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# *Streptococcus pseudopneumoniae* as an emerging pathogen from patients with respiratory diseases

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***Streptococcus pseudopneumoniae*** **is a mischaracterized species of Streptococci that is usually overlooked during examination of sputum samples of patients with chest infections. The association of this organism with lower respiratory tract diseases is still unclear and its isolation and description is underestimated in our locality. To our knowledge, there are no published studies on the isolation of *S. pseudopneumoniae* from pathological specimens in Egypt. The aims of this study were to isolate *S. pseudopneumoniae* from sputum specimens of patients admitted to Chest Department of Mansoura University Hospitals (MUHs) and to differentiate it from *Streptococcus pneumoniae* and other viridans group Streptococci, also to determine its prevalence and associated risk factors. Sixteen isolates of *S. pseudopneumoniae* were diagnosed phenotypically by optochin susceptibility and bile solubility tests followed by genotypic characterization by multiplex polymerase chain reaction (PCR). All of the isolates were subjected to antibiotic susceptibility testing using the disk diffusion method. The prevalence of *S. pseudopneumoniae* among studied patients was 4.9% (16/329). All of the examined isolates were found to be positive for *aliB*-like ORF2 and negative for *cpsA* and *lytA* genes by multiplex PCR. Elevated resistance rate of the isolates was recorded for erythromycin, penicillin and co-trimoxazole. Infection by *S. pseudopneumoniae* was found to be significantly associated with Chronic Obstructive Pulmonary Diseases (COPD). Although, *S. pseudopneumoniae* recorded a low prevalence in this study, their elevated antibiotic resistance together with the association with COPD and their pure isolation from sputum samples underline the necessity of spending more effort for their detection and characterization in the microbiology laboratories.**

**Key words:** Chronic obstructive pulmonary diseases (COPD), optochin, respiratory diseases, *Streptococcus pseudopneumoniae*.

## INTRODUCTION

*Streptococcus pseudopneumoniae* as suggested by DNA-DNA homology studies is recorded as a species of the *Streptococcus mitis/oralis* members of viridans group Streptococci; they have some similar features to *Streptococcus pneumoniae* (Arbique et al., 2004). The principle characteristics of *S. pseudopneumoniae* are the lacking of pneumococcal capsule, bile insolubility, indeterminate sensitivity or resistance to optochin after

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incubation in $CO_2$ (5%), while sensitivity to it after incubation in ambient air, they give positive results with antigen detection and DNA probe hybridization tests (Keith et al., 2006).

*S. pseudopneumoniae* was firstly described in sputum samples of patients with lower respiratory tract infection, particularly those with Chronic Obstructive Pulmonary Diseases (COPD) (Laurens et al., 2012). The clinical relevance and pathogenic potential of this organism has not been clearly determined except in few studies that were conducted on mouse models with sepsis and peritonitis, so the correct identification and rapid detection of this emerging pathogen in clinical setting is very important for determination of its disease potential (Harf-Monteil et al., 2006).

*S. pseudopneumoniae* is usually misidentified in our laboratories as we usually rely on optochin sensitivity testing with 5% $CO_2$ for detection of *S. pneumoniae* and miss the incubation in ambient air which is the key point for identification of *S. pseudopneumoniae.* Biochemical reactions are usually used to differentiate typical *S. pneumoniae* from *S. pseudopneumoniae* and other related Streptococci; several molecular techniques have also been used (Rolo et al., 2013).

There are several polymerase chain reaction (PCR)-based assays that target specific pneumococcal virulence factors for the typical *S. pneumoniae*, such as autolysin A (*lytA*) which is the major pneumococcal autolysin (Simo˜es et al., 2010) and the capsular polysaccharide biosynthesis gene A (*cpsA*) which is a conserved pneumococcal capsular polysaccharide gene (Bentley et al., 2006), also *aliB*-like ORF2 (a gene that usually found in the capsular region of non-capsulated *pneumococci*) (Hathaway et al., 2004); these genetic markers have been used to differentiate *S. pneumoniae* from other related species (Rolo et al., 2013).

Unknown putative genes, specific intergenic DNA sequences, or specific regions of the 16S rRNA, have also been proposed to be pneumococcal species-specific (El Aila et al., 2010).

To the best of our knowledge, there are no published studies on the isolation of *S. pseudopneumoniae* from pathological specimens in Egypt. So the aims of the present study were to isolate *S. pseudopneumoniae* from sputum samples of patients admitted to Chest Department of Mansoura University Hospitals (MUHs) and to differentiate it from *S. pneumoniae* and other viridans group Streptococci by phenotypic and genotypic tests as well as to determine the prevalence of this overlooked pathogen and the risk factors of its acquisition.

