International Journal of Medical and Health Research
ISSN: 2454-9142, Impact Factor: RJIF 5.54
www.medicalsciencejournal.com
Volume 3; Issue 8; August 2017; Page No. 103-107

Prevalence of *Streptococcus pneumoniae* in Egyptian children

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Abstract
Streptococcus pneumoniae is the leading bacterial cause of paediatric community acquired pneumonia. The life threatening illnesses caused by this organism have been declined significantly after the use of pneumococcal conjugate vaccines. Our herein study was conducted to determine the prevalence of *S. pneumoniae* isolated from one hundred mechanically ventilated children with severe community-acquired pneumonia in the intensive care unit of Assiut University Paediatric Hospital. Endotracheal aspirates were taken from the cases and were subjected for culture. Discrimination of thirty six *S. pneumoniae* from closely related Streptococcus species was developed by various phenotypic tests. Our findings strongly recommend the incorporation of vaccine in national immunization schedule which would effectively reduce the incidence of pneumoniae in Egypt.

Keywords: *Streptococcus pneumoniae*, pneumonia, optochin sensitivity, bile solubility, vaccines

1. Introduction
S. pneumoniae, a capsulated gram positive diplococci, is a member of normal flora of upper respiratory tract and can cause life-threatening illnesses. This pathogen is responsible for meningitis, sepsis, pneumonia and otitis media especially in children. The polysaccharide capsule is the main virulence factor and protects *S. pneumoniae* against phagocytosis, and helping attachment of bacterium to the upper airways [1].
S. pneumoniae, is the most frequently pathogen causing community-acquired pneumonia (CAP) among children. In addition, CAP is an important type of pneumonia and it is a major public health problem and a principal cause of morbidity and mortality in children under 5 years old [2]. World Health Organization (WHO) estimates 156 million new cases of childhood pneumonia occur annually worldwide, and the majority of these cases occurring in developing countries [3]. By 2015 pneumonia ranked the first cause of neonatal deaths (13%) [4]. The mortality rate of young children under five years in most developing countries ranges from 60 to 100 per 1000 live births, 21% of these deaths are due to pneumonia [5]. Globally, about half million children die from pneumonia yearly. Half the world's deaths due to pneumonia in children under the age of five years occur in Africa [6].
Various vaccines composed of common invasive serotypes were developed for prevention of pneumococcal diseases [1]. Namely, PCV-7, PCV-10 and PCV-13 are available globally and licensed in Egypt but, haven't involved in national immunization schedule yet.

2. Materials and Methods
2.1 Study population
A total of 100 consecutive children with CAP admitted to ICU of Assiut University Paediatric Hospital were studied prospectively from February 2014 to February 2017. Eligibility criteria included age between 1 month and 3 years, preceding subjective fever or documented temperature of 37.8°C, compatible clinical signs (cough, sputum, tachypnea, chest retractions or abnormal auscultation findings), and radiographic abnormalities consistent with LRI. Children were excluded if they had proven a diagnosis that invoked a presumptive viral etiology. None of the children had previously received the pneumococcal conjugate or polysaccharide vaccines [7].

2.2 Data Collection
The following information was recorded prospectively on ICU admission: age; sex; cause of ICU admission; location prior to ICU admission; comorbidities; diagnosis; route of feeding and duration of mechanical ventilation [8].

2.3 Sample collection
Endotracheal aspiration was collected under aseptic conditions from each patient in a mucus extractor sterile tubes. The suction tube was blindly introduced through the intubation and was wedged into the tracheobronchial tree before suction [8]. The collected samples were transported to infection control laboratory at Assiut university hospital, as soon as possible, to avoid autolysis of micro-organism [9].

2.4 Isolation of *S. Pneumoniae* from endotracheal aspirate samples
Samples were cultured for 24 hours at 37 °C in a humidified atmosphere supplemented with 5% CO_2 on Columbia blood agar plates (CBA), supplied with 10% defibrinated sheep blood. Selectivity of media was verified by adding 5 µg/mL gentamicin. Each sample was inoculated on blood agar plate, examined by Gram stain, and then identified by the conventional laboratory procedures including different biochemical tests. Bacteria were stored on Skim milk, tryptone, glucose, and 10% glycerol (STGG) preservative media at -80 °C for further experiments.

