OPEN ACCESS Freely available online

Clinical Microbiology: Open Access

Reaserch Article

Characterization of Streptococcus Pneumoniae Strain Isolated From Pneumonia Cases of Child Patients in Dinajpur, Bangladesh

Nayemul Bari*, Dinajpur, Bangladesh[1], Aoulad Hosen[1], Nazmi Ara Rumi[1]. Mostafizer Rahman[1]. Abdul Khalek[2]

[1]*Department of Microbiology, Hajee Mohammad Danesh Science & Technology University,*

[2] *Department of Microbiology, Primeasia, Dhaka, Bangladesh*

ABSTRACT

Aim: Pneumonia is a common disease in child patients caused by streptococcus pneumoniae. The aim of this research was determined for the identification of Streptococcus pneumonia and their antibiotic sensitivity profile.

Methodology And Results : A total of 40 samples were randomly collected from three main hospitals in the Dinajpur district of Bangladesh and analyzed through different bacteriological, biochemical, molecular and antibiotic susceptibility testing. Out of 40 samples, positive cases of pneumonia were found to be 37.5% & 15 isolates were isolated. The Frequency of pneumonia in relation to age were 3-5yrs (50%), 6-8yrs (33.33%), 9- 11yrs (25%) & 12-15 (20%). The present study reveals that the study area had no significant effect ($P > 0.05$), but age ($P < 0.05$) & socio-economic status ($P < 0.05$) had significant effects on the prevalence of pneumonia in pneumonia patients. Among the age group, the prevalence of pneumonia was highest (50%) in the 3-5 years age group. The Highest prevalence of pneumonia was found in poor socio-economic status (54.54%). Streptococcus pneumoniae was characterized by 16S rRNA Sequencing & the identified strain was Streptococcus pneumoniae NBRC102642. The antibiotic study revealed that all of the isolates of Streptococcus pneumoniae were resistant to most of the drugs, but found sensitive to Neomycin, Kanamycin & Streptomycin followed by Erythromycin, Azithromycin & Bacitracin.

CONCLUSION, SIGNIFICANCE, AND IMPACT OF STUDY: Streptococcus pneumonia create critical complication to child patients and need to prevention for overcome this situation using vaccination, development new effective antibiotic against pneumonia and also need public awareness.

Keywords: pneumonia;antibioticsensitivity;PCR;nasopharyngealswab; blood sample; Child patients

INTRODUCTION

Streptococcus pneumoniae remains a leading cause of serious infections and deaths in young children and the elderly globally (O'Brien et al., 2018). As global efforts to prevent and control pneumococcal disease gain momentum, a good understanding of the population structure of Streptococcus pneumoniae, including the circulating pneumococcal serotypes and genotypes would be critical in guiding the development of appropriate interventions. Some studies have reported changes in circulating serotypes causing invasive disease and nasopharyngeal carriage in different parts of the world (Ubukata et al., 2015; Devine et al., 2017). The estimated incidence of community-acquired pneumonia among children under five years of age in developing countries is approximately 151.8 million new cases per year, 11-20 million of which require hospitalization (Rudan et al., 2004). In developing countries, pneumonia occurs more often and is more severe than in developed nations, and carries higher incidence and mortality rates; pneumonia accounts for one- fifth of under-five deaths within the developing world (Bryce et al., 2005). Regarding antibiotic drug susceptibility of S. pneumoniae, minimum repressing concentration (MIC)

***Corresponding author:** Bari N, Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Bangladesh; Email:nayeembari919@gmail.com

Received date: March 24, 2021 **Accepted date**: September 7, 2021; **Published date**: September 17, 2021

