CHARACTERIZATION AND SENSITIVITY PATTERN OF BACTERIAL PATHOGENS ISOLATED FROM PNEUMONIA PATIENTS UNDER 5 YEARS OF AGE: A HOSPITAL-BASED STUDY

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Accepted for publication: October 25, 2008

ABSTRACT

Das S., Khan M. S., Neogi P. K., Akhter N. and Islam M. J. 2008. *Characterization and Sensitivity Pattern of Bacterial Pathogens Isolated from Pneumonia Patients Under 5 Years of Age: A Hospital-Based Study.* **j. innov.dev.strategy 2(3): 59-64**

A surveillance study on pneumonia was undertaken at Chittagong Maa-Shishu-O-General Hospital, Bangladesh during February 2007 to June 2007. It was conducted in order to isolate, identify and determine the sensitivity pattern of this disease causing agents from blood and cerebrospinal fluid (CSF) of hospitalized children less than 5 years of age to document the burden of these agents in Chittagong; Bangladesh. A no. of tests including cytological, biochemical, cultural and antimicrobial susceptibility test by Kirby-Bauer disc diffusion method was performed for their confirmative identification and for concerning their sensitivity pattern. Out of 547 blood samples, 52 (9.51%) yielded bacterial growth of which *K. pneumoniae,* 29 (55.77%) was the most frequent offender. It was followed by *Serratia spp.,* 07 (13.46%); *Acinetobacter spp.,* 5 (9.62%); *Staphylococcus aureus,* 4 (7.69%) etc. Only 01 (1.92%) *Haemophilus influenzae* was isolated from blood. From 53 CSF samples, number of bacterial isolates were 3 (5.66%) of which *Streptococcus pneumoniae* were 02 (66.67%) and the rest was *pseudomonas aeruginosa* (33.33%). Studies on age-dependent variation of bacterial isolates revealed that neonates (13.56%) are particularly vulnerable to infections because of weak immune barrier. Imipenem was found to be more active against *Klebsiella, Serratia,* and *Proteus spp.* than other antibiotics. Again *Acinetobacter spp*. was found to be more sensitive against imipenem and ciprofloxacin. Other Enterobacteriaceae family such as *E. coli* and *Pseudomonas spp.* offered sensitivity towards extended spectrum ciprofloxacin, imipenem as well as co-trimoxazole. The fluorinated quinolones, in particular ciprofloxacin and β-lactams, in particular ceftriaxone are still active against *Streptococcus Pneumoniae*. Pneumococcal isolation rate was very low both from blood and CSF. This study will provide local information on the common serotypes so that the future formulation of pneumococcal and other vaccines based on the local prevalent serotypes causing disease could be suggested.

Keywords: Multidrug resistant, *Klebsiella pneumoniae*, neonatal age, sensitivity pattern

INTRODUCTION

Pneumonia is defined as inflammation and consolidation of the lung tissue due to an infectious agent. World Health Organization (WHO) statistically showed pneumonia as the leading cause of death in children (under 5 years of age) worldwide and the most of them in developing countries like Bangladesh. While it is difficult to know which pathogen is responsible for a specific child's death from pneumonia, infections caused by *Streptococcus pneumoniae* (pneumococcus) are believed to be a major cause of fatal childhood pneumonia and meningitis worldwide. Even in ideal situation pneumococci are difficult to isolate, and so the vast majority of pneumococcal infections are unrecognized. Among 90 serotypes of *S. pneumoniae,* 4 strains are actually responsible for most disease and drug-resistant *S. pneumoniae* (DRSP). These are strains 6B, 14, 19 and 23F. Most strains are resistant to co-trimoxazole. Penicillin resistant strains are also recognized which are resistant to other drugs such as chloramphenicol, erythromycin, tetracycline etc. In Bangladesh, the most common serotypes of pneumococcus isolated among hospitalized patients are different from the serotypes that are included in the available vaccines (Saha *et al.,* 2003). Besides pneumococcal pneumonia, reports from different studies reveal that G (-ve) pneumonia may also contribute to the higher percentage in nosocomial pneumonia. Newborn infants are particularly vulnerable to gram-negative bacteria including *Klebsiella pneumoniae, Serratia marcescens,* etc. Various extrinsic and intrinsic factors are the main cause of G (-ve) pneumonia. However, the incidence of *Staphylococcus aureus* lower ultimate fate of G (-ve) pneumonia continues to be a common causes of neonatal mortality in Bangladesh (Darmstadt *et al.,* 2005). Besides the risk factors identified both in the neonates and in the mother, neonatal age is particularly vulnerable to infections because of weak immune barrier. Moreover, the G (-ve) organisms isolated are often resistant to multiple antimicrobials which make the treatment difficult. Resistant may be acquired by the loss or deficiency of specific porins, emergence of extended spectrum β-lactamases (ESBLs) producing strains, presence of R-factors that carry one or more genes that encode resistance, mutation in chromosomally-encoded genes or by the horizontal gene transfer of antibiotic resistance determinants. So, this study was designed to isolate, identify and determine the sensitivity pattern of pneumococcus and other pneumonia causing agents from the suspected cases to document the burden of these agents in Chittagong, Bangladesh.