## METHODOLOGY

### Design of the study

Cross sectional descriptive study was conducted on 381 patients showing signs of respiratory tract infection (Horan et al., 2008) during period extending from the first of September, 2015 to the end of August, 2016. All patients, enrolled in this study, were admitted to the Chest Department of MUHs. The protocol of this research was approved by the ethical committee in the Faculty of Medicine, Mansoura University, with code number R/16.10.08.

### Sample collection

Sputum samples were collected from all studied patients (one sample from each patient) under complete aseptic condition. The samples were processed in the Unit of Microbiology Diagnostic and Infection Control in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University.

### Microscopic examination

Prepared films stained with Gram from sputum specimens were microscopically examined. Specimens with >25 leukocytes and <10 squamous epithelial cells/×100 microscopic field were incorporated in this study and subjected to advance processing, whereas specimens with >10 epithelial cells/×100 microscopic field were not included in the research (Goyal et al., 2007).

After microscopic examination of the collected 381 sputum samples, 329 samples fulfilling aforementioned criteria were subjected to further processing and 52 samples were excluded.

### Culture and identification

The 329 sputum specimens were cultivated on 5% sheep blood agar, chocolate agar (Oxoid, UK), and MacConkey's agar (HiMedia Laboratories, India) plates by taking a loopful from the purulent portion of each sputum specimen using calibrated bacteriological loop that carry 0.01 ml. Cultivation on blood agar and chocolate agar plates were done in duplicate; one was incubated in $CO_2$ enriched atmosphere using a candle jar for 24 h at 37°C and the other was incubated aerobically at 37°C for 24 h. MacConkey's agar plates were incubated aerobically at 37°C for 24 h (Zhou et al., 2011). Significant count of bacteria has been determined as being $\geq 10^5$ CFU/ml, whereas bacteria < $10^5$ CFU/ml was considered to be non-significant count of normal flora (Amissah and Pappoe, 2014). Isolated organisms were identified according to standard microbiological methods. Alpha hemolytic colonies were selected for Gram staining, colonies that showed Gram positive cocci arranged in pairs or chains were subjected to further identification by pneumococcal capsule detection test (capsules were detected by observing a halo around Pneumococci with India ink at ×400 magnification) (Harf-Monteil et al., 2006), optochin susceptibility and bile solubility tests.

### Optochin susceptibility test

The test was done in duplicate; one plate was incubated in 5% $CO_2$ and the other in ambient air ($O_2$ atmosphere) at 35°C for 18 to 24 h. Inhibition zones around the disks were measured and interpreted according to the recommended standards of CLSI (2014). Positive test showed a zone of inhibition of 14 mm or more around the disk (Koneman et al., 2006).

### Bile solubility test

The test was conducted with bile solubility reagent (10%) (Remel, USA). Clearing of the test suspension within 3 h was considered to

**Table 1.** Primers used in the multiplex PCR of the studied isolates

| Gene | Primer name | Primer sequence | Size, bp | References |
|---|---|---|---|---|
| *cpsA* | *cpsA* F2 | AGCAGTTTGTTGGACTGACC | 613 bp | Simo˜es et al. (2011) |
| | *cpsA* R2 | GTGTGAATGGACGAATCAAC | | |
| *lytA* | *lytA* 1145 | AATCAAGCCATCTGGCTCTA | 395 bp | Messmer et al. (1997) |
| | *lytA* 750 | GGCTACTGGTACGTACATTC | | |
| *aliB*-like ORF2 | 104_FI3.6 | AGATGCCAAATGGTTCACGG | 290 bp | Hathaway et al. (2004) |
| | 104_b832.10 | GAAACTCTTCGTTTACTGGG | | |
| *16S rRNA* | 16S rRNA F2 | ACATGCTCCACCGCTTGTG | 522 bp | Simo˜es et al. (2011) |
| | 16S rRNA R2 | GCTCTGTTGTAAGAGAAGAACG | | |

bp, Base pair.

be positive test (Wessels et al., 2012).

All *S. pseudopneumoniae* isolates included in this study were phonotypically suspected as being capsule free, sensitive to optochin in ambient air but intermediate or resistant by incubation in 5% $CO_2$ and bile insoluble.

#### Maintenance of the selected alpha hemolytic Streptococci

For isolates preservation, skim-milk tryptone glucose glycerol (STGG) medium was used (O'Brien et al., 2001). Pure growth of each isolate was collected and dispensed into 1.5 ml vials containing 1.0 ml of STGG medium. After that, the suspension was stored at -70°C for further testing. Before starting any further experiment, subculture of the isolates on blood agar was done twice to allow isolates to restore their viability (Charalambous et al., 2003).