Citation: *Bari N* (2021) Characterization of Streptococcus pneumoniae strain isolated from pneumonia cases of child patients in Dinajpur, Bangladesh. Clin Microbiol10: p263

breakpoints for the antibiotic drug were initial established within late 1976, in response to the necessity for making certain roaring treatment of diplococcus infectious disease. Over the past three decades, increasing MICs to penicillin have emerged in pneumococci, and the percentage of penicillin-intermediate and penicillin-resistant strains have risen (Berezin et al., 1996; Whitney et al., 2000). In a study conducted by the Regional immunizing agent System Project (Projeto First State Sistema Regional First State Vacinas, SIREVA) between 2000 and 2005, of a complete of eight,993 diplococcus isolates (36.4% of that obtained from patients with pneumonia), 37.8% were antibiotic drug (Castaned et al., 2009). *Streptococcus pneumoniae* is the most common cause of community-acquired respiratory tract infections such as otitis media, sinusitis, and pneumonia (Ishida, et al., 2008). Globally, diplococcus diseases account for one to a pair of million deaths annually in each extreme older. (Mulholland, 1999). It is supposed to be a very sensitive organism to routine antibiotics, especially penicillins. However, with the isolation of the primary clinically vital antibiotic penicillin-resistant pneumococcus (PRP) in 1967, several studies from totally different elements of the planet have reported AN increasing emergence of PRP (Collignon and Bell, 1996). At present, there are not only reports of resistant strains of *S. pneumoniae* to the beta-lactam group of antibiotics, but there is also an emergence of multidrug-resistant strains (Lalitha and Manoharan, 2002). *Streptococcus pneumoniae* remains a major cause of morbidity and mortality worldwide. Pneumococcus is communal of the top breathing tract of humans. However, it is also a human pathogen responsible for several respiratory tract infections & serious invasive pneumococcal diseases, such as sepsis and meningitis (O'Brien et al., 2009). The pneumococcal disease has a higher incidence among young children, the elderly, and the immunocompromised of all ages. As the epidemiology of pneumococcal disease varies with time and place, periodic reassessment with monitoring of prevalent serotypes and patterns of resistance is required for better therapeutic guidance and definition of control strategies. With that in mind, the most objectives of this study were: to spot the S. pneumoniae serotypes most often isolated from children hospitalized for invasive pneumonia; compare these serotypes with those included in conjugate vaccines; analyze their susceptibility to the antimicrobial agents most frequently utilized in pediatric practice.

Materials and Methods

Isolation of a bacterial strain

A total of 40 Blood & Nasopharyngeal swab samples of pneumonia patients were collected at different intervals during January-November 2018 from three hospitals of Dinajpur district in Bangladesh for identification of *Streptococcus pneumonia.* Out of 40 samples 20 samples were collected from M. Abdur Rahim Medical College Hospital. 15 samples were collected from Aurobindo Shishu Hospital of Dinajpur & the rest of the 5 samples were collected from Islami Bank Community Hospital, Dinajpur (IBCH). Samples were plated on nutrient agar and blood agar then incubated at 37 °C for 24hours for microbiological growth formation. Further subculture for pure isolation in blood agar that can be utilized for determining hemolytic reactions Streptococcus pneumoniae gives alpha hemolysis and then identified the bacterial strain by using a biochemical test (Catalase, Oxidase, MR, VP, Indole, Lactose Fermentation, TSI and Bile Solubility) based on Clin. Path (1951) and Cheesbrough, (1984).

Antibiotics susceptibility test

Once the bacteria is isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates according to the recommendations of the National Committee for Clinical Laboratory Standards (CLSI 2013). Bacterial inoculum was prepared by suspending the freshly grown bacteria in 4–5 ml sterile nutrient broth and the turbidity was adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium, Antibiotic disks were applied using sterile forceps. Agar plates were incubated at 37°C for 18 hours. After overnight incubation at 37 °C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant or sensitive following company recommendations. All isolates were tested for sensitivities to 10 of routine and practical antibiotics.