2.4.1 Biochemical reactions
Catalase test [10], Inulin fermentation [11] and Bacitracin test [12] were carried as in previous studies to differentiate of *S. pneumoniae* from other gram positive organisms.

2.4.2 Phenotypic pneumococcal identification
2.4.2.1 Optochin sensitivity test
Optochin, a paper disc containing 5 µg of the Optochin drug, (ethylhydrocupreine hydrochloride) (oxoid, UK) was used. A zone of growth inhibition around the disk (14 mm or greater) indicates Optochin sensitivity [13].

2.4.2.2 Bile solubility test
Bile solubility was determined by growing the pneumococcal culture in tryptic soy broth (TSB) (Oxoid, UK) for 2 hours, under 5% CO_2 supplied air and the cell density was adjusted by diluting bacterial culture in saline to obtain turbidity equivalent to that of the 0.5 McFarland opacity standard (10^8 CFU/mL). The culture was divided into two tubes, tube a (test) and tube B (control). A 0.5 ml of 2% solution of sodium deoxycholate (Bioshop, Canada) was added to the test tube A, and 0.5 ml saline was added to the control tube B. Both tubes were incubated for 30 min at 37° C. A decrease in turbidity and increase in viscosity in tube A indicated that the strain was bile soluble [14].

2.4.2.3 Bacitracin sensitivity test
Bacitracin, a paper disc containing 0.04 Units of the drug, Bacitracin Disks (Oxoid, UK) are used in the presumptive identification of group A β-hemolytic streptococci and allow for their differentiation from other β-hemolytic streptococci. A zone of inhibition greater than or equal to 14 mm indicates susceptibility to bacitracin and is presumptive of group a streptococci [12].

3. Results
3.1 Isolation of *S. pneumoniae*
Of 100 clinical samples, *S. pneumoniae* isolated from 36 samples.

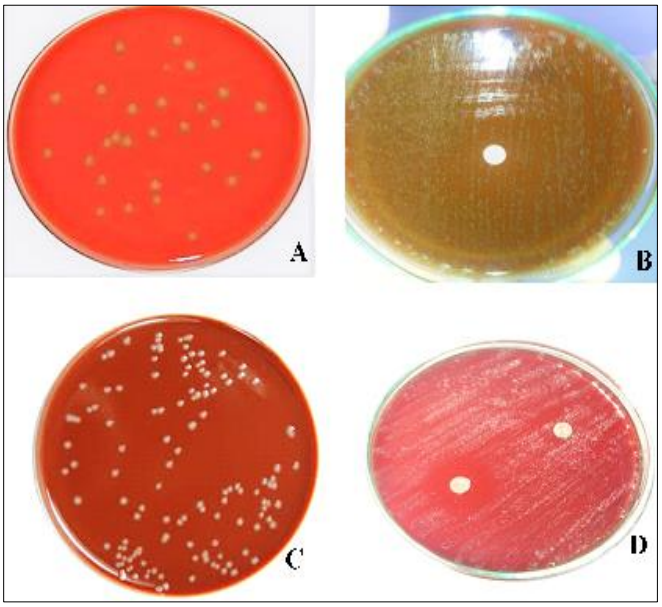

Fig 1: A-D different colonies shapes due to different serotype isolates

3.2 Phenotypic tests results
3.2.1 Optochin sensitivity
Isolates were cultured and colonies identified as *S. pneumoniae* appeared on Columbia blood agar plate as small, grey, shiny or mucoid almost with a zone of alpha-hemolysis. The percentage of patients with positive *S. pneumoniae* is 36%. Out of 36 *S. pneumoniae* isolates, only10 isolates (27.8%) were found to be optochin sensitive to the optochin disks with inhibition zones (> 14 mm) (Figure 2A). While, 26 samples were found to be optochin resistant (Figure 2B).