MATERIALS AND METHODS

Patient selection

Children of less than 5 years of age who were admitted in the Chittagong Maa-Shishu-O-General Hospital, Chittagong, Bangladesh either with the manifestations of pneumonia, severe pneumonia, meningitis/febrile convulsion or very severe disease were included in this study. On fulfilling the clinical criteria, blood and/or CSF were collected following standard operating procedure (SOP) with prior parental consent.

Subculture of blood culture bottle

About 1-3 ml blood was drawn and aseptically added to the blood culture bottle containing 10 ml of trypticase soya broth. After 14-17 hrs of incubation, the bottles were examined for the appearance of any turbidity or lysis of the erythrocytes and then everyday for up to 7 days. Subcultures from the positive growth had been performed ordinarily on Chocolate agar plates (Oxoid, UK), Blood agar plates (Oxoid, UK) and MacConkey agar plates (Oxoid, UK). Next day after proper incubation, media were examined for any bacterial growth and subsequently gram stain followed by different biochemical tests (e.g. KIA, Citrate, Catalase, Coagulase, Oxidase, Satellitism test etc) were performed (Cheesebrough, 2000).

CSF collection and analysis

CSF was collected by lumber puncture and was inoculated directly on to supplemented chocolate agar plates (CAP), blood agar plates (BAP) and MacConkey agar plates (MAP). In the laboratory, Blood agar (BA) and Chocolate agar (CA) plates were placed in the incubator with 5-10% carbon-dioxide (CO_2) containing environment. On the 2nd day, incubated media were examined for any bacterial growth and subsequently gram stain followed by different biochemical tests (KIA, Citrate, Optochin susceptibility test etc.) for their confirmative identification. Besides microbiological, cytological (to determine the number and types of cells) and biochemical tests (to measure protein and sugar content) of CSF had also been done.

Antibiogram by disc diffusion method

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966) as per the NCCLS (presently CLSI) recommendations. In brief, the tests were performed on Mueller-Hinton agar supplemented with 5% sheep blood for all identified G (-ve) and G (+ve) pathogens except *Haemophilus influenzae* (antibiogram on Chocolate agar). There were the discs (Oxoid, UK) of ampicillin (10µg/disc), ceftriaxone (30µg/disc), co-trimoxazole (25µg/disc), ciprofloxacin (5µg/disc), gentamicin (10µg/disc), amikacin (30µg/disc), imipenem (10µg/disc), optochin (5µg/disc) and oxacillin (1µg/disc). Finally results were interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI-defined breakpoints.

RESULTS AND DISCUSSIONS

In this study, among 600 cases (from few patients blood and CSF both were obtained) total 55 (9.17%) cases exhibited bacterial infection of which 52 (9.51%) were from blood samples and 03 (5.66 %) were from CSF samples (Table 1). Similarly Mamishi *et al.,* (2005) in Iran described 9.13% isolation rate from blood stream infection. But Dewanjee (2000) and Saha *et al.,* (1992) in Bangladesh reported that the rate of isolation were 20.5% and 23.5% respectively.

Table 1. Representation of total case definitions

Total cases	Numbers	Percentages
A. Disease cases:		
a) Total pneumonia cases	322	53.67 %
b) Total meningitis cases	50	8.33 %
c) Total severe cases	228	38.00 %
B. Total sample:	600	
a) Total blood sample	547	91.17 %
b) Total CSF sample	53	8.83 %
C. Total positive cases	55	9.17 %
a) Blood	52	9.51 %

b) CSF 3 5.66 %

In the present study, *K. pneumoniae* (55.77%) and *Serratia marcescens* (13.46%) were the most common isolates in blood (Table 2, Figure 1). Levy *et al.,* (1996) in Israel also found *K. pneumoniae*, *E. coli* and *P. aeruginosa* as the predominant isolates in blood. Study on septicaemic patients done by Dewanjee (2000) in Bangladesh found four common isolates as *E. coli*, *Staph. aureus*, *K. pneumoniae* and *Enterobacter* species.