Bacterial genomic DNA isolation (extraction)

Bacteria from saturated liquid culture (5 ml of LB broth overnight growth) are lysed and proteins are removed by digestion with proteinase-k. Cell wall debris, polysaccharides, and remaining proteins are removed by Phenol-chloroform extraction and high-molecular-weight DNA is recovered from the resulting supernatant by isopropanol precipitation. The DNA extraction was performed based on the manufacturer's instructions and use a DNA extraction kit. The extracted DNA was validated by spectrophotometer and determine the absorbance.

Polymerase chain reaction (PCR)

PCR assay was done by victimization microorganism universal primers. The sequences of the forward and reverse primers are 5'TG ATC GTT TAC GGC GTG GAC3' and 5'A ATA CCA AGT CTC AAG AGT GT3'.The desoxyribonucleic acid fragment of 16S ribosomal RNA was created victimization DNA polymerase enzyme of Thermo Scientific™ Phusion™ HighFidelity (Fisher scientific). PCR was administered in an exceeding reaction mixture that contains 25 µL of 2× Phusion Master combine, 0.5 µM for each of the primers, 100 ng/ 50 µL of DNA. The ultimate volume of reactions was selected to be 50 µL. The thermal cycler was run PCR was set as a denaturation for 30 sec at 95 °C, tempering at 56 °C for 30 sec, extraction at 72 °C for an amount of 1.5 min, and a final extension at 72 °C for an amount of 10 min. The DNA band was detected by using 1% of agarose gels. The agarose gels were run using electrophoresis and stained with ethidium bromide stain. The bands were then extracted from the gel by the Thermo scientific Gene Extraction Kit.

DNA sequencing method

Then the extracted 16S rRNA PCR fragment of streptococcus pneumoniae was sent for sequencing. The ester sequence 16S rRNA cistron region inform ation was submitted toNCBI ester sequence info. Using BLAST tool, phylogenic tree, primer pairs were designed from the NCBI info search tool. Sequences were aligned exploitation clustal Omega electronic computer, and biological process tree analysis was done exploitation neighbor-joining technique.

Statistical analysis

Data were analyzed using SPSS version 21. The chi-square ($\chi 2$) test was used to assess statistical differences between the groups. A p-value less than 0.05 was statistically considered significant.

RESULTS AND DISCUSSION

bacterial identification

A total number of 40 samples were collected from pneumonia patients (3-15 years) for this study from the different hospitals in Dinajpur, Bangladesh. Out of 40 pneumonia patients, positive cases of pneumonia were found to be 37.5% (15 out of 40 samples) and no grown was identified in 63.5% (25 samples out of 40). The result of morphological, staining, cultural, biochemical, and percentage of incidence of isolated bacteria are presented in different tables. Table 1 summarizes the Frequency of pneumonia in pneumonia patients in relation to age of 3-5yrs (50%), 6-8yrs (33.33%), 9-11yrs (25%) and 12-15 (20%). Table 2 summarizes the distribution of organisms based on age difference Streptococcus pneumoniae of 3-5 years (40%), 6-8 years (26.66%), 9-11 years (20%), and 12-15 years (13.33%). The present study reveals that the study area had no significant ($P > 0.05$) effect but age ($P < 0.05$) & socio-economic status ($P < 0.05$) had significant effects on the prevalence of pneumonia in pneumonia patients that was summarized in table 3. In the study area, the highest prevalence was found in M Abdur Rahim Medical College Hospital (40%), followed by Aurobindo Shishu Hospital (33.33%) & Islami Bank Community Hospital (40%) respectively. Among the age group, the prevalence of pneumonia was highest (50%) in 3-5 years age group, then 33.33% in 12-15 years age group, 25% in 6-8 years age group & 20% in 9-11 years age group. The highest prevalence of pneumonia was found in poor socio-economic status (54.54%), followed by medium (16.66%) & rich (16.66%) socio-economic conditions respectively. Blood and nasopharyngeal swab samples were plated on nutrient agar and blood agar at 37 °C for 24 h and finally observed Convex, smooth & gray-white colonies in nutrient agar, the alpha-hemolytic colony on blood agar. Then bacterial colonies were identified by using Gram's stain which showed spherical chain shape cocci cells and purple color. Table 4 summarizes the phenotypic and biochemical properties of the isolates. In line with microscopic appearance, cultural characteristics, and biochemical tests, the isolated bacterial strain was associated with, *Streptococcus pneumoniae*.

