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Antibiotic susceptibility and capsular genes of *Streptococcus pneumoniae* colonizing children with chronic respiratory diseases

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ABSTRACT

Background: *Streptococcus pneumoniae (S. pneumoniae)* is a main pathogen causing acute infectious exacerbations in chronic lung diseases (CLD) children. Determining the local antibiotic susceptibility pattern of colonizing strains in these patients is crucial for empirical therapy. Moreover, identifying prevalent types is important to evaluate the effectiveness of available vaccines. This study aimed to detect antibiotic susceptibility and searching for some capsular gene types among *S. pneumoniae* isolates colonizing respiratory tract in CLD children. **Methods:** Bronchoalveolar lavage (BAL) samples were collected from 51 CLD children undergoing bronchoscopy in Ain Shams University Pediatric Hospital. All identified *S. pneumoniae* isolates were tested for antibiotic susceptibility. Two-steps sequential multiplex PCR technique was performed to detect capsular gene types: [3/ 22f/(22A) / 19A / 6A/6B /4 / 14/ 12F/(12A) / 9V/(9A). **Results:** From 51 BAL samples, 32 (62.75%) pneumococcal strains were isolated. Most of the isolates had capsular gene type 6A/6B (65.6%). Capsular gene type 14 was detected in 25% of isolates. In 9.4% of strains, capsular type could not be identified. All isolates were sensitive to vancomycin. The lowest resistance rate was to levofloxacin (6.3%) and linezolid (9.4%), while the highest rates were to clindamycin (71.9%) and erythromycin (68.8%). **Conclusion:** *Streptococcus pneumoniae* colonizing CLD children showed high resistance to clindamycin and erythromycin thus highlighting the importance of antimicrobial stewardship programs in all levels of healthcare. Capsular gene type 6A/6B was the commonest colonizing type suggesting that CLD children can benefit from the currently available PCV13 vaccine.

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is considered as one of the major infectious causes of morbidity and mortality around the world, with an estimated 1.6 million deaths annually. Most of the recorded mortalities are in children under 5 years old [1,2]. *Streptococcus pneumoniae* is considered as one of the commonest causes of infective exacerbations in patients with chronic lung diseases [3].

In children, pneumonia caused by *S. pneumoniae* is considered the most common pneumococcal disease worldwide. Otitis media caused by *S. pneumoniae* usually is more severe than other bacterial causes and it is considered a

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leading cause of bacterial meningitis in infants and children [2,4].

Respiratory tract colonization is an essential step in *S. pneumoniae* pathogenesis which is followed by either spread and transmission to another host or progression into invasive disease [5].

Results of several studies contradicted the old known concept of lower respiratory tract sterility, with revealed core pulmonary microbiota including, *Streptococcus, Fusobacterium, Porphyromonas, Haemophilus, Prevotella,* and *Veillonella* [6,7].

Pneumococcal colonization plays important roles in chronic lung diseases; it increases the risk of recurrent wheezes and asthma early in life and is also linked to acute infective exacerbation in patients with asthma [6], interstitial lung diseases [8], and chronic obstructive pulmonary disease (COPD) [3]. Moreover, *S. pneumoniae* is linked to the immunopathogenesis of bronchiectasis [9].

As for many pathogens, *S. pneumoniae* expresses rising resistance to previously effective antimicrobials, therefore determining the local pattern of susceptibility of *S. pneumoniae* colonizing the respiratory tract can guide empirical therapy during acute exacerbation in patients with chronic lung diseases [10].

The polysaccharide capsule is the prominent virulence factor of *S. pneumoniae*. It helps in establishing infection and the evasion of the immune response. Over than 97 capsular types are recognized, and association exists between some capsular types and the resultant disease type e.g., serotypes 1 and 14 are responsible for most cases of pneumococcal pneumonia in children while, higher mortality rates are noticed with serotypes 6A, 19F,6B, 9N, and 3 [11].

