Bangladesh Crit Care J September 2014; 2 (2): 60-64

Original Article

Bacterial Isolates from Tracheal Aspirates and their Anti-microbial Susceptibility in Mechanically-Ventilated Children with Pneumonia Admitted to an Urban Critical Care Ward

K. M. Shahunja[1], Mohammed Abdus Salam[2], Tahmeed Ahmed[3], Pradip Kumar Bardhan[4], Shafiqul Alam Sarker[5], Hasan Ashraf[6], Abu SG Faruque[7], Md Iqbal Hossain[8], Md Munirul Islam[9], Sumon Kumar Das[10], Sharifuzzaman[11], Abu Sadat Mohammad Sayeem Bin Shahid[12], Ehsanul Huq[13], Mohammad Habibur Rahman Sarker[14], Mohammod Jobayer Chisti[15]

Abstract

Background and Aims: *Data on Bacterial isolates from tracheal aspirates in children with severe pneumonia requiring intubation and mechanical ventilation especially in developing countries are very limited. We examined the microbial spectrum of bacteria isolated from tracheal aspirate of those children. The antibiotic susceptibility profiles of those bacteria were also examined.*

Methods: *We evaluated the data of all mechanically ventilated children aged 0-59 months admitted to Intensive Care Unit (ICU) of "Dhaka Hospital" of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) between August 2009 and July 2013 having their tracheal aspirate culture done. Data were extracted from electronic medical records of the Dhaka Hospital.*

Results: *Among 836 admitted pneumonia children in the ICU, we identified 35 children who fulfilled the inclusion criteria. Among them 34 (97%) had positive bacterial growths: Klebseilla species in 14 (40%), Escherichia coli in 11 (31%), Acinetobacter in 8 (23%) and Streptococcus species in 8 (23%). Additionally, Enterococcus, Staphylococcus aureus, Pseudominas and Proteus species were identified in 6 (17%), 4 (11%), and 2 (6%) of the children respectively. The susceptibility of the gram-negatives, except Klebseilla, to ampicillin, cotrimoxazole, gentamycin, ciprofloxacin, azithromycin, and ceftriaxone ranged from 0- 54%, while that for ceftazidime and amikacin ranged from 12-80%. The sensitivity of Klebseilla to these antibiotics ranged from 0-100%.*

Conclusions: *Our data suggests that gram-negative bacteria, Klebseilla followed by Escherichia coli, and Acenetobacter are the predominant bacteria associated with severe pneumonia in ventilated children. The increased number of infections caused by Gram-negative bacteria is being accompanied by rising rates of multi-drug resistance which underscores the importance of aggressive antimicrobial therapy in the management of such children.*

Key Words: *Pneumonia, Respiratory Infections, Tracheal Aspirate, Respiratory Organisms*

Introduction

Pneumonia is the leading cause of deaths among under-five children globally [1,2], with an estimated 1.4 million out of the total 7.6 million deaths in this population in 2010 [3]. In the critical care medicine the case-fatality is even higher [4]. A number of initiatives leading to improvement in the

1. Dr. K. M. Shahunja, MBBS, Medical Officer[a]
2. Dr. Mohammed Abdus Salam, MBBS, Director[b]
3. Dr. Tahmeed Ahmed, MBBS, PhD; Director[a]
4. Dr. Pradip Kumar Bardhan MBBS, MD, Scientista & Chief Physicianc
5. Dr. Shafiqul Alam Sarker, MBBS, MD, PhD, Senior Scientista & Consultant physician[c]
6. Dr. Hasan Ashraf, MBBS, MCPS, MD, Senior Scientista & Clinical Lead, ARI Ward[c]
7. Dr. Abu Syeed Golam Faruque, MBBS, MPH, Consultant[a]
8. Dr. Md Iqbal Hossain, MBBS, DCH, PhD, Senior Scientista & Clinical Lead, Nutrition Ward[c]
9. Dr. Md Munirul Islam, MBBS, PhD, Scientista & Consultant physician[c]
10. Dr. Sumon Kumar Das, MBBS, Assistant Scientist[a]
11. Dr. Sharifuzzaman, MBBS, Medical Officer [a, c]
12. Dr. Abu Sadat Mohammad Sayeem Bin Shahid, MBBS, Medical Officer[a]
13. Dr. Ehsanul Huq, MBBS, MPH, Senior Medical Officerd
14. Dr. Mohammad Habibur Rahman Sarker, MBBS, MPH, Medical Officer[a]
15. Dr. Mohammod Jobayer Chisti, MBBS, MMed (Paed) , Scientista & Clinical Lead, ICU[c]