antibiotics susceptibility

An antibiotic sensitivity test was carried out using the disc diffusion technique on Mueller Hinton agar for all the bacterial isolates to the most common antibiotic agents. 20 different antibiotics were used against identified Streptococcus pneumoniae (Table 5). The antibiotic study revealed that all of the isolates of Streptococcus pneumoniae were sensitive to Neomycin, Kanamycin & Streptomycin followed by Erythromycin, Azithromycin & Bacitracin. The isolates were resistant to Penicillin, Amoxicillin, Ampicillin, Methicillin, Cloxacillin, Clindamycin, Ciprofloxacin, Vancomycin, Amikacin & Co-trimoxazole followed by Cefradin, Cefixime, Cefaclor, Cephalexin, and Cefepime.

Table 1: Frequency of pneumonia in pneumonia patients concerning age

AGE	NO. EXAMINED	NO. POSITIVE	PERCENTAGE (%)
3-5yrs	12	6	50
6-8yrs	10	4	33.33
9-11	8	2	25
12-15	10	15	20
Total	40	27	128.33

Table 2: Distribution of organism based on age difference

AGE	ORGANISMS (STREPTOCOCCUS PNEUMONIAE)	PERCENTAGE (%)
3-5yrs	6	40
6-8yrs	4	26.66
9-11	3	20
12-15	2	13.13
Total	15	100

Table 3: Prevalence of pneumonia based on the study area, age & socio-economic status

PARAMETER		NO. EXAMINED	NO. POSITIVE	PERCENTAGE (%) P-VALUE
Study area		20	8	40
Community	Medical College	15	5	33.33 0.061
	Hospital	5	2	40
	Aurobindo Shishu			
	Hospital			
	Islami Bank			
Age	Hospita	20	10	50
	3-5 years	12	3	25 0.018
	6-8 years	5	1	20

	9-11years	3	1	33.33
	12-15 years			

Note: P > 0.05 means not significant,

P < 0.05 means statistically significant

Table 4: Phenotypic and biochemical properties of the isolated bacteria.

TESTS	REACTIONS
Growth on blood agar	alpha-hemolytic colony
Gram staining	Gram-positive spherical chain shape
cocci	
Catalase	-
Oxidase	-
MR	+
VP	-
Indole	-
Lactose fermentation	+
TSI	A/A
Bile Solubility	+

(+): positive reaction; (-): negative reaction; A/A: acid / acid(yellow slant/yellow but) reaction

Table 5: Result of antibiotic sensitivity test for Streptococcus pneumonia

NAME OF ANTIBIOTIC	INTERPRETATION
Penicillin (10)	R
Amoxicillin (30)	R
Ampicillin (25)	R
Methicillin (5)	R
Cloxacillin (1)	R
Clindamycin (2)	R
Ciprofloxacin (5)	R
Vancomycin (30)	R
Amikacin (30)	R
Cefradin (25)	R
Cefixime (5)	R
Cefaclor (30)	R
Cephalexin (30)	R
Cefepime (30)	R
Co-trimoxazole (25)	R
Neomycin (30)	R
Kanamycin (30)	S
Streptomycin (10)	S
Erythromycin (15)	S
Azithromycin (30)	S
Bacitracin (10)	S

Legends: S: Sensitive, R: Resistant and I: Intermidiate Detection of 16S rRNA gene and sequence analysis

16S rRNA gene region was amplified with the universal primers, Forward primer- 27F (5'AGAGTTTGATCCTGGCTCAG 3') Reverse primer- 1492R (5' TACCTTGTTACG ACTT 3'). PCR Amplification band was found at 1466 bp which is shown in figure 1. Streptococcus_pneumoniae_strain _NBRC102642 was identified and the BLAST results of the NCBI–GenBank database were only 92% identical.