Production of the capsule is controlled by capsular polysaccharide synthesis genes located at the cps locus, flanked by conserved genes. The first five genes are conserved in almost all serotypes, while the central parts of the loci contain the serotype-specific genes that serve as the basis for differentiation of pneumococci by PCR-based approaches [12].

Pai and his colleagues reported that nearly 15 strains are responsible for most cases of invasive pneumococcal diseases [13]. Several protein conjugate vaccines (PCV) were developed to cover these serotypes. PCV7 covers a limited number of invasive serotypes . PCV10 and PVC13 were developed to cover a wider range of strains.

Pneumococcal vaccines have not been introduced among compulsory vaccination program of infants and children in Egypt, but their use is increasing recently. The change of circulating serotypes upon introduction of pneumococcal vaccine warrant the necessity of frequent surveillance of the prevalent strains and their potential coverage by the available vaccines [14,15]. There is a limited numbers of studies monitored the prevalent *S. pneumoniae* capsular types in Egypt, and none investigated strain types as well as the antibiotic susceptibility of *S. pneumoniae* colonizing chronic lung disease (CLD) pediatric patients.

This study aimed to detect antibiotic susceptibility patterns and searching for some capsular gene types known to be associated with invasive diseases among *S. pneumoniae* isolates colonizing respiratory tract in Egyptian children with chronic lung diseases. This might help to guide the empirical treatment of *S. pneumoniae* infections among this high-risk group and allow the recommendation of a suitable vaccine for prevention of infection by invasive pneumococcal strains.

Material and Methods

This cross-sectional study was conducted during the period from September 2019 till February 2020. The fifty-one included children (≥2 years old) had chronic lung diseases including cystic fibrosis bronchiectasis, non-cystic fibrosis bronchiectasis, and interstitial lung disease, were attending chest clinic at Ain Shams University Pediatric Hospital for clinical evaluation and follow-up. They have undergone bronchoscopy for different clinical indications. Children with suspected or confirmed acute exacerbation of infection and/ or receiving antimicrobials in the preceding 30 days were excluded. None of the patients enrolled in the study has received pneumococcal vaccine.

The study was approved by the ethical committee of Ain Shams Faculty of Medicine. Informed consents were obtained from the parents of children. All demographic and other relevant data were recorded.

Sample collection and processing

Bronchoalveolar Lavage samples were collected from all patients and transferred immediately to microbiology laboratory of the Medical Microbiology and Immunology Department at Ain

Shams Faculty of Medicine for further processing. The samples were inoculated into Columbia agar medium (Oxoid, England) supplemented with 5% horse blood, incubated overnight at 37ºC in CO_2 enriched environment using candle jar method. Suspected colonies were identified through colony morphology, microscopic examination of Gram-stained films, a negative result to catalase reaction, and susceptibility to Optochin [16,17].

Antimicrobial susceptibility testing of identified *S. pneumoniae* isolates

The antimicrobial susceptibility of identified *S. pneumoniae* isolates was determined using disk diffusion method according to CLSI 2019. The following antibiotics were tested: Oxacillin (1µg), Vancomycin (30µg), Erythromycin (15µg), Levofloxacin (5µg), Clindamycin (2µg), Rifampicin (5µg), Chloramphenicol (30µg), Tetracycline (30µg) and Linezolid (30 µg) (Bioanalyze, Turkey). Results were interpreted and reported according to inhibition zone diameters of clinical and laboratory standards institute 2019 [18].

Determination of capsular gene types of *S. pneumoniae* isolates

Multiplex PCR based technique was done for detection of some pneumococcal capsular gene types [3/ 22f/(22A) / 19A / 6A/6B /4 / 14/ 12F/(12A) / 9V/(9A)], in two sequential reactions [13]. DNA extraction was performed using DNA extraction kit (Qiagen, USA) according to the manufacturer's instructions. Primers used in Multiplex 1 reaction were (3/ 22F/ 19A / 6A/6B /*cpsA*) and the primers used in Multiplex 2 reaction were (4 / 14/ 12F/ 9V/ *cps A*). The *cpsA* primer pair targeting the conserved *cpsA* gene locus common for all *S. pneumoniae* isolates was served as an internal control in both reactions. **Table 1** includes the sequences of all used primers (Oligo, Korea) used in the study.

