotics will have better health outcomes in terms of development of nosocomial pneumonia during their hospital stay.

As a result of existing studies demonstrating probiotics' potential in up regulating immune defenses and reducing bacterial translocation, there has been a rapidly growing interest in the clinical application of probiotics. A few clinical trials have already begun to look at the incidence of infections with probiotic use and have demonstrated promising results. However, current evidence and opinions suggest that data to conclusively determine whether probiotics can be safely used in prevention of nosocomial infections, particularly reduction of incidence of ventilator-associated pneumonia is still insufficient.

Although there is theoretical plausibility shown by current literature in the use of probiotics for infection prevention, most of which are inconsistent in results and utilized different sets of population. In addition, a bench research is yet to be performed to determine the most appropriate probiotic formulation for various clinical applications as specific strains are thought to be effective in certain disease states.

In this respect, this study was conducted to contribute to the knowledge of whether probiotics can be used in the prevention of nosocomial infection, particularly of nosocomial pneumonia, in critically ill children admitted in pediatric intensive care units. The study intended to re-evaluate the present knowledge or hypothesis that administration of probiotics in critically ill children may reduce the incidence of ventilator-associated pneumonia. In this light, it is hoped that a promising alternative may be used to lessen the incidence of nosocomial pneumonia, thereby decreasing hospital stay, preventing morbidities and mortalities, reducing cost needed for treatment, lessening side effects common to antibiotic use, and combating antibiotic resistance.

This study aimed to serve as a guide in deciding whether to supplement probiotics among pediatric patients admitted at PICU of any Pediatric Hospital and needing antibiotic therapy. In addition, it is possible that there are other hospitals/health care institutions that are having the same dilemma regarding ventilator-associated pneumonia and antibiotic resistance. This study can serve as a blueprint on how to manage such issues with new potential alternatives.

## METHODOLOGY

To identify studies for inclusion in this review, literature search was conducted in PubMed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews to identify all relevant randomized controlled trials (RCTs) published between 1980 and April 2016.

The search was limited to studies conducted in humans. The literature search used search terms containing "randomized", "clinical trial", "probiotics", "nosocomial infection", "health care associated pneumonia", "ventilator associated pneumonia", "pediatrics", "intensive care". No language and publication restrictions were applied. Personal files, reference lists of relevant review articles, and proceedings of major relevant conferences were also be reviewed as secondary searches. Excluded trials included those with no specific disease being studied, non- RCTs, and trials on animals other than humans.

The author used titles and abstracts to already exclude trials which clearly did not meet the set inclusion criteria. The common reasons encountered for exclusion of the articles from the electronic search were non-human studies, the non-use of probiotics, measurement of outcomes other than incidence of VAP. Full articles were retrieved for further assessment if the abstracts indicated that there was a possibility that the study fulfilled the inclusion criteria. The journals were screened, and peer reviewed by another reviewer to assess study eligibility. Analysis was restricted to double-blind, randomized controlled trials (RCTs). For this meta-analysis, those RCTs that compared administration of probiotic vs. placebo in critically ill patients, and that reported the incidence of VAP, were considered.

The investigator and another peer reviewer independently reviewed and assessed inclusion criteria and quality of trials. Three potentially eligible papers were identified and reviewed.

Data concerning details of study population, intervention and outcomes were extracted independently by the reviewers using a specifically designed data extraction form of "The Cochrane Collaboration" (Cochrane Library). From each paper, the researcher extracted information related to

- General Information: published/unpublished, title, authors, year of publication, number of patients
- Trial Characteristics: method of randomization and allocation concealment, blinding (participants, clinician, outcome assessor, loss of participants to follow up, intention to treat analysis)
- Intervention: doses, frequency of probiotic supplementation
- Participants characteristics: inclusion and exclusion criteria, age group, number of patients in each intervention
- Outcomes: the primary outcome was the incidence of patients that developed ventilator-associated pneumonia (VAP) following probiotic/control. The researcher used the authors' definition of NP or VAP if they included clinical, microbiologic and radiologic criteria. Secondary outcomes were mortality, length of stay in the ICU and in hospital, and reports of adverse outcomes.
- Results: continuous data were expressed as weighted mean differences and standard deviation, use of intention to treat a analysis.

Differences in data extraction were resolved by discussion and consensus. When necessary, additional information was sought from the authors of the studies.

Each included study was assessed based on the following indicators of risk of bias as listed below. A verdict of LOW RISK meant low risk of bias, a HIGH RISK meant high risk of bias; and UNCLEAR RISK for unknown risk of bias—were used as the criteria for judging risk.