Detection of 16S rRNA gene and sequence analysis

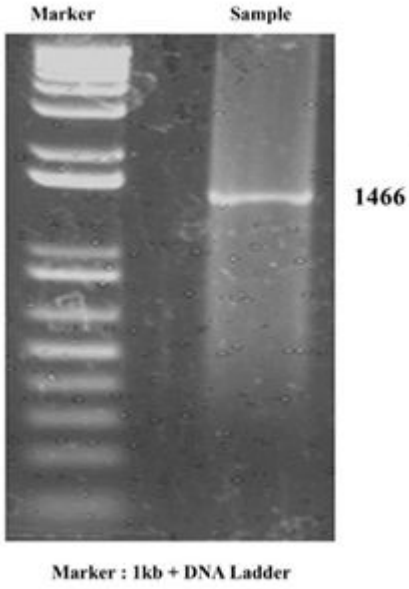

Figure 1: Amplification of 16S rRNA gene region of Streptococcus pneumoniae. The target amplification of 16S rRNA gene (size 1466 bp). M: 2000 bp marker of DNA.

PHYLOGENETIC TREE ANALYSIS

Phylogenetic tree analysis was done by using 16S rRNA gene fro m Streptococcus_pneumoniae_strain _NBRC102642 that is closed to Streptococcus pneumoniae__strain _ATCC33400 and far from Streptococcus_ mitis _ATCC 49456 and Streptococcus_ mitis _ATCCNS51

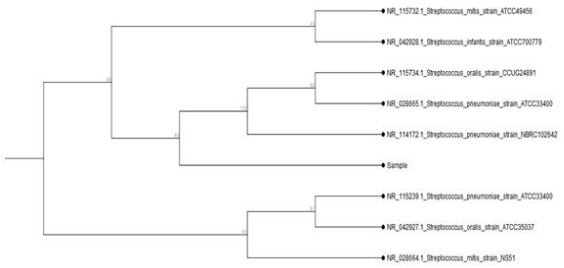

Figure 2: Phylogenetic tree analysis of the sequenced 16S rRNA gene of Streptococcus_pneumoniae_strain _NBRC102642, accession number (NR_1144172.1).