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by amplification 30 cycles of 94º C for 45s, 54º C for 45s, and 65º C for 2min and 30s. The reactions were finally stored at 72º C for 10 min.

The PCR products were analyzed by gel electrophoresis. The sizes of the PCR products were determined by comparison with the molecular size standard [DNA ladder (50bp) mix was used as standard DNA with molecular weights of 1500, 1200,1000,900, 800, 700,600, 500, 450, 400, 350, 300, 250,200, 150, 100 and 50 bp]. **Figure 1** summarizes the interpretation of multiplex PCR results.

Figure 1. Algorithm for multiplex PCR result interpretation.

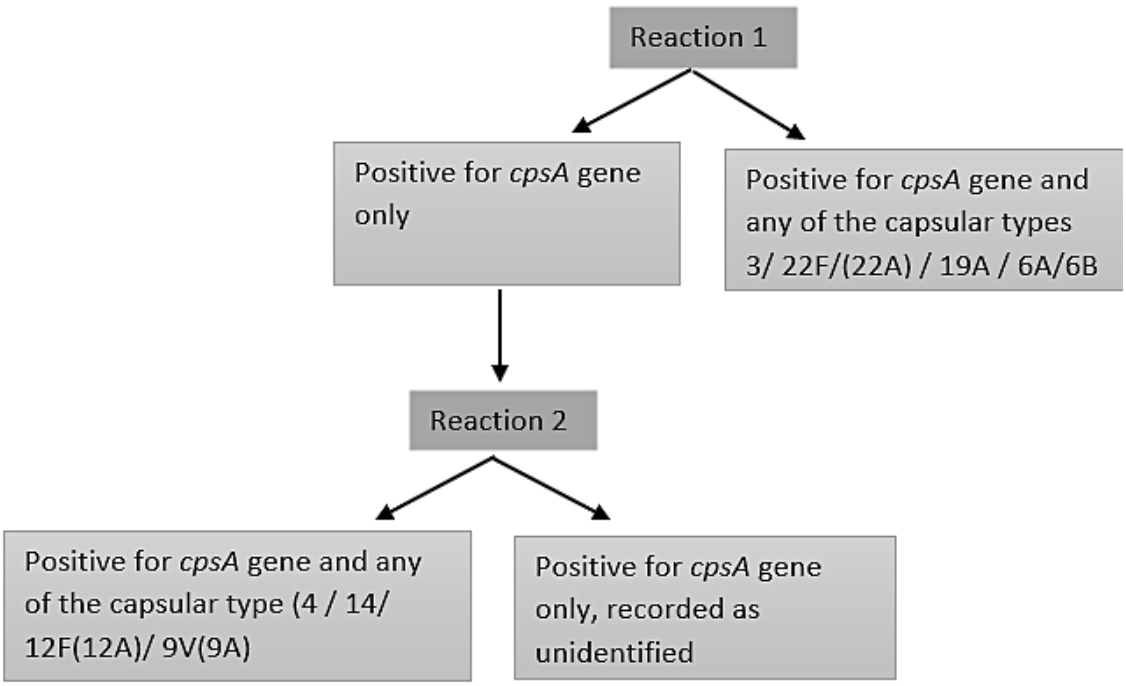


Table 1. List of the primers used in multiplex PCR and sizes of their products [13].