[a]Centre for Nutrition and Food Security (CNFS), International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh
[b]Research and Clinical Administration and Strategy (RCAS), icddr,b, Dhaka, Bangladesh
[c]Clinical Service (CS), icddr,b, Dhaka, Bangladesh
[d]Centre for Food and Waterborne Diseases, icddr,b, Dhaka, Bangladesh

Corresponding author:
Dr. Mohammod Jobayer Chisti, MBBS, MMed (Paed), Scientist, Centre for Nutrition and Food Security & Clinical Lead, ICU, Dhaka Hospital, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)., 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh, E-mail: chisti@icddrb.org

nutritional status and rate of exclusive breast-feeding, case management practices including better access to antibiotics, care-seeking behaviour and referral practices, along with reductions in the indoor air-pollutions and housing overcrowding have contributed to reductions in the global burden of deaths from pneumonia [5,6,7], the United Nations Millennium Development Goal 4 (MDG 4) that aims to reduce child mortality by two-thirds by 2015 still remains in dream. Preventive strategies and appropriate and prompt management of pneumonia are integral part of the strategy to reduce the mortality from pneumonia [8]. Appropriate antimicrobial therapy requires evidence base, most importantly the etiology in particular patient population and their antimicrobial susceptibility. Pneumonia is frequently caused by a varieties of viruses, bacteria, fungus or mycobacterium tuberculosis [9,10]; however, their isolation and identification in resource poor settings is not only expensive but also very intriguing. Moreover, the etiology varies by age groups, nutritional status, geographical locations and settings in which they occur [9,11,12]. In most reported studies, the organisms were isolated from blood, sputum, throat swab or nasopharyngeal swab [9,13,14], which either did not reflect the actual incidence or isolation of the causative organism; isolation of organisms from tracheal aspirates is more likely representative of the actual pathogens [15,16,17]. However, there is limited data on isolation of bacterial pathogens from tracheal aspirates in mechanically ventilated children admitted to ICU with pneumonia, especially from developing countries. Therefore, we aimed at evaluating the bacterial isolations from tracheal aspirates in mechanically ventilated children admitted to intensive care unit (ICU) of the Dhaka Hospital of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) with community acquired pneumonia.

Methods

Study design:

This is a retrospective analysis of data excerpted from electronic medical records system of the "Dhaka Hospital" (named SHEBA) of icddr,b. We collected data of all children of both sex, aged 0-59 months, who were admitted to the ICU of the hospital between August 2009 and July 2013 and required mechanical ventilation, and also had their tracheal aspirate collected for bacteriological culture. The indication of ventilation was respiratory failure, defined by severe respiratory distress and severe hypoxemia (arterial oxygen saturation < 90% at sea level) despite receiving non-invasive continuous positive airway pressure (such as by bubble CPAP) and / or severe hypercarbia (Ph < 7.2 and PCO_2 > 60 mm of water). Tracheal aspirates were sent for bacterial culture and antimicrobial susceptibility testing of the isolates within an hour of intubation and ventilation. Collection of samples was made directly in a sterile, closed vacuum container using aseptic techniques inserting a tracheal aspiration probe up to carina. The containers were sealed by the physicians and sent to the microbiology laboratory at icddr,b for the further processing.

Procedure of isolation:

The tracheal aspirates specimens were seeded in BACTEC™ Peds Plus culture media, which contains resins for neutralization of antibiotics. Then they were placed into BACTEC™ FX in which the microorganisms, if present in the samples, metabolize nutrients in the BACTEC culture vial and release CO_2 into the medium or utilize the oxygen in the medium. The instrument monitors the fluorescence of the vial sensor that is activated when CO_2 is produced or O_2 is utilized. Analysis of the rate and amount of CO_2 produced or O_2 utilized enables the instrument to determine if the vial is positive; i.e., the presumptive presence of viable organisms [18].

Diagnosis of pneumonia and Clinical care:

Pneumonia diagnosis was done following the World Health Organization (WHO) criteria for under-five children [19]; the study children were managed in accordance with the protocolized guidelines of the hospital that is based on local and global evidence [9,20,21]

Data analysis:

Clinical and laboratory data were collected and entered onto a personal computer using Statistical Package for Social Sciences (SPSS), Windows (Version 17.0; Chicago, IL) and Epi Info (Version 1.0.3, USD, Stone Mountain, GA). The variables analyzed include demographic data, presence of diarrhea (three or more abnormally loose or watery stools in previous 24 hours) [20], severe malnutrition (weight for length z score of <-3 of the median of the WHO reference or nutritional edema) [20], results of tracheal aspirates, the bacterial isolates and their antimicrobial susceptibility with multi drug resistant bacteria (if the bacteria is >30% resistant to any antibiotics).