Table 2. Rate of type specific bacterial Isolates from blood and CSF

Sample	Bacterial isolates	No. of isolates	Percentage
Blood	*K. pneumoniae*	29	55.77%
	S. marcescens	7	13.46%
	A. baumannii	5	9.62%
	Staph. aureus	4	7.69%
	E. coli	2	3.85%
	P. aeruginosa	2	3.85%
	Proteus spp.	2	3.85%
	H. influenzae	1	1.92%
CSF	*Strep. pneumoniae*	2	66.67%
	P. aeruginosa	1	33.33%

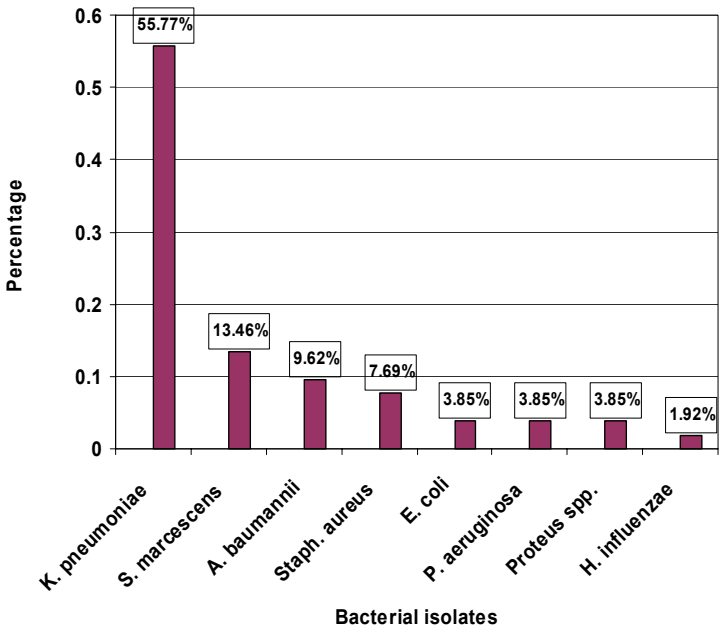


Figure 1. Rate of bacterial isolates from blood

In another study from Trinidad, Orrett and Changoor (2006) found *Staph. aureus* as the commonest isolates followed by *P. aeruginosa , K. pneumoniae* and *Enterobacter* species. Among five most common bacterial isolates in blood, Weinstein *et al.,* (1997) in the USA described coagulase negative *Staphylococci* (CoNS) as the third commonest organism. However, the etiological agents of septicaemia may vary from country to country, from hospital to hospital and from one community to another.

From the statistical representation of age-dependent distribution of bacterial pathogens, it is very much clear that neonate (13.56%) (Figure 2) are particularly vulnerable to infections because of weak immune barrier. Roy *et al.,* (2002) from India also isolated and depicted on the neonatal septicaemia in a tertiary care hospital of Northern India. Several risk factors have been identified both in the neonates and in the mother, which make them susceptible to infections. The varying microbiological pattern of neonatal septicaemia warrants the need for an ongoing review of the causative organisms and their antibiotic sensitivity pattern. Some reports from home and abroad also showed the incidence of neonatal septicaemia to vary from 36% to 55% (Ako-Nai *et.al.,* 1999 and Das *et.al.,* 1999).