Name	Primer pair sequence (5'-3')		Product size (bp)
3	ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G	CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G	371
22F	GAG TAT AGC CAG ATT ATG GCA GTT TTA TTG TC	CTC CAG CAC TTG CGC TGG AAA CAA CAG ACA AC	643
19A	GTT AGT CCT GTT TTA GAT TTA TTT GGT GAT GT	GAG CAG TCA ATA AGA TGA GAC GAT AGT TAG	478
6A/6B	AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG	TTA GCG GAG ATA ATT TAA AAT GAT GAC TA	250
4	CTG TTA CTT GTT CTG GAC TCT CGA TAA TTG G	GCC CAC TCC TGT TAA AAT CCT ACC CGC ATT G	430
14	CTT GGC GCA GGT GTC AGA ATT CCC TCT AC	GCC AAA ATA CTG ACA AAG CTA GAA TAT AGC C	208
12F	GCA ACA AAC GGC GTG AAA GTA GTT G	CAA GAT GAA TAT CAC TAC CAA TAA CAA AAC	376
9V	CTT CGT TAG TTA AAA TTC TAA ATT TTT CTA AG	GTC CCA ATA CCA GTC CTT GCA ACA CAA G	753
CpsA	GCA GTA CAG CAG TTT GTT GGA CTG ACC	GAA TAT TTT CAT TAT CAG TCC CAG TC	160

Statistical analysis

The data collected were tabulated and analyzed by SPSS (statistical package for social science) version 25 (Armonk, NY: IBM Corp) on IBM compatible computer. The data were tested for normality using Kolmogorov–Smirnov, and Shapiro–Wilk tests. Continuous variables are expressed as mean and standard deviation (SD). Categorical variables are expressed as frequencies and percentages.

Student t-test was used for comparison between two groups having quantitative variables with normal distribution. Chi-square, Fisher exact tests were used to study comparison and association between two qualitative variables. *p*-value of < 0.05 was considered statistically significant for two-tailed tests.

Results

Fifty-one children, 30 (58.9%) females, and 21(41.1%) males were enrolled in the study. Their ages ranged between 3-12 years with a mean ±SD of 6.47±2.69. Twenty eight (54.9%) of the children were in preschool age (3-5years). Bronchiectasis was the pre-existing lung disease in most of the children enrolled in the study (42 out of 51; 82.3%) including cystic fibrosis bronchiectasis in 22 out of 51 (43.1%), non-cystic fibrosis bronchiectasis in 20 (39.2%). Interstitial lung diseases were the underlying disease in 9 out of the 51 patients (17.7%).

Thirty-two (62.75%) pneumococcal strains were isolated. Accordingly, the included children were categorized into two groups: the colonized group including 32 out of 51 (62.75%), and the non-colonized group including 19 (37.25%). **Table 2** demonstrates the demographic characteristics and pre-existing lung disease of the patients in the two groups. No statistically significant difference was found between pneumococcal colonizers and non-colonizers as regards gender, mean age, or school status. **Table 2** shows that about two-thirds (28/42; 66.6%) of bronchiectasis children were colonized with *S. pneumoniae* and 4 (44.5%) out of the 9 patients with interstitial lung diseases were colonized. No statistically significant association was found between *S. pneumoniae* colonization and any of the pre-existing lung diseases.

Capsular gene type studying of the isolated strains using a two-steps multiplex PCR revealed that 21 (~66%) of isolates had the 6A/6B type and 8 (25%) of isolates had the 14 capsular gene type.

Three strains (~9%) could not be identified as any of the main eight capsular types searched by the used PCR technique. **Table 3** demonstrates demographic characteristics, and pre-existing respiratory conditions of the patients colonized with different capsular gene types. **Table 3** shows that 75 % of patients colonized with isolates containing gene for capsular type 14 had bronchiectasis as an underlying chronic lung disease and this represents a statistically significant relationship ($p < 0.05$).

As regards the antimicrobial susceptibility pattern of the isolates, no resistance was detected to vancomycin and the least resistance rates were to levofloxacin (6.3%) and linezolid (9.4%), while the highest resistance rates were to clindamycin (71.9%) and erythromycin (68.8%) as shown in **figure (2)**. None of the erythromycin-resistant isolates showed inducible clindamycin resistance.