Results:

Among 836 admitted pneumonia children in the ICU, we identified 35 children who had their tracheal aspirate assayed for bacterial culture and antimicrobial susceptibility tests. Out of these children 34 (97%) had bacterial isolates: 12 (35%) had single isolates (Table 1) and the rest 22 (65%) had poly-microbial growths (Table 2), and there were a total of 60 isolates (Table 3).

Table 1. Single bacterial isolates from tracheal aspirates of under-five children mechanically-ventilated for pneumonia.

Bacteria	n = 12 n (%)
Klebseilla species	4 (33)
Escherichia coli	4 (33)
Enterococcus species	1 (8)
Staphylococcus aureus	2 (17)
Coagulase negative *Staphylococcus*	1 (8)

Figures represent n (%), unless specified

Median age (inter-quartile range) of the study children was 7.5 (5.0, 12.0) months. Male and female distribution was almost equal 18 and 17, 10 children of available 29 data (35%) were

non-breast-fed, and 27 children of available 30 data (90%) were vaccinated. Diarrhea and severe malnutrition was present in 31/34 (91%) and 21/34 (62%) children respectively.

Table 2. Multiple bacterial isolates from tracheal aspirates of under-five children mechanically-ventilated for pneumonia.

Organism	n(%)
***Klebseilla* species**	10 (45)
Escherichia coli	7 (32)
***Acinetobacter* species**	8 (36)
***Streptococcus* species**	8 (36)
***Enterococcus* species**	5 (23)
Staphylococcus aureus	2 (9)
Pseudomonas species	3 (14)
Coagulase negative *Staphylococcus*	2 (9)
***Proteus* species**	2 (9)
Staphylococcus hemolyticus	1 (4)

Forty-four of the 60 (73%) isolates were gram negatives - 14 (23%) were *Klebseilla* species, 11 (18%) were *Escherichia coli,* 8 (13%) were *Acenatobacter* species and 4 (7%) were *Pseudominas* species. Among the gram positives, 8 (13%) were *Streptococcus* species and 4 (7%) were *Staphylococcus aureus* (Table 3).

Discussion:

This perhaps is the first study from Bangladesh that examined the prevalence of various bacterial pathogens isolated from mechanically ventilated under-five children admitted to ICU of an urban hospital in Dhaka with pneumonia, nearly all of whom also had diarrhea. There were three major observations of our study: high rates of bacterial growth from the tracheal aspirates in mechanically-ventilated under-five children with pneumonia, predominance of gram negatives in this population, and multi-drug resistance among the Gram-negatives.

Isolation of bacterial agent in nearly all children indicate an association of bacterial pathogens in pneumonia in mechanically-ventilated, under-five children who also have diarrhea. However, the isolation of multiple bacterial agents from individual tracheal aspirates makes it difficult to define a causal relationship The observation is likely in developing world where environmental contamination and poor hygiene practice is likely. In acute watery diarrhea in children such co-pathogens or infection with polymicrobial pathogen are not uncommon [22]. This observation with polymicrobial pathogens deserves further attention in selecting antibiotic in case management in children with pneumonia necessitating ventilation. Nevertheless, the possibilities of contamination during tracheal aspirate collection yielding multiple pathogens cannot be ruled out. However, the observation is consistent with a number of recent studies involving tracheal aspirate in ventilated children [15,23].

Table 3: Bacterial isolates from tracheal aspirates and their sensitivity

Bacterial isolates	n (%)	Sensitivity of antibiotics (%)							
		AMX	SXT	GEN	CIP	CRO	CAZ	AMK	AZM
Klebseilla species	14 (40)	0	40	100	20	80	100	-	40
Escherichia coli	11 (31)	0	10	54	20	10	20	80	0
Acenetobacter species	8 (23)	0	25	36	13	0	17	50	8
Streptococcus species	8 (23)	-	-	100	100	-	50	50	-
Enterococcus species	6 (17)	0	28	12	12	0	12	25	33
Staphylococcus aureus	4 (11)	0	0	33	-	-	-	-	-
Pseudomonas spacies	3 (8)	-	0	0	0	-	-	-	-
Coagulase negative Staphylococcus	3 (8)	-	0	50	100	50	50	50	-
Proteus species	2 (6)	28	-	14	12	-	-	-	-
Staphylococcus hemolyticus	1(3)	67	-	67	-	50	-	-	-