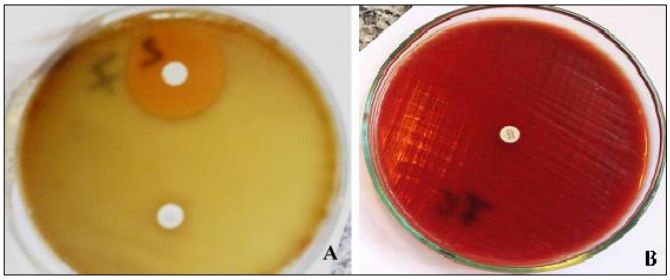

Fig 2: Optochin sensitive (A) and optochin resistant (B) strains of *S. pneumoniae*.

3.3.2 Bile solubility
Out of thirty six samples, thirty two (88.9%) are bile-soluble.

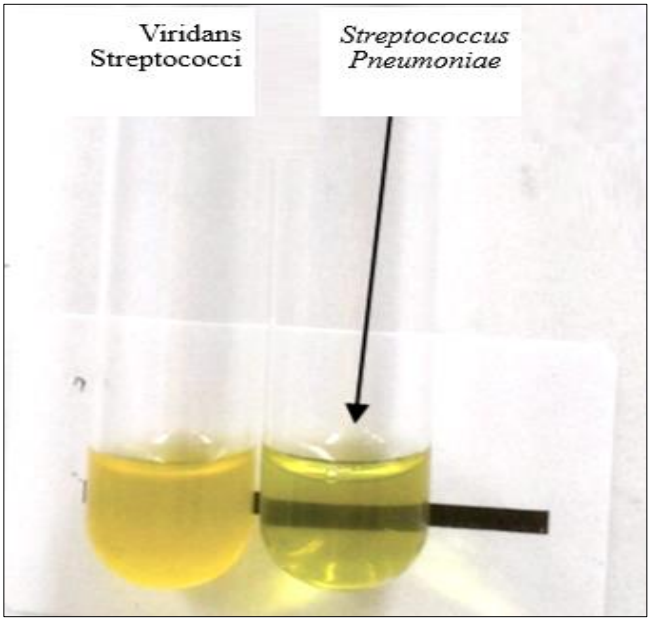

Fig 3: Bile solubility test for *S. pneumoniae.*

3.3 Gram stain

Fig 4: Gram stain for colonies on CBA showing *S. pneumoniae* cells as Gram positive, lanceolate, diplococci and short chains.

3.4 Patients with *S. pneumoniae* infection

Of 100 children within the age range 1 month – 3 years were admitted to ICU of Assiut University Pediatric Hospital, each of them diagnosed as CAP case, *Streptococcus pneumoniae* was isolated from 36 patient. 56% (20/36) of those positive cases were males and 44% (16/36) were females (Figure 5).

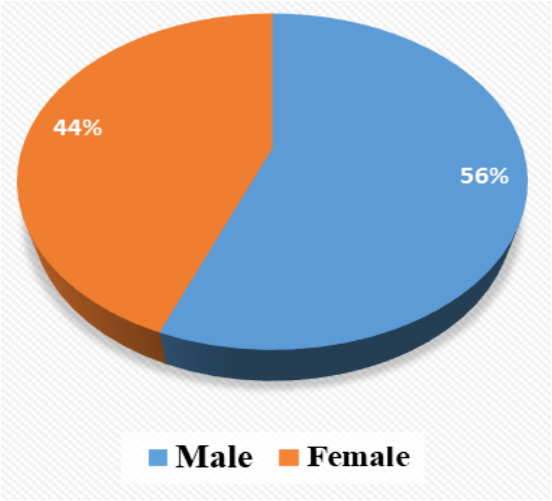

Fig 5: Distribution of *S. pneumoniae* isolates in pneumonic children according to sex.

Table1: Distribution of *S. pneumoniae* isolates in pneumonic children according to age

Age	Children with CAP	*S. pneumoniae* infections	% of *S. pneumoniae* infections
< 2 months	14	7	50%
2–12 months	73	23	31.5%
< 3years	13	6	46.15%
Total	100	36	36%

In each age group, it is obvious that the highest percentage [50% (7/14)] was among infants from 30 days to 53 days age. The following percentage [46.1% (6/13)] was for those from 1 to 3 years, while the lowest percentage [31.5% (23/73)] was among infants with 2 to 12 months age.

Of the 36 patients with CAP caused by *S. pneumoniae* in this study, [25/36 (69.5%)] were died in hospital while, [31/64 (48%)] of non–pneumococcal children were died.