Studying the distribution of antimicrobial susceptibility patterns in isolates with different capsular gene types, no statistically significant differences were detected as shown in **table (4)**. Most of the strains carrying genes of capsular type 6A/6B and 14 are sensitive to levofloxacin (90.5% and 87.5% respectively) and to linezolid (90.5% and 100% respectively). High rates of non-susceptibility to erythromycin and clindamycin were detected in the isolates of both capsular gene types.

Table 5 shows that almost 50% of isolates have multi non-susceptibility patterns to different antimicrobials (more than three antimicrobials). No statistically significant differences were detected in rates of combined non-susceptibility in relation to capsular gene types.

Table 2. Demographic characteristics and pre-existing lung diseases of the colonized and non-colonized patient groups.

Patients / **Characteristics**	**Colonized Group (No = 32)**	**Non-colonized Group (No = 19)**	**Total (No = 51)**	***p*- value**
Gender No. (%)				0.917
• Female	19 (59.4)	11 (57.9)	30 (58.9)	
• Male	13 (40.6)	8 (42.1)	21 (41.1)	
Age (years)				0.910
• Range	3 –12	3 –12	3 –12	
• Mean ± SD.	6.44±2.70	6.57±2.74	6.47±2.69	
School status No. (%)				0.741
• Pre-school	17 (53.1)	11 (57.9)	28 (54.9)	
• School	15 (46.9)	8 (42.1)	23 (41.1)	
Non-Cystic Fibrosis Bronchiectasis No. (%)	14 (43.8)	6 (31.6)	20 (39.2)	0.389
Cystic Fibrosis Bronchiectasis No. (%)	14 (43.8)	8 (42.1)	22 (43.1)	0.909
Interstitial Lung Disease No. (%)	4 (12.4)	5 (26.3)	9 (17.7)	0.211

Table 3. Demographic characteristics and pre-existing lung diseases among patients colonized with isolates with different capsular gene types .

Patient colonization type / **Characteristics**	6A/6B (No = 21)	14 (No = 8)	Other unidentified types (No=3)	*p*- value
	No. (%)	No. (%)	No. (%)	
Gender				
• Female	13 (61.9)	3 (37.5)	3 (100)	0.237
• Male	8 (38.1)	5 (62.5)	0 (0.0)	
School status				
• Pre-school	12 (57.1)	3 (37.5)	2 (66.7)	0.550
• School	9 (42.9)	5 (62.5)	1 (33.3)	
Non-Cystic Fibrosis Bronchiectasis	8 (38.1)	6 (75)	0 (0.0)	0.049
Cystic Fibrosis Bronchiectasis	10 (47.6)	2 (25)	2 (66.7)	0.475
Interstitial Lung Disease	3 (14.3)	0 (0.0)	1 (33.3)	0.234

Table 4. Distribution of individual antimicrobial susceptibility patterns of isolates in relation to different capsular gene types.

Isolate capsular gene type / **Antibiotic susceptibility**	6A/6B (No=21)	14 (No=8)	Other unidentified types (No=3)	*p*-value
	No. (%)	No. (%)	No. (%)	
Oxacillin				
S+	14 (66.7)	4 (50)	3(100)	0.329
NS++	7 (33.3)	4 (50)	0(0)	
Erythromycin				
S	3 (14.3)	2(25)	2(66.7)	0.116
NS	18 (85.7)	6 (75)	1(33.3)	
Clindamycin				
S	2(9.5)	2(25)	0(0)	0.538
NS	19(90.5)	6(75)	3(100)	
Tetracycline				
S	7(33.3)	2(25)	2(66.7)	0.539
NS	14(66.7)	6(75)	1(33.3)	
Rifampicin				
S	17 (81)	6 (62.5)	2(66.7)	0.461
NS	4 (19)	3 (37.5)	1 (33.3)	
Linezolid				
S	19(90.5)	8(100)	2(66.7)	0.393
NS	2(9.5)	0(0)	1(33.3)	
Chloramphenicol				
S	12(57.1)	3(37.5)	0(0)	0.198
NS	9(42.9)	5(62.5)	3(100)	
Levofloxacin				
S	19(90.5)	7(87.5)	3(100)	1
NS	2(9.5)	1(12.5)	0(0)	