DISCUSSION

Every year in developing countries, some 4.5 million persons, most of them under 5 years of age, die of acute respiratory infections (Berman 1991). This study was conducted in January-November 2018 at different hospitals of the Dinajpur district of Bangladesh. Out of 40 pneumonia patients, positive cases of pneumonia were found to be 37.5% & 15 isolates were isolated. The Frequency of pneumonia in pneumonia patients in relation to age of 3-5yrs (50%), 6-8yrs (33.33%), 9-11yrs (25%), 12-15 (20%). Distribution of organism based on age difference Streptococcus pneumoniae of 3-5 years (7%), 6-8 years (20%), 9-11 years (6.67%), and 12-15 years (30%). The present study reveals that the study area had no significant ($P > 0.05$) effect but age ($P < 0.05$) & socio-economic status ($P < 0.05$) had significant effects on the prevalence of pneumonia in pneumonia patients. In the study area, the highest prevalence was found in M Abdur Rahim Medical College Hospital (40%), followed by Aurobindo Shishu Hospital (33.33%) & Islami Bank Community Hospital (40%) respectively. Among the age group, the prevalence of pneumonia was highest (50%) in 3-5 years age group, then 33.33% in 12-15 years age group, 25% in 6-8 years age group & 20% in 9-11 years age group. The highest prevalence of pneumonia was found in poor socio-economic status (54.54%), followed by medium (16.66%) & rich (16.66%) socio-economic conditions respectively. An antibiotic sensitivity test was carried out using the disc diffusion technique on Mueller Hinton agar for all the bacterial isolates to the most common antibiotic agents. The antibiotic study revealed that all of the isolates of Streptococcus pneumoniae were sensitive to Neomycin, Kanamycin & Streptomycin followed by Erythromycin, Azithromycin & Bacitracin. The isolates were found resistant to Penicillin, Amoxicillin, Ampicillin, Methicillin, Cloxacillin, Clindamycin, Ciprofloxacin, Vancomycin, Amikacin & Co-trimoxazole followed by Cefradin, Cefixime, Cefaclor, Cephalexin & Cefepime which is related to Huang et al., 2015, Staceviciene, et al., 2016. In Rio de Janeiro, Barroso et al., (2012) isolated, from patients with pneumonia, penicillin-resistant S. pneumoniae (clone Spain9V-ST156) linked to serotype. For the other non-meningeal pneumococcal infections, our research detected 3% of strains with intermediate resistance and 0.1% with full resistance to penicillin. According to Negrini (2010), in the municipality of Ribeirão Preto, children younger than 5 years presented 3.5% of S. pneumoniae with intermediate resistance, and none presented full resistance to penicillin. In 2012, in Brazil, full resistance was not observed to penicillin either, and intermediate resistance was 7.5% and 3.9% for children younger and older than 5 years, respectively. S. pneumonia was highly resistant to Clindamycin, Co-trimoxazole which result was similar to Khan et al., 2017 and some author showed that azithromycin and erythromycin are commonly prescribed for resistant antibiotics (Thummeepak, et al., 2015, Sabrina et al., 2012). Where our research found azithromycin and erythromycin are highly sensitive. (Rapee et al., 2015) found in their study all isolates were sensitive to ceftriaxone but we found resistant. In Argentina, for children younger than 5 years, 100% of the strains were susceptible to penicillin. In this study, among the pneumococci isolated from patients with pneumonia, just 0.1% were resistant to penicillin and 3% of the isolates from non-meningeal infections presented intermediate resistance to penicillin., In our study, we identified Streptococcus_pneumoniae_strain _NBRC102642 that is closed to Streptococcus pneumoniae__strain _ATCC33400 and far from Streptococcus_ mitis _ATCC 49456 and Streptococcus_ mitis _ATCCNS51. The results of our research study showed that the currently isolated bacterial strain was multi-drug resistant antibiotics, and our research suggests streptomycin, erythromycinand neomycin for the treatment of pneumonia.

CONCLUSION

Streptococcus pneumoniae is a leading cause of bacterial pneumonia in children worldwide. In the present study, it was observed that pneumonia was most commonly found in the 3-5 years age group & in poor socio-economic conditions. The use of antibiotics without the recommendation of registered physicians is one of the most important causes of this proble

REFERENCES

1. Barroso, D.E., Godoy, D. and Castiñeiras, T.M. (2012). β-lactam resistance, serotype distribution, and genotypes of meningitis-causing Streptococcus pneumoniae, Rio de Janeiro, Brazil.The Pediatric Infectious Disease Journal 31(1), 30-36.
2. Berezin, E.N., Carvalho, E.S., Casagrande, S., Brandileone, M.C., Mimica, I.M. and Farhat, C.K. (1996). Streptococcus pneumoniae penicillin-nonsusceptible strains in invasive infections in Sao Paulo, Brazil. The Pediatric Infectious Disease Journal 15(11), 1051-1053.
3. Berman, S. (1991). Epidemiology of acute respiratory infections in children of developing countries. Reviews Infectious Diseases 13(6), 454–462.
4. Bryce, J., Boshi-Pinto, C., Shibuya, K. and Black, R.E. (2005). WHO Child Health Epidemiology Reference Group. WHO Child Health Epidemiology Reference Group 365, 1147-1152.
5. Castaneda, E., Agudelo, C.I., Regueira, M., Corso, A., Brandileone, M.C. and Brandao, A.P. (2009). Laboratory-based surveillance of Streptococcus pneumoniae invasive disease in children in 10 Latin American countries: a SIREVA II project, 2000-2005. The Pediatric Infectious Disease Journal 28(9), 265-270.
6. Cheesbrough, M. (1984). Medical laboratory manual for tropical countries. English Language Book Society, London 35(2), 40-57.
7. Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) 2013: Performance standar for antimicrobial susceptibility testing. 17th Informational Supplement document M100-S17: 1. Wayne, Pennsylvania 32-50.