- Adequate sequence generation
- Allocation concealment
- Blinding of participants, personnel, and assessors
- Incomplete outcome data
- Selective outcome reporting

Meta-analysis was conducted using Review Manager 5.3 (Cochrane Collaboration, UK). A fixed-effects method (Mantel-Haenszel method) was used. The author computed pooled odds ratios (ORs) and 95% confidence intervals (CIs) from adjusted ORs and 95% CIs reported in the observational studies.

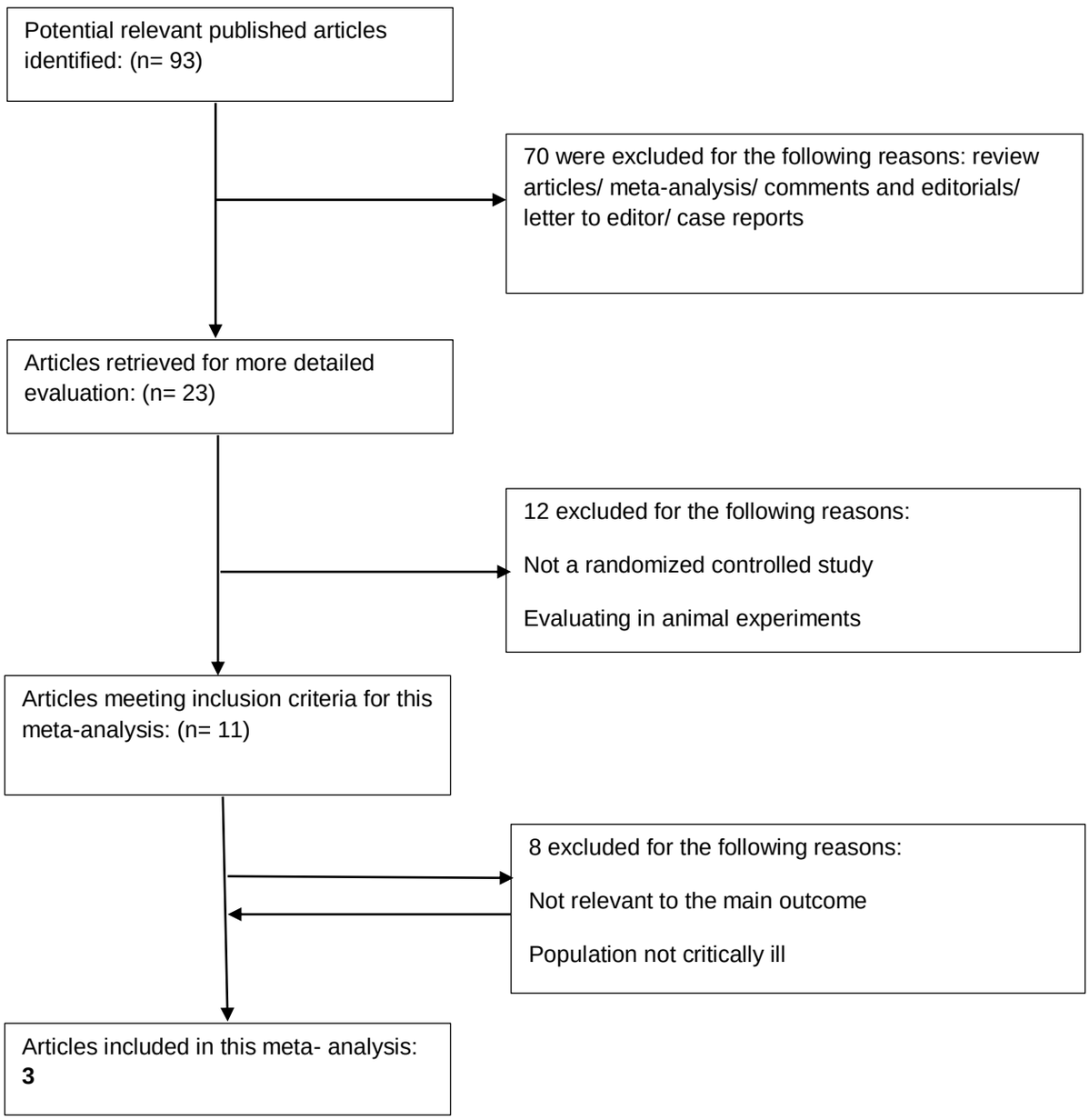
If the researcher was unable to extract all the information with regards to the details of

the study, the plan was to contact the authors. Fortunately, all numerical results in the published reports were adequate to proceed with the study. Quantification of the effect of heterogeneity was assessed by means of $I^2$. We predefined heterogeneity as low, moderate or high with $I^2$ values > 25%, 50%, and 75%, respectively. In the analysis of heterogeneity, we considered a *P-value* < 0.10 statistically significant. Both positive and negative results were reported among the studies. Publication bias was assessed by a funnel plot using the occurrence of VAP as an endpoint.