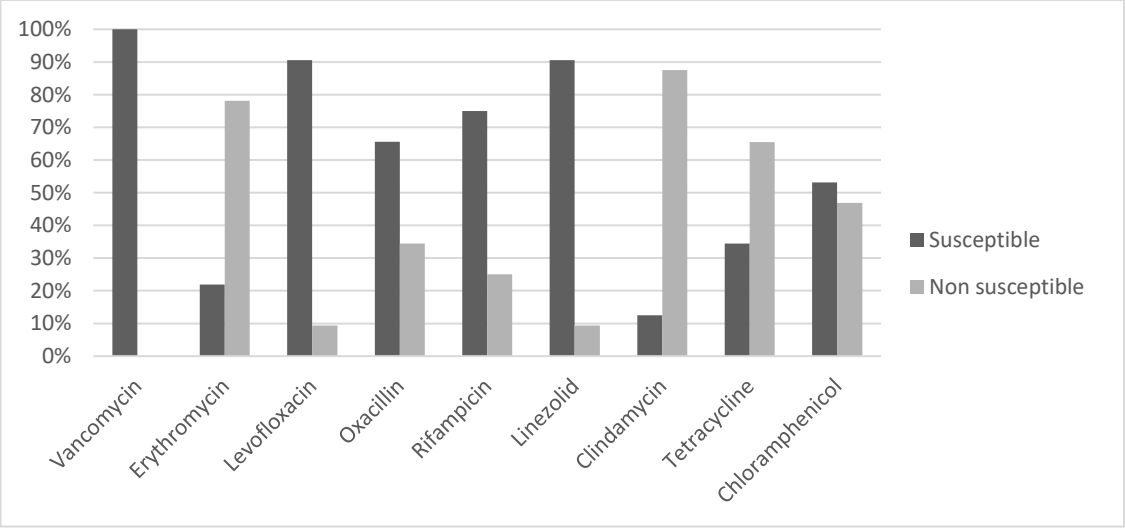
+S: susceptible ++NS: non-susceptible (intermediate and resistant)

Table 5. Distribution of combined antibiotic non-susceptibility patterns of isolates in relation to different capsular gene types.

Isolate capsular gene type / Combined antibiotic non susceptibility	6A/6B (No=21)	14 (No=8)	Other unidentified types (No=3)	*p*-value
	No. (%)	No. (%)	No. (%)	
Oxacillin and Erythromycin	7 (33.3)	4 (50)	0(0)	0.294
Oxacillin and Clindamycin	6 (28.6)	2 (25)	0(0)	0.565
Oxacillin, Erythromycin and Clindamycin	6 (28.6)	2(25)	0(0)	0.565
More than three antimicrobials	12(57.1)	4 (50)	1(33.3)	0.726

NS: non-susceptible (intermediate plus resistant)

Figure 2. Antimicrobial susceptibility of isolated strains.



Discussion

The results showed that 32 out of the 51 CLD pediatric patients enrolled in the study were colonized with *S. pneumoniae*. About 50% of the colonized patients were in the preschool age. Previous studies reported that colonization by *S. pneumoniae* occurs usually at a young age with a peak in children of preschool age [11,19,20].

All children enrolled in the study had chronic lung diseases and they have undergone bronchoscopy for different clinical indications as bronchial toilet and bronchoalveolar lavage (BAL) collection for early pseudomonal colonization detection [21]. Accordingly, BAL samples were collected and used for the detection of *S. pneumoniae* colonization. The results of some previous studies using molecular methods to characterize the microbiome of the lower airways accepted BAL as a candidate sample for detection of pneumococcal colonization [22]. They concluded that the lower airways of healthy subjects are not sterile, and the colonizing community is mainly made up of bacterial genera that are also common in the upper respiratory tract including *Streptococcus pneumoniae*. Several studies correlated the culture results of nasopharyngeal and BAL samples as regards the detection of *S. pneumoniae* [23]. The detection of multiple strains of *S. pneumoniae* in the lower respiratory tract of children with bronchiectasis suggested their accumulation due to recurrent aspiration and failure to clear previous colonizing strains [9]. This finding supports the importance of BAL in the detection of *S.*

pneumoniae colonization in this category of patients.