8. Collignon, P.J. and Bell, J.M. (1996). Drug-resistant Streptococcus pneumoniae: the beginning of the end for many antibiotics?. The Medical Journal of Australia 164, 64–67.
9. Devine, V.T., Cleary, D.W., Jefferies, J.M., Anderson, R., Morris, D.E., Tuck, A.C., Gladstone, R.A., O'doherty, G., Kuruparan, P., Bentley, S.D., Faust, S.N. & Clarke, S.C. (2017). The rise and fall of pneumococcal serotypes carried in the PCV era. Vaccine 35, 1293-1298.
10. Huang, S., Liu, X., Lao, W., Zeng,S., Liang, H., Zhong, R., Dai1, X., Wu, X., Hongyu Li, H. and Yao, Y. (2015). Serotype distribution and antibiotic resistance of Streptococcus pneumoniae isolates collected at a Chinese hospital from 2011 to 2013. BMC infectious disease. DOI 10.1186/s12879-015-1042-5.
11. Ishida, T., Maniwa, K., Kagioka, H., Hirabayashi, M., Onaru, K., Tomioka, H., HAYASHI, M.,TOMII, K., GOHMA, I.and HIRAI, T.(2008). Antimicrobial susceptibilities of Streptococcus pneumoniae isolated from adult patients with community-acquired pneumonia in Japan. Respirology 13(2), 240– 246.
12. Johnson, B.I. (1951). Paradoxical Embolism. Journal of Clinical Pathology 4(3), 316-332.
13. Khan, F.Z., Baig, S., Zameer, S. and Sharafat, S. (2017). Prevalence and antibiotic resistance pattern of Streptococcus pneumoniae, isolated from invasive and non-invasive infections in a tertiary care hospital of karachi. International Journal of Advanced Research 5(4), 825-831.
14. Lalitha, M.K., Pai, R.and Manoharan, A. (2002). Multidrug-resistant Streptococcus pneumoniae from India. The Lancet 359, 445.
15. Mulholland, K. (1999). Strategies for the control of pneumococcal diseases. Vaccine 17, 79–84.
16. O' Brien, K.L., Wolfson, L.J., Watt, J.P., Henkle, E., Deloriaknoll, M., Mccall, N., Lee, E., Mulholland, K., Levine, O.S. and Cherian, T. (2009. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. The Lancet 374, 893-902.
17. Rudan, I., Tomaskovic, L., Boschi-Pinto, C. and Campbell, H. (2004). On behalf of WHO Child Health Epidemiology Reference Group. Global estimate of the incidence of clinical pneumonia among children under five years of age. Bull World Health Organization 82, 895-903.
18. Staceviciene, I., Petraitiene, S., Vaiciuniene, D., Alasevicius, T., Kirsliene, J. and Usonis, V. (2016). Antibiotic resistance of Streptococcus pneumoniae, isolated from nasopharynx of preschool children with acute respiratory tract infection in Lithuania. BMC infectious disease. DOI 10.1186/s12879-016-1544-9.
19. Thummeepak, R., Leerach, N., Kunthalert, D., Tangchaisuriya, U., Thanwisai, A. and Sitthisak, S. (2015). High Prevalance of multi-drug resistant Streptococcus pneumoniae among healthy children in Thailand. Journal of Infection and Public Health 8(3), 274–281.
20. Ubukata, K., Chiba, N., Hanada, S., Morozumi, M., Wajima, T., Shouji, M. and Iwata, S. (2015). Serotype Changes and Drug Resistance in Invasive Pneumococcal Diseases in Adults after Vaccinations in Children, Japan, 2010-2013. Emerging Infectious Disease 21, 1956-1965.