## RESULTS

Our search retrieved a total of 131 references. After screening them against the inclusion criteria. Three studies were included in this meta-analysis. A flowchart diagram for the studies evaluated and the reasons for exclusion are shown in **Figure 1.**

**Figure 1.** Flow Chart of Study Selection. Pooled ORs were calculated using Mantel-Hanszel (M-H) Estimator. Study-level data were pooled using fixed-effects model.



Characteristics of the included studies are summarized in **Table 1**. A total of 332 critically ill patients were included in these three studies. The trials were carried out at a single center or hospital. All three trials recruited patients in pediatric intensive care units (PICU), both medical and surgical cases included. The patients recruited were those needing mechanical ventilation as deemed necessary by the expert clinicians. Two studies excluded patients with evidence of perforated intestine, mechanical GI obstruction, ANC < $0.5x\ 10^9$, admitted at PICU >72 hours, used probiotics in the week before study accession, and if there was lack of parental presence/consent.

**Table 1**. Characteristics of the study population in various studies

| **Study, Year** | **Study Design** | **Population** | **Disease Severity Score** | **Regimen Used** | **Route of Administration/ Duration of Intake** |
|---|---|---|---|---|---|
| **Banupriya *et al.*, 2014 [24]** | Open-Label Randomized Controlled Trial (RCT) | All children aged 12 years or less admitted to PICU and who were likely to need mechanical ventilation for more than 48 hrs were recruited | Pediatric Risk of Mortality (PRISM III) Score 11.61 ± 5.63 vs 11.25 ± 6.58 | Probiotic capsules containing 2 billion CFU of Lactobacillus, 1 billion CFU of Bifidobacterium, and 300 million CFU of Streptococcus thermophilus were used in this study. One capsule was administered twice a day mixed with milk (or 5 ml of 5 % dextrose solution if enteral feeding had not been started). | Given through a nasogastric tube. A total of 6.6 billion CFU of probiotic organisms per day was administered to each child in the probiotic group for the initial 7 days or till discharge, whichever was earlier. |
| **El-Wakeel *et al.*, 2016 [25]** | Double Blinded Randomized Placebo-Controlled Trial (DBRCT) | Patients admitted at the Pediatric Intensive Care Unit (PICU) of a University's Childrens' Hospital requiring MV > 48 hrs were said to be eligible | Pediatric Risk of Mortality (PRISM) Score 33.9±13.9 vs 34.2±15.6 | One Lactobacillus rhamnosus strain GG capsule once a day (Culturelle, $10\times10^9$ cells/capsule, ConAgra Foods, Omaha, NE) was used. Probiotic capsules were prepared in a suspension of (5 ml) of 5% dextrose. | Administered by orogastric, nasogastric tube or by mouth in patients who could be fed orally for the duration of hospitalization. |
| **Honeycutt *et al.*, 2007 [9]** | Double Blinded Randomized Placebo-Controlled Trial (DBRCT) | Children admitted at the medical-surgical PICU of a university-affiliated hospital requiring MV > 48 hours | N/A | One capsule of Lactobacillus rhamnosus strain GG (Culturelle, $10x10^9$ cells/ capsule, ConAgra Foods, Omaha, NE) once a day. The probiotic and placebo capsules were prepared in a suspension of 5 mL of 5% dextrose. An appropriate normal saline flush was administered in patients with an orogastric/naso-gastric tube. | Administered by mouth in those able to orally feed or by orogastric/nasogastric tube. Patients continued the protocol until discharge from the hospital, parental request to withdraw from the study, or the death of the patient. |

The quality assessment of these studies were summarized in a table that can be found in the appendix section as table 3. Results of the meta-analyses that explored the effects of probiotic prophylaxis in the primary and secondary clinical outcomes are shown in **Table 2.**

**Table 2.** Outcome data of all randomized controlled trials included in the meta-analysis (comparison of probiotic versus control)

| Study | Incidence of VAP (n/N) | | Length of ICU Stay, median days (range) | | Length of Hospital Stay, median days (range) | | ICU Mortality (n/N) | |
|---|---|---|---|---|---|---|---|---|
| | *Placebo* | *Probiotic* | *Placebo* | *Probiotic* | *Placebo* | *Probiotic* | *Placebo* | *Probiotic* |
| Banupriya *et al.*,* 2014 [24] | 35/73 | 12/73 | 12.54 ± 9.91 | 7.7± 4.6 | 19.17 ± 13.51 | 13.13 ± 7.1 | 23/73 | 17/73 |
| El-Wakeel *et al.*, 2016 [25] | 15/50 | 7/75 | 15.6 ± 11.6 | 14.8 ± 11.8 | N/A | N/A | 15/50 | 10/75 |
| Honeycutt *et al.*, 2007 [9] | 0/30 | 2/31 | 7 ± 2.5 | 12.2 ± 2.5 | 11.1 ± 3.3 | 17.6 ± 3.2 | 4/30 | 2/31 |