The results of this work showed that two-thirds of bronchiectasis children were colonized with *S. pneumoniae.* Several previous studies found that patients with chronic lung diseases as bronchiectasis are persistently colonized by *S. pneumoniae* [24]. **Pizzutto and his colleagues** in their review article stated that *S. pneumoniae* is the second most common pathogen associated with bronchiectasis in children after *Haemophilus influenzae* [9]. The difference in colonization rates for different bacterial organisms can be attributed to geographical distribution and local differences in vaccine coverage rates. Some studies found that higher incidence of antimicrobial intake especially macrolides and β-lactam antibiotics in children with chronic lung diseases resulted in decreased detection rate of *S. pneumoniae* carriage [23,25,26]. This may explain that about one-third of bronchiectasis children in our study were not colonized by *S. pneumoniae.* Colonization by other common bacteria may be another explanation of this finding as mentioned before.

As regards the antimicrobial susceptibility pattern of the isolates, no resistance was detected to vancomycin and the least resistance rates were to levofloxacin (6.3%) and linezolid (9.4%), while the highest resistance rates were to clindamycin (71.9%) and erythromycin (68.8%). The resistance rate to beta-lactam drugs (oxacillin) was 34% as it was detected in 11 out of the 32 isolates. Comparable results to the present study were obtained by previous Egyptian studies where susceptibility to penicillin, macrolides, clindamycin, tetracyclines, chloramphenicol, fluoroquinolones, and linezolid ranged between (15%-85%), (50%-78.9%), (64.5%-94.7%), (16.1%-68.4%), (75%-100%), (85%-100%), and (84%-100%) respectively [27–30].

Two metanalyses addressed susceptibility of pneumococci isolated from children below 16 years of age in Africa showed that, for most antibiotics, susceptibility towards tested antibiotics fluctuated among several studies but generally decreased gradually between 1995 and 2015. In northern Africa, penicillin resistance reached (66.7%), while recorded resistance to erythromycin was (22%). The overall resistance to chloramphenicol was (9.2%) and for tetracycline was (49 %) [31,32].

In the Middle East highest resistance to penicillin and erythromycin was detected in Jordan and Riyadh reaching up to (90%), (77%) respectively. While, highest resistance to tetracycline and clindamycin (77%), (46%) was reported in Lebanon and Palestine respectively [33].

The difference in resistance rates among isolates from different geographical regions reflected the difference in the consumption of antibiotics and the rate of selection pressure exerted by their overuse. This may explain the relative moderate resistance rate of the isolates towards beta-lactam drugs in contrast to macrolides and clindamycin in our study. This result may be attributed to the high selection pressure exerted by the overuse of macrolides and clindamycin in our community.

Another reasonable explanation of the difference in resistant patterns of colonizing strains of *S. pneumoniae* is the difference in the local prevalence of different serotypes.

In our study we utilized two sequential multiplex PCR method (as described earlier by Pai and his colleagues) for detection of serotypes 3/ 22F/ 19A / 6A/6B /4 / 14/ 12F/ and 9V. Primers for serotypes 9V,12F, 22F are not serotypes specific and can detect other serotypes e.g. (9A), (12A.44,46), (22A) respectively. Also, primers for serotype 6B can cross-react with the common serotype 6A cps operon sequence 13. Although serotyping of pneumococcal strains is considered the gold standard method, however, it is limited by the relatively very high cost [34]. PCR based typing is currently used and continuously developed for best outcomes [17].