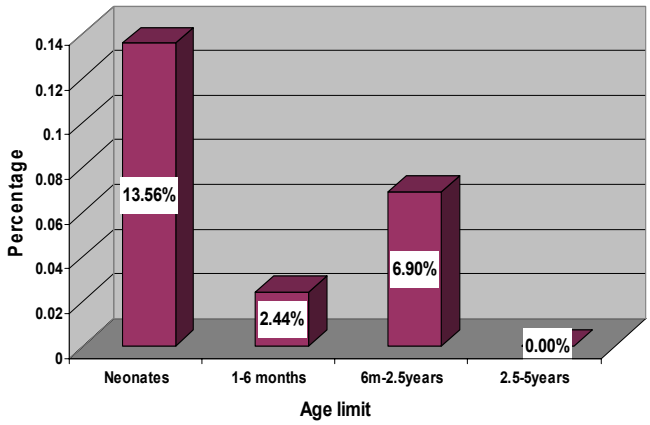


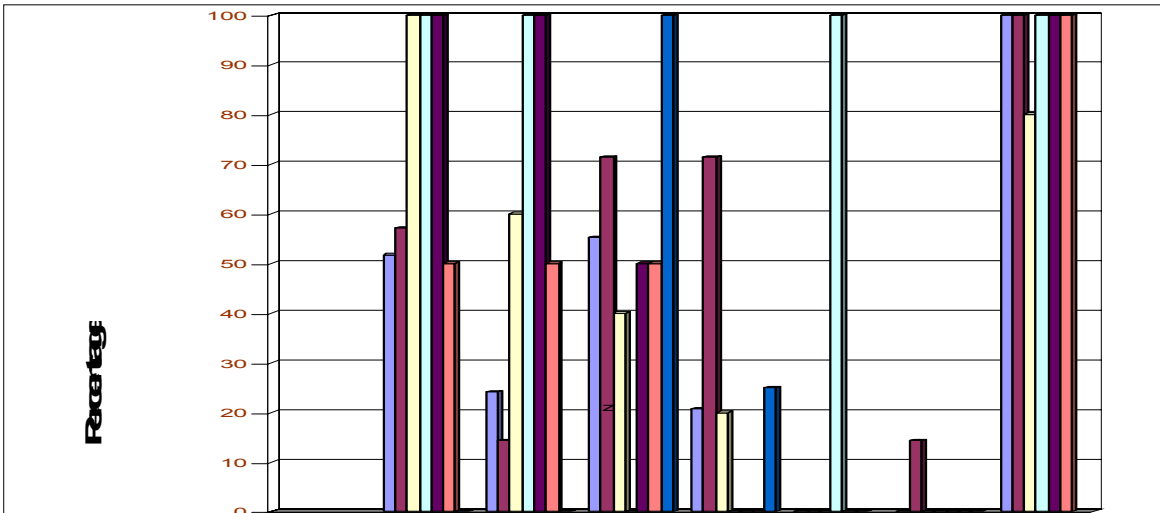
Figure 2. Prevalence of bacterial pathogens at neonatal age

Antibiogram of bacterial pathogens showed that maximum 100% *K. pneumoniae* were sensitive to imipenem followed by amikacin (55.17%), ciprofloxacin (51.72%) etc. In the study undertaken by Dewanjee (2000) in Bangladesh found 28.58% *K. pneumoniae* were sensitive towards chloramphenicol (Table 3, Figure 3). So, culture and antimicrobial susceptibility tests should always be sought before instituting any empirical antimicrobial therapy.

Table 3. Antimicrobial sensitivity (%) pattern among the bacterial isolates

Sample	Name of Isolate	Sensitivity pattern (%)	Name of antibiotics						
			CIP	TS	AK	GM	AMP	CRO	IPM
Blood	*K. pneumoniae*	S	51.7	24.1	55.2	20.7	0	0	100
	S. marcescens	s	57.1	14.3	71.4	71.4	0	14..3	100
	A. baumanii	S	100	60	40	20	0	0	80
	E. coli	S	100	100	0	0	100	0	100
	P. aeruginosa	S	100	100	50	0	0	0	100
	Proteus spp	S	50	50	50	0	0	0	100
	Stap aureus	S	0	0	100	25	X	0	X
CSF	*Strep. pneumoniae*		100	100	50	0	100	100	

An important striking feature found in this study was increased resistance to ampicillin by *Pseudomonas aeruginosa*. Alarming increase in resistance of *Pseudomonas spp*. to various antimicrobial agents has also been reported by many workers (Paul, *et al.*, 1992). Area based knowledge of the bacteriological spectrum is essential because the first antibiotic administered will not wait for the culture results and keeping in mind the high morbidity and mortality associated with neonatal sepsis, a right choice for such empiric therapy is of utmost importance. In western countries, antibiotics of choice are directed towards group B *Streptococcus* and *E. coli*. But in tropical areas, early onset neonatal infections may be caused by multi resistant hospital acquired bacteria, which are transmitted during delivery by lack of hygiene. During study, most causes of death displayed seasonal fluctuation, and sex differentials were marked with female deaths exceeding male deaths for all ages after the neonatal period. Malnourished children from low socioeconomic status families had higher mortality rates than their better nourished and weather counterparts. Overall, the data suggest that the delivery of a few basic health measures (oral hydration and immunization) could result in substantial reduction of less than 5 years age mortality.



	CIP	TS	AK	GM	AMP	CRO	IPM
K.pneumoniae	51.72	24.14	55.17	20.69	0	0	100
S.marcescens	57.14	14.29	71.43	71.43	0	14.29	100
A. baumannii	100	60	40	20	0	0	80
E.coli	100	100	0	0	100	0	100
P.aeruginosa	100	100	50	0	0	0	100
Proteus spp.	50	50	50	0	0	0	100
Staph.aureus	0	0	100	25		0	

Figure 3. Sensitivity pattern of 7 isolates against 7 antibiotics

Finally, the important feature of this study is the resistance of bacterial pathogens to a variety of antimicrobial agents that is emerging in throughout the world at the present time. Therefore, to combat this problem, efforts should be made to isolate and characterize plasmids responsible for resistance in multi-drug resistant (MDR) organism from all over the country and a nation wide antibiotic policy should be defined after evaluating the effectiveness of the regime so that misuse of antibiotics is minimized and also the emergence of multi-drug resistant organism can be restricted. The present study will remain as a pioneering milestone for any future study to understand the burden of pneumococcal and other infections of pneumonia in Chittagong; Bangladesh.

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