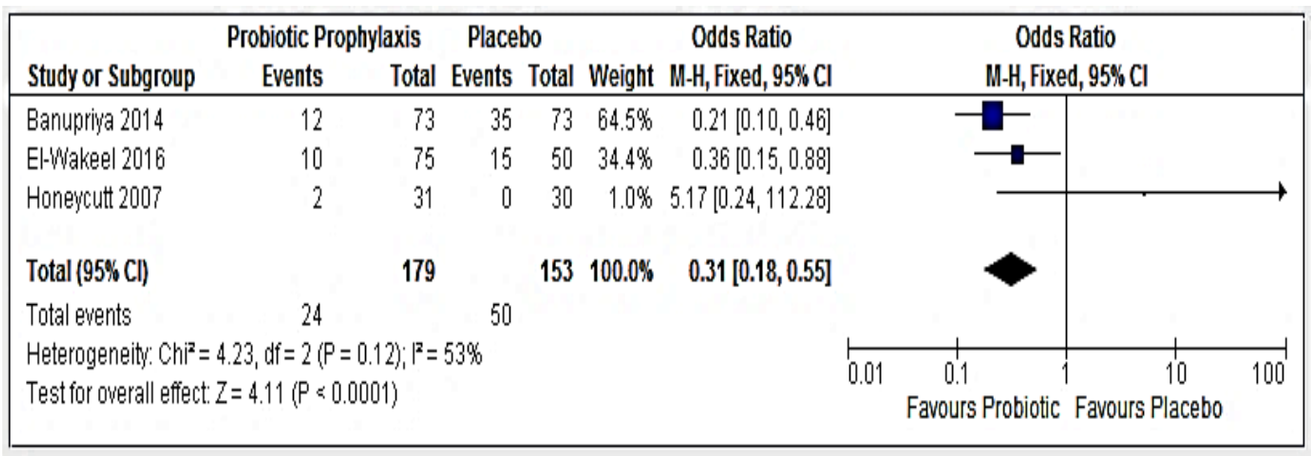
ICU: intensive care unit; NA: not available; VAP: ventilator-associated pneumonia

***** The control group did not receive any placebo

### A. Nosocomial Pneumonia and Subgroup Analyses

Results from the three trials (332 patients) were available to examine the effects of oral probiotics on the incidence of VAP [9,24,25]. A moderate level of heterogeneity was found among the identified comparisons ($I^2$ = 53%, $P$ = 0.12). Pooled analysis showed that the use of probiotics was associated with a statistically significant reduction in the incidence of NP in critically ill patients (OR = 0.31, 95% CI 0.18 to 0.55, $P<$ 0.0001) (**Figure 2**)

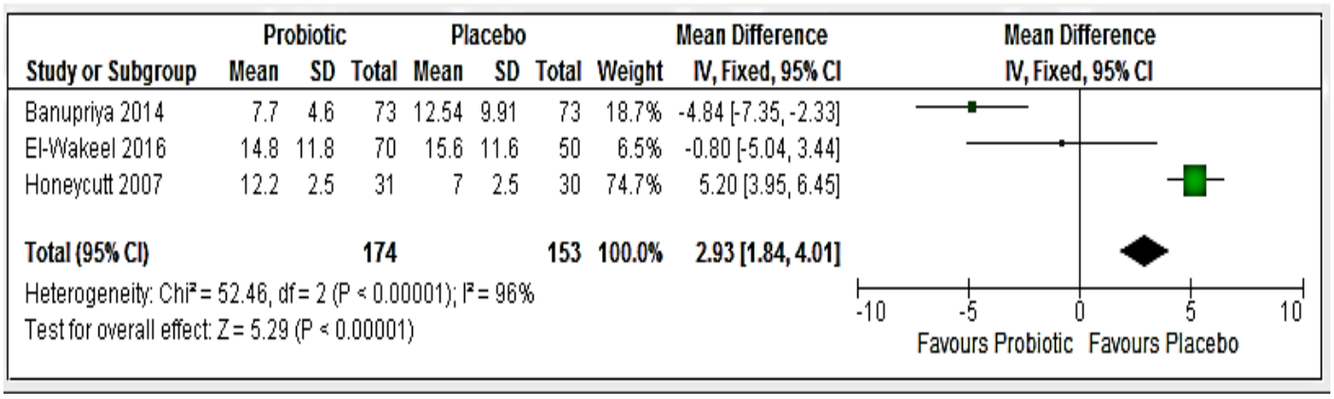
**Figure 2.** Forest plot showing the effect of probiotics on the occurrence of ventilator associated pneumonia (VAP) in critical ill patients