In our study 21 of the 32 (65.6%) pneumococcal isolates were identified as having the genes for 6A/6B capsular types and 8 (25%) were identified as having the gene for the 14 capsular type. Three isolates (9.4%) could not be identified by the applied PCR technique suggesting that they carry genes of other capsular types different than the types targeted in our work. Previous studies found that serogroup 6 and serotype 14 are common causes of invasive pneumococcal disease in unvaccinated populations [35,36]. This may explain the high prevalence of these types in our study as the introduction of pneumococcal vaccine is not compulsory in Egypt. Other previous studies noted relative resistance of serotype 14 to antibiotics and this may also explain the identification of this type among isolates from the study group of chronic

respiratory patients with high exposure to antibiotics due to repeated episodes of exacerbating infections [37,38].

Our results support previous work results of **Badawy and his colleagues** who reported that serogroup 6 is considered one of the most common colonizers in asymptomatic children in Africa and the Middle East **[**28]. Our results also supported the results of some earlier Egyptian studies where they found that types 6A/6B were the most common isolated capsular type in Egyptian children [29,30]. In contrast, another study done in Egypt in 2020, found that serotype 1 predominates in clinical and nasopharyngeal samples of Egyptian children [27]. The latter study did not target high-risk patients as our patients and included clinical isolates from invasive pneumococcal infections and this may explain the predominance of a different serotype.

Studying the distribution of antimicrobial susceptibility patterns in isolates with different capsular gene types, no statistically significant differences were detected. Most of the strains carrying genes of capsular type 6A/6B and 14 are sensitive to levofloxacin (90.5% and 87.5% respectively) and to linezolid (90.5% and 100% respectively). High rates of non-susceptibility to erythromycin and clindamycin were detected in the isolates of both capsular gene types. Almost 50% of isolates have multi non-susceptibility patterns to different antimicrobials (more than three antimicrobials). These results highlighted the overuse of antibiotics in our community particularly among high-risk children with chronic lung diseases. No statistically significant differences were detected in rates of combined non-susceptibility in relation to capsular gene types.

Our results supported the recommendation of the use of PCV-13 vaccine as it includes serotypes 6A, 6B, and 14 as recommended by other studies [39]. (Serotype 6A is not included in PVC 7 or PVC 10 vaccines). According to current guidelines for vaccinating high-risk children including chronic lung disease patients, it is recommended to administer PPSV23 after completing administrating doses of PCV13 [40] in order to cover as much as possible of the different serotypes which can colonize this high-risk group of patients. Although low number of our patients (3 out of 32) had serotypes different than the targeted prevalent serotypes; yet we do recommend the use of PPSV23 in these high-risk patients.

One of the limitations faced in the present study is the small subject numbers that lead to limited statistical power in some subgroups. Also, we did not culture BAL samples quantitatively, due to discrepancies among previous studies in determining a clear cut-off to differentiate contamination from lower airway infection in CLD pediatric patients as the cut-off differs according to underlying disease [25]. The patients included in the study were stable with no signs of exacerbation increasing the probability of isolated *S. pneumoniae* being colonizers rather than pathogens. Another limitation is the very high cost for searching of genes of all capsular types among isolates. This testing necessitates the use of seven sequential multiplex reactions which was not applicable in our study.

Conclusion

Streptococcus pneumoniae colonizing chronic lung disease patients showed high resistance to clindamycin (71.9%) and erythromycin (68.8%), thus highlighting the importance of implementing antimicrobial stewardship programs in all levels of healthcare. Capsular gene type 6A/6B was the most common pneumococcal type colonizing children with chronic lung disease suggesting that these patients can benefit from the currently available PCV13 vaccine.

Future large-scale studies are needed to keep ongoing surveillance of pneumococcal serotypes and their susceptibility pattern in carriage as well as in invasive diseases in children particularly those with chronic lung diseases.

Declaration of interest and funding information

No funding was received. The authors report no conflicts of interest.

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