AMX = ampicillin, SXT = trimethoprime-sulfamethoxazole, GEN = gentamycin, CIP = ciprofloxacin; CRO = ceftriaxone, CAZ = ceftazidime, AMK = amikacin, AZM = azithromycin;

The antimicrobial susceptibility of the isolates are provided in Table 3; it can be seen that, the susceptibility of the gram-negatives, with the exception of *Klebseilla,* to ampicillin, cotrimoxazole, gentamycin, ciprofloxacin, azithromycin, and ceftriaxone ranged from 0- 54%, and that to ceftazime and amikacin ranged from 12-80%. The susceptibility of *Klebseilla* species, *Escherichia coli*, *Acenatobacter* species and *Pseudomonas* species to imipenem and meropenem ranged from 25 – 90%. The susceptibility of the gram positives to these drugs ranged from 0-100% (Table 3)

The predominance of gram negatives from tracheal aspirates of our study children is a new finding. Our isolated bacterial agents significantly differ from the common bacterial etiology of pneumonia in this age group of children [24,25]. High proportion of children with severe malnutrition (62%), a common co-morbidity of pneumonia and diarrhea in developing countries may be a factor that explains our observation- [26] - the etiology of pneumonia in under-five children is different in such children compared to those without severe malnutrition [9,27]. Our isolated bacterial

agents are common in ventilation-associated pneumonia (VAP) or in nosocomial pneumonia [28,29]; however, we collected tracheal aspirates from children with community acquired pneumonia and we collected the sample immediately following their intubation and ventilation. The remote chance of the possibility of nosocomial infection in our study population could not be ruled out since the median duration of hospital stay of those children, before intubation and ventilation, was 5.0 (inter-quartile range 1-24) days.

With the exception of Klebsiella species, all of the gram-negatives were frequently resistant to all commonly used antibiotics such as ampicillin, gentamycin, trimethoprime-sulfamethoxazole, ciprofloxacin, and ceftriaxone. The susceptibility of these isolates to ceftazidime and amikacin were a bit better but still very low. Our findings underscores the importance of considering carbapenem such as imipenem or meropenem as first-line therapy for pneumonic children who develop respiratory failure- the historical and local susceptibility pattern of the most of the gram negatives to these two drugs are still very encouraging [29].

In conclusion, the results of our data suggest that tracheal aspirate was highly sensitive for bacterial pneumonia in under-five children who required mechanical ventilation. Gram negative and multi-drug resistant bacteria were the predominant essential etiology of pneumonia in under-five ventilated children especially in children with diarrhea which underscores the importance of initiation of intravenous carbapenem drugs in such population to reduce potential ramification. However, long term prospective studies with greater sample may provide more robust information.

Acknowledgement:

We gratefully acknowledge the donors for their support and commitment to icddr,b's research efforts. icddr,b acknowledges the following donors which currently provide unrestricted support to the centre's research efforts: Government of the People's Republic of Bangladesh, Canadian International Development Agency (CIDA), Embassy of the Kingdom of the Netherlands (EKN), Swedish International Development Cooperation Agency (Sida) and the Department for International Development, UK (DFID). We offer our sincere gratitude to all physicians including clinical fellows, nurses, members of the feeding team and cleaners of of the Dhaka Hospital of icddr,b for their invaluable contribution. We also express our sincere thanks to the SHEBA team members for their help in providing robust data set for this analysis.

References:

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, et al. (2012) Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet.
2. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, et al. (2010) Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet 375: 1969-1987.
3. Nair H, Simoes EA, Rudan I, Gessner BD, Azziz-Baumgartner E, et al. (2013) Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. Lancet.
4. Chisti MJ, Pietroni MA, Smith JH, Bardhan PK, Salam MA (2011) Predictors of death in under-five children with diarrhoea admitted to a critical care ward in an urban hospital in Bangladesh. Acta Paediatr 100: e275-279.
5. Sazawal S, Black RE (1992) Meta-analysis of intervention trials on case-management of pneumonia in community settings. Lancet 340: 528-533.
6. Dherani M, Pope D, Mascarenhas M, Smith KR, Weber M, et al. (2008) Indoor air pollution from unprocessed solid fuel use and pneumonia risk in children aged under five years: a systematic review and meta-analysis. Bull World Health Organ 86: 390-398C.
7. Mulholland K (2007) Childhood pneumonia mortality--a permanent global emergency. Lancet 370: 285-289.
8. Graham SM, English M, Hazir T, Enarson P, Duke T (2008) Challenges to improving case management of childhood pneumonia at health facilities in resource-limited settings. Bull World Health Organ 86: 349-355.
9. Chisti MJ, Tebruegge M, La Vincente S, Graham SM, Duke T (2009) Pneumonia in severely malnourished children in developing countries - mortality risk, aetiology and validity of WHO clinical signs: a systematic review. Trop Med Int Health 14: 1173-1189.
10. Chisti MJ, Ahmed T, Pietroni MA, Faruque AS, Ashraf H, et al. (2013) Pulmonary tuberculosis in severely-malnourished or HIV-infected children with pneumonia: a review. J Health Popul Nutr 31: 308-313.
11. Stuckey-Schrock K, Hayes BL, George CM (2012) Community-acquired pneumonia in children. Am Fam Physician 86: 661-667.
12. McIntosh K (2002) Community-acquired pneumonia in children. New England Journal of Medicine 346: 429-437.
13. Chisti MJ, Salam MA, Sharifuzzaman, Pietroni MA (2009) Occult pneumonia: an unusual but perilous entity presenting with severe malnutrition and dehydrating diarrhoea. J Health Popul Nutr 27: 808-812.
14. Larsson M, Kronvall G, Chuc NT, Karlsson I, Lager F, et al. (2000) Antibiotic medication and bacterial resistance to antibiotics: a survey of children in a Vietnamese community. Trop Med Int Health 5: 711-721.
15. Aly H, Badawy M, El-Kholy A, Nabil R, Mohamed A (2008) Randomized, controlled trial on tracheal colonization of ventilated infants: can gravity prevent ventilator-associated pneumonia? Pediatrics 122: 770-774.
16. Golia S, K TS, C LV (2013) Microbial profile of early and late onset ventilator associated pneumonia in the intensive care unit of a tertiary care hospital in bangalore, India. J Clin Diagn Res 7: 2462-2466.
17. Navaneeth BV, Belwadi MR (2002) Antibiotic resistance among gram-negative bacteria of lower respiratory tract secretions in hospitalized patients. Indian J Chest Dis Allied Sci 44: 173-176.
18. Jakribettu RP, Boloor R Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care center in Mangalore. Saudi J Anaesth 6: 115-119.
19. WHO (2005) Pocket book for hospital care of children: guidelines for the management of common illness with limited resources. World Health Organization, Geneva.
20. WHO (2013) Pocket book for hospital care of children: guidelines for the management of common childhood illness 2nd ed. Geneva: World Health Organization.
21. Ahmed T, Ali M, Ullah MM, Choudhury IA, Haque ME, et al. (1999) Mortality in severely malnourished children with diarrhoea and use of a standardised management protocol. Lancet 353: 1919-1922.
22. Sarker SA, Jakel M, Sultana S, Alam NH, Bardhan PK, et al. (2013)

Anti-rotavirus protein reduces stool output in infants with diarrhea: a randomized placebo-controlled trial. Gastroenterology 145: 740-748 e748.

23. Zaidi AK, Reller LB (1996) Rejection criteria for endotracheal aspirates from pediatric patients. J Clin Microbiol 34: 352-354.
24. McIntosh K (2002) Community-acquired pneumonia in children. N Engl J Med 346: 429-437.
25. Morar P, Singh V, Makura Z, Jones AS, Baines PB, et al. (2002) Oropharyngeal carriage and lower airway colonisation/infection in 45 tracheotomised children. Thorax 57: 1015-1020.
26. Chisti MJ, Duke T, Robertson CF, Ahmed T, Faruque AS, et al. (2011) Co-morbidity: exploring the clinical overlap between pneumonia and diarrhoea in a hospital in Dhaka, Bangladesh. Ann Trop Paediatr 31: 311-319.
27. Chisti MJ, Ahmed T, Faruque AS, Abdus Salam M (2010) Clinical and laboratory features of radiologic pneumonia in severely malnourished infants attending an urban diarrhea treatment center in Bangladesh. Pediatr Infect Dis J 29: 174-177.
28. Erdem I, Ozgultekin A, Inan AS, Dincer E, Turan G, et al. (2008) Incidence, etiology, and antibiotic resistance patterns of gram-negative microorganisms isolated from patients with ventilator-associated pneumonia in a medical-surgical intensive care unit of a teaching hospital in istanbul, Turkey (2004-2006). Jpn J Infect Dis 61: 339-342.
29. Ning BT, Zhang CM, Liu T, Ye S, Yang ZH, et al. (2013) Pathogenic analysis of sputum from ventilator-associated pneumonia in a pediatric intensive care unit. Exp Ther Med 5: 367-371.