### B. Duration of Stay in the Intensive Care Unit

Data from the three studies were included in the analysis of the duration of stay in the intensive care unit [9,24,25]. There was significant heterogeneity in the length of PICU stays ($I^2$ = 96%, $P$ = <0.00001) (**Figure 3).** There was significant difference between the compared groups regarding this outcome (MD in days = 2.93, 95% CI 1.84 to 4.01, *P < 0.00001).*

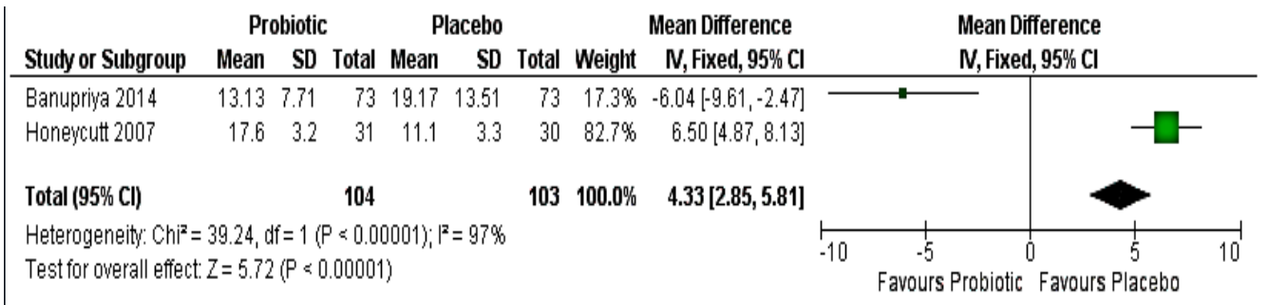
**Figure 3.** Forest plot showing the effect of probiotics on length of ICU stay (in days). Mean differenceswere estimated by the inverse variance (IV) approach.




### C. Duration of Stay in the Hospital

Two of the studies were included in the analysis of the length of stay in the hospital [9,24]. Again, there was a high level of heterogeneity in the length of hospital stay found between this comparison ($I^2$ = 97%, $P$ = <0.00001) There was apparent effect of probiotics therapy on the duration of stay in the hospital, with a mean difference (MD) of 4.33 days (95% CI 2.85 to 5.81, $P$ < .00001) (**Figure 4).**

**Figure 4.** Forest plot showing the effect of probiotics on length of hospital stay (in days). Mean differences were estimated by the inverse variance (IV) approach.



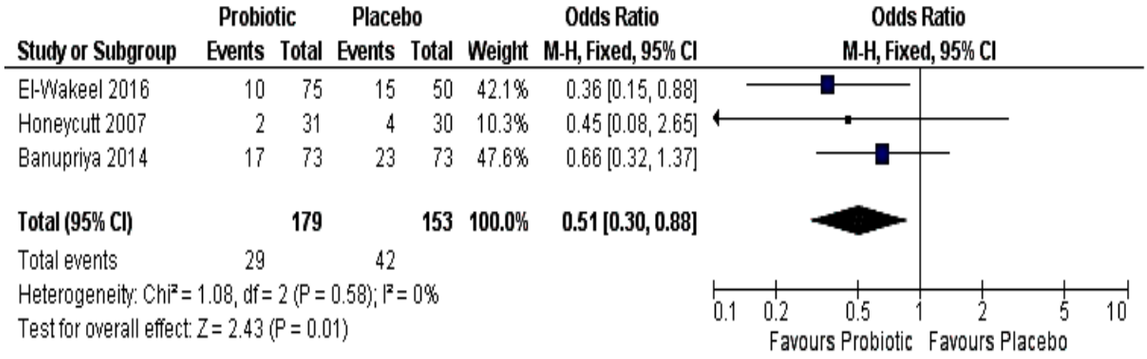

*Caption*

Forest plot of comparison: 1 Probiotic Prophylaxis versus Placebo, outcome: 1.3 Length of Hospitalization (days).

### D. Overall Mortality

Results of all three trials were available for the analysis of mortality during the entire ICU stay [9, 24, 25]. A meta-analysis of these trials found that probiotic administration had an effect on overall mortality during the hospital stay or had a significant difference in ICU mortality between probiotics group and placebo group (OR =0.51 , 95% CI 0.30 to 0.88, $P$ = 0.01) (**Figure 5).** There was no heterogeneity between trials ($I^2$ = 0%).

**Figure 5.** Forest plot showing the effect of probiotics on ICU mortality. Pooled ORs were calculated using the Mantel-Haenszel (M-H) Estimator.