Table 2: Prognosis of pneumonic patients in ICU

Group	No of non-pneumococcal children	No of pneumococcal children	Odds (95%CI)	P-value
Improved	33	11	1	
Died	31	25	2.4 (1.02–5.7)	0.045*

Table 3: Clinical characteristics of CAP caused by *S. pneumoniae*

Characteristic Findings at admission to ICU	*S. pneumoniae* infection	Positive cases	95% CI	P- value
Fever ≥ 38°C	36	33 (91.6%)	83.3–100	<0.001
Infiltration in chest	36	30 (83.3%)	69.4–94.4	<0.001
Cough	36	34 (94.4%)	86.1–100	<0.001
Sputum	36	32(88.8%)	77.8–97.2	<0.001

Children whom were positive for *S. pneumoniae* as causative pathogen of CAP had highly significant incidence of fever, infiltration in chest, cough and sputum (P <0.001). High 95% confidence intervals and P values indicate how much sever is the pneumonia caused by *S. pneumonia.*

4. Discussion

The CAP caused by the bacterium *S. pneumoniae* is one of the most lethal public health problem in developing countries including Egypt. It is widely reported that carriage of *S. pneumoniae* is a precursor for developing any invasive pneumococcal disease (IPD) and children represent its main reservoir with colonization rates of up to 50% [15]. The reduction in pneumococcal disease from vaccine serotypes (VT) following widespread implementation of the pneumococcal conjugate vaccine (PCV) is believed to be through the direct immunogenic protective effect of immunized individuals as well as indirectly through herd immunity; diminishing the incidence of disease in non–immunised individuals [16]. In Egypt, although PCVs have been licensed, they have not been included in the national immunization programmes (NIP). So that, the vaccine is only available privately and the majority of the population is not able to afford it [17]. Here, clinical isolates were collected from endotracheal aspirations of children at ICU of Assiut Paediatric Hospital with CAP. By isolation the *S. pneumoniae* on CBA selective media it was gave different shapes of colonies due to different serotypes prevalent in ICU of paediatric hospital. These colonies varied from large mucoid to small glossy colonies, almost with α haemolysis in 5% CO_2, but sometimes

it gave no haemolysis in ambient anaerobic air. We found that (72%) (26/36) of *S. pneumoniae* strains are optochin resistant. Interestingly, it was found that sensitivity test failed for all samples after freezing (100% optochin resistant), and this is consistent with previous study [18].

Bile solubility test has been shown to have a high level of accuracy and is frequently used for the identification of *S. pneumoniae* [19]. In this work, bile solubility by tube method was relatively sensitive (88.9%). Out of 36 *S. pneumoniae* positive samples, there were only four isolates had been bile insoluble. Moreover, only seven typical samples gave positive results for both tests simultaneously and all other 29 pneumococcal samples had non–typical optochin sensitivity and/or bile solubility. Our results consistent with previous studies which were reported that optochin resistant and/or bile insoluble *S. pneumoniae* strains are being isolated more frequently, and the bile solubility is still considered the gold standard phenotypic test [19, 20].

Males are more likely to develop lower respiratory tract infections by *S. pneumoniae* (figure 5). The greater resistance found in females can be explained by their enhanced Th1 immune response [2].

It was observed that the infection by *S. pneumoniae* was significantly high among extreme young children. As 50% of children less than 2 months with CAP were acquired *S. pneumoniae* (table 1).

Of the 36 patients with CAP caused by *S. pneumoniae* in this study, [25/36 (69.5%)] were died in hospital while, [31/64 (48%)] of non–pneumococcal children were died. Increased percentage of mortality between pneumococcal children ensure that *S. pneumoniae* causing severe CAP as invasive pneumococcal disease (IPD) (table 2).

From clinical aspect view, children with positive *S. pneumoniae* have more highly significant fever, sputum, dyspnoea and infiltration in chest than those negative for Pneumococcal pneumonia (table 3).

5. Conclusion

S. pneumoniae causing 36% of sever CAP between children less than five years old in Egypt. And we are in urgent need to incorporate pneumococcal conjugated vaccines (PCVs) in national immunization program to reduce the incidence of pneumonia between Egyptian children.

6. References

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