*Caption*

Forest plot of comparison: 1 Probiotic Prophylaxis versus Placebo, outcome: 1.4 Mortality.

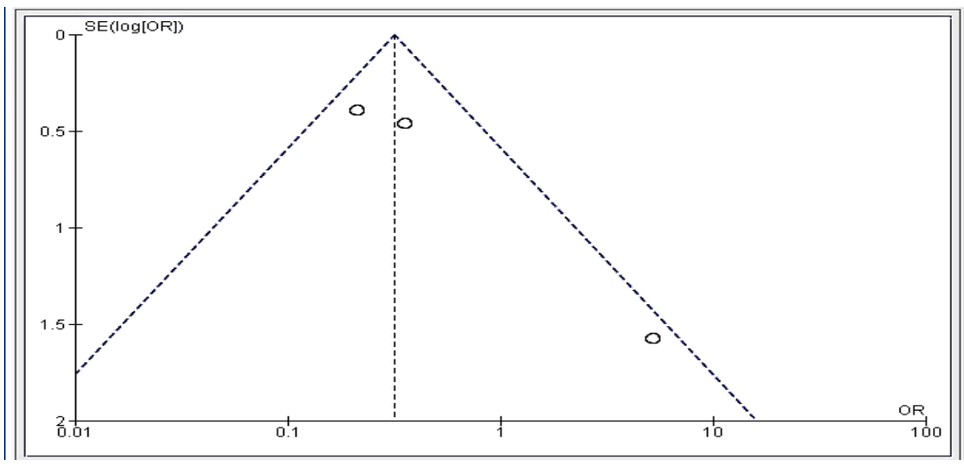
## E. Adverse Events

For the study done by Banupryia *et al.*, the probiotics were administered for the initial 7 days or until discharge, whichever was said to be earlier. While for the study of El Wakeel *et al.*, the probiotics were administered for the duration of the hospitalization. For these two studies, there were no adverse events such as cases of bacteremia caused by *Lactobacillus* were observed in the patients of the intervention group. On the other hand, in the study of Honeycutt *et al.*, the probiotics were given during the hospitalization until they are discharged, withdrew from the study, or died. In the study period, there were no cases of L. bacteremia in the study population reported and no known serious adverse effects occurred in any subject. However, because of recent safety concerns regarding the administration of Lactobacillus GG in critically ill pediatric patients and a lack of benefit in their analysis, the study was eventually terminated by the study investigators.

## F. Publication Bias

Upon visualization of the funnel plot for the primary outcome, there is a possibility of publication bias (absence of small studies, shown in the left lower corner of **Figure 6.** But this may also be attributed to the small number of studies included in the meta-analysis. As well as presence of heterogeneity in the correlation between study size and intervention effects.

**Figure 6.** Funnel plot showing possibility of a small publication bias. SE, standard error: OR, odds ratio.



## DISCUSSION

The meta-analysis found that probiotics administration was associated with statistically significant reduction in the incidence of ventilator-associated pneumonia among critically-ill patients. The pooled results also showed that probiotics affect the other secondary endpoints of the study (ie. overall mortality, length of stay in the hospital, and length of stay in the ICU). However, due to a large heterogeneity in the length of hospital stay and ICU stay, the effects found in these sub-studies may be non-comparable that one cannot confidently say that the combined estimate will be a meaningful description of the outcome produced.

The current meta-analysis is different from previous reviews in several aspects. One, it includes more eligible studies than previous reviews on probiotics administration that recruited only selected populations (ie. surgical patients only).[26] Trials done before were mostly that of critically adult patients [27], while this study focused on critically ill pediatric patients, and therefore results are more applicable across clinical situations encountered with critically ill children.

In order to diminish the number of confounding factors, the study also excluded studies that used interventions known to be effective in preventing nosocomial pneumonia, namely the use of chlorhexidine and antibiotic decontamination of the digestive tract as control groups [28,29].

The results of this study appear like previous studies done by Siempos *et al.* [30] and Pitsouni *et al.* [26], but inconsistent with the results of the systematic review by Watkinson *et al.* [27]. Siempos *et al*. found that administration of probiotics was beneficial in the incidence of both VAP and NP, length of stay in the ICU and colonization rates of *Pseudomonas aeruginosa* in the respiratory tract. The reasons for inconsistent results, even in this study, may be partly due to differences in focused clinical outcomes.

The results of this meta-analysis should be interpreted with caution based on other considerations. As the diagnosis of pneumonia may be more subject to bias due to it being a more subjective outcome as compared to mortality or length of ICU stay. And this may in part explain the marked reduction in pneumonia found in these studies. In addition, the presence of an effect on secondary outcomes, but that of presence of high heterogeneity across studies, may be from the small number of pooled RCTs and small number of total patients. Lastly, the treatment duration in some studies were likely too short to demonstrate maximum benefits. Consequently, it may be difficult to derive conclusive results based on this meta-analysis due to the lack of standard protocols and insufficient number of included patients.

Our analysis has several limitations. First, as mentioned above, there is presence of heterogeneity among the study categories and variables- both clinical and statistical, that were used for establishing NP or VAP. Some factors that may not be comparable in the trial might have affected the clinical outcomes derived. The moderate level of heterogeneity seen from the comparison on the incidence of ventilator associated pneumonia may be attributed to differences among the local hospitals' PICU set-up, their practices, and the way the probiotics were administered. These differences may explain the statistical heterogeneity in some of the secondary outcomes investigated. Second, although we have pooled similar data across all trials, the number of participants per trial may be not sufficient to exclude significant clinical benefit. Thirdly, the setting of most trials was done only in single hospital centers and may have inherent bias related to their local practice habits. Finally, a possibility of publication bias based on funnel plot may discount our extensive search for relevant studies using multiple search items and removing language restriction. Finally, although initial results seem to be promising for the use of probiotics to prevent nosocomial pneumonia, there is still insufficient evidence to conclude to pediatricians that administration of probiotic prophylaxis is associated with lower incidence of VAP in critically ill patients. In addition, studies that investigate which particular probiotic strain has more benefit including superiority in terms of dose, preparation, duration, safety, and route, are still lacking. These are areas that are yet to be ventured when it comes to evaluation of probiotics use in large-scale randomized controlled trials for nosocomial pneumonia.

## CONCLUSIONS AND RECOMMENDATIONS

The use of probiotics was associated with statistically significant reduction in the incidence of Ventilator-Associated Pneumonia in critically ill children. However, there is lacking evidence to support claims of beneficial effects on other clinically important outcomes such as length of hospital or ICU stay. Larger and well-designed, multi-center, RCTs are needed to establish the effects of probiotic prophylaxis in the pediatric population of critically ill children who are at risk of developing nosocomial infection.

## BIBLIOGRAPHY


1. McNabb B, Isakow W. Probiotics for the prevention of nosocomial pneumonia: current evidence and opinions. Curr Opin Pulm Med. 2008 May;14(3):168-75.

2. Simon AG, Rogacion J. A Randomized Placebo-Controlled Trial on the Use of probiotics in the Prevention of Nosocomial Infection in Pediatric Patients with Hematologic and Oncologic Diseases. PIDSP Journal. 2005; 9 (2). http://www.pidsphil.org/pdf/Journal_02050821/jo31_ja03.pdf

3. Estes RJ, Meduri GU: The pathogenesis of ventilator-associated pneumonia: I. Mechanisms of bacterial transcolonisation and airway inoculation. Intensive Care Med 1995, 21:365-383

4. Oudhuis GJ, Bergmans DC, Dormans T, Zwaveling J-H, Kessels A, Prins MH, Stobberingh EE, Verbon A: Probiotics versus antibiotic decontamination of the digestive tract: infection and mortality. Intensive Care Med 2011, 37:110-117

5. Walker WA: Mechanisms of action of probiotics. Clin Infect Dis 2002, 46(Suppl 2):S87-S91.

6. Safdar N, Dezfulian C, Collard HR, Saint S: Clinical and economic consequences of ventilator-associated pneumonia: A systematic review. Crit Care Med 2005, 33:2

7. Coconnier M, Bernet M, Kerneis S, et al: Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by Lactobacillus acidophilus strain LB decreases bacterial invasion. FEMS Microbiol Lett 1993; 110:299 –305

8. Agarwal K., Bhasin S., Faridi M., Mathur M., Gupta S. Lactobacillus cassei in the control of Acute Diarrhea: A Pilot Study. Indian Pediatrics 2001; 38: 905 – 910.

9. Honeycutt TC, Khashab ME, Wardrop RM, McNeal-Trice K, Honeycutt AL, Pharm D, et al. Probiotic administration and the incidence of nosocomial infection in pediatric intensive care: A randomized placebo-controlled trial. Pediatric Crit Care Med 2007 Vol. 8, No.5.

10. Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W. Efficacy of Lactobacillus GG in prevention of Nosocomial Diarrhea in infants. Journal of Pediatrics 2001, March; 138(3): 361-365.

11. Kaila M, Isolauri E, Soppi E, et al: Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain. Pediatr Res 1992; 32: 141–144

12. Hattaka K. et. al. Effect of long term consumption of probiotic milk on infections in children attending day care centers: double blind, randomized trial. British Medical Journal 2001; 322:1327-1329.

13. Minocha A. Probiotics for preventive health. Nutrition in Clinical Practice. PubMed. 05/2009; 24(2):227-41

14. Oudhuis G, Bergmans D, Annelies V. Probiotics for prevention of nosocomial infections: efficacy and adverse effects. Curr Opin Crit Care 2011; 17:487–492.

15. Walker WA: Mechanisms of action of probiotics. Clin Infect Dis 2002, 46(Suppl 2):S87-S91.

16. Navoa-Ng JA, et al. Device-associated infections rates in adult, pediatrics, and neonatal intensive care units of hospitals in

the Philippines: International Nosocomial Infection Control Consortium (INICC) findings. American Journal of Infection Control. 2011. Sep; 39 (7):548-54. Doi: 10.1016/j.ajic2010.10.018. Epub 2011 May 26.

17. Morrow LE, Kollef MH, Casale TB: Probiotic prophylaxis of ventilatorassociated pneumonia: a blinded, randomized, controlled trial. Am J Respir Crit Care Med 2010, 182:1058-1064

18. Barraud D, Blard C, Hein F, Marçon O, Cravoisy A, Nace L, Alla F, Bollaert PE, Gibot S: Probiotics in the critically ill patient: a double blind, randomized, placebo-controlled trial. Intensive Care Med 2010, 36:1540-1547.

19. Snydman D. The safety of probiotics. Oxford Journals, Medicine & Health, Clinical Infectious Diseases. 2008; 46 (2): S104-S111.

20. Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University. Prebiotic and probiotic fortified milk in prevention of morbidities among children: community-based, randomized, double-blind, controlled trial. PLoS ONE (Impact Factor: 3.53). 08/2010; 5(8):e12164. PubMed

21. Katelaris P. Probiotics control of diarrheal disease. Asia Pacific Journal of Clinical Nutrition1996;5:39-43

22. Elward AM, Warren DK, Fraser VJ. Ventilator-associated pneumonia in pediatric intensive care unit patients: risk factors and outcomes. Division of Infectious Diseases, Department of Pediatrics, St. Louis, Missouri, USA. 2002 May; 109 (5):758-64.

23. Hulley SB, Cummings SR, Browner WS, Grady D, Newman TB. Designing clinical research: an epidemiologic approach. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2013. Appendix 6B, page 75.

24. Banupriya, B., Biswal, N., Srinivasaraghavan, R. et al. Probiotic prophylaxis to prevent ventilator associated pneumonia (VAP) in children on mechanical ventilation: an open-label randomized controlled trial. Intensive Care Med (2015) 41: 677. https://doi.org/10.1007/s00134-015-3694-4

25. El-Wakeel Niveen, Wakeel Angi, El-Assmy Mohamed, El-Ghada, Barakat & Soliman, Wafaa. Role of probiotics in prevention of hospital acquired pneumonia in Egyptian children admitted to Pediatric Intensive Care Unit of Mansoura University Childrens Hospital. African Journal of Microbiology Research (2016). 10. 1240-1247. 10.5897/AJMR2016.8085.

26. Pitsouni E, Alexious V, Saridakis V, Peppas G, Falagas ME. Does the use of probiotics/synbiotics prevent postoperative infections in patients undergoing abdominal surgery? A meta-analysis of randomized controlled trials. Eur J Pharmacol. 2009;65:561–570. doi: 10.1007/s00228-009-0642-7.[PubMed]

27. Watkinson PJ, Barber VS, Dark P, Young JD. The use of pre-pro- and synbiotics in adult intensive care unit patients: systemic review. Clin Nutr. 2007;26:82–192. [PubMed]

28. Oudhuis GJ, Bergmans DC, Dormans T, Zwaveling J-H, Kessels A, Prins MH, Stobberingh EE, Verbon A. Probiotics versus antibiotic decontamination of the digestive tract: infection and mortality. Intensive Care Med. 2011;37:110–117. doi: 10.1007/s00134-010-2002-6. [PMC free article][PubMed]

29. Klarin B, Molin G, Jeppson B, Larsson A. Use of the probiotic *Lactobacillus plantarum* 299 to reduce pathogenic bacteria in the oropharynx of intubated patients: a randomized controlled open pilot study. Crit Care. 2008;12:R136. doi: 10.1186/cc7109. [PMC free article] [PubMed]

30. Siempos II, Ntaidou TK, Falagas ME. Impact of the administration of probiotics on the incidence of ventilator-associated pneumonia: a meta-analysis of randomized controlled trials. Crit Care Med. 2010;38:954–962. doi: 10.1097/CCM.0b013e3181c8fe4b. [PubMed]

31. Sun S, Yang K, He X, Tian J, Ma B, Jiang L. Probiotics in patients with severe acute pancreatitis: a meta-analysis. Langenbecks Arch Surg. 2008;394:171–177. [PubMed]