#### Multiplex PCR for genotypic characterization of *S. pseudopneumoniae* isolates

##### *Design of specific gene primers for multiplex PCR assay*

A multiplex PCR was performed for all of studied alpha hemolytic Streptococci in order to detect internal fragments of the following four genes: *cpsA, lytA*, *aliB*-like ORF2 and 16S rRNA gene as a positive internal control (Rolo et al., 2013). The primer sequences and expected band sizes have been used in accordance with previous studies (Table 1).

##### *DNA extraction*

DNA was extracted using QIAamp® DNA Mini kits, QIAGEN (Germany) according to the manufacturer's instructions.

##### *Multiplex PCR technique*

The multiplex PCR was done in a 10 µl volume with 1×PCR buffer (10 mM Tris-HCl, pH 8.3/50 mM KCl/1.5 mM $MgCl_2$), 0.12 mM of dNTPs Mix (2 mM each), 1 U/µl of Taq DNA polymerase (Fermentas) and 0.5 *p*mol/µl of each primer (Simo˜es et al., 2011). 1 µl of extracted DNA was added as DNA template. PCR was done with the following reaction conditions: a pre-denaturation step of 4 min at 94°C; 35 cycles at 94°C for 30 s, 52°C for 45 s, and 72°C for 1 min; a final extension step of 5 min at 72°C; and soaking at 16°C. The PCR products were analyzed after running on 2% agarose gel containing ethidium bromide at 80 volts for about 2 h on comparison to DNA standard marker: #SMO373, 50 bp DNA Ladder, Thermo Scientific Inc., USA (Richter et al., 2013; Hajia et al., 2014). Distilled water was incorporated in every run of the multiplex PCR as a negative control.

#### Testing for antimicrobial sensitivity

*S. pseudopneumoniae* isolates were subjected to antibiotic sensitivity testing as recorded by the recommendations of CLSI using disk diffusion method on Muller-Hinton agar (Oxoid, UK) supplemented with 5% sheep blood using the following antibiotics: penicillin, amoxicilline/clavulinic acid, erythromycin, clindamycin, cefotaxime, co-trimoxazole, ciprofloxacin, linezolid, cefepime, vancomycin, meropenem and teicoplanin (Forbes et al., 2007). The inhibitory zone limits of the tested antimicrobials were referred to CLSI (2014) guidelines.

#### Analysis of data

The data were entered and analyzed statistically with Statistical Package of Social Science (SPSS) using software version 21 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were described as mean, standard deviation (SD), minimum, maximum and percentage. Inter-group comparison of categorical data was done using Chi square test ($\chi 2$-value). Odds ratio (OR) and confidence interval (CI) were used to calculate the risk factors. For all of the previous statistical tests, the threshold of significance is fixed at 5% ($p$-value <0.05 was considered to be statistically significant).

## RESULTS

Out of the studied 329 sputum samples, 286 yielded single pathogen whereas 13 yielded more than one pathogen (2 pathogens) and mixed insignificant growth of normal respiratory tract flora was detected in the remaining 30 samples. The total number of the isolated respiratory pathogens was 312. Sixty two isolates (62/312, 19.9%) were alpha hemolytic Streptococci whereas the remaining 250 isolates were *Klebsiella*

**Table 2.** Multiplex PCR results of the examined alpha hemolytic Streptococcal isolates.

| Gene | Alpha hemolytic Streptococci (number=62) | | |
|---|---|---|---|
| | ***S. pseudopneumoniae*** **(number=16)** **% (number positive/screened)** | ***S. pneumoniae*** **(number=21)** **% (number positive/screened)** | **Viridans group Streptococci (number=25)** **% (number positive/screened)** |
| *cpsA* | 0.0 (0/14) | 90.5 (19/21) | 0.0 (0/20) |
| *lytA* | 0.0 (0/14) | 100 (21/21) | 0.0 (0/20) |
| *aliB*-like ORF2 | 100 (14/14) | 14.3 (3/21) | 20 (4/20) |
| *16S rRNA* | 87.5 (14/16) | 100 (21/21) | 80.0 (20/25) |

species (59/312, 18.9%), *Candida* species (50/312, 16.0%), *Staphylococcus aureus* (41/312, 13.1%), *Escherichia coli* (23/312, 7.4%), *Pseudomonas aeruginosa* (21/312, 6.7%), *Streptococcus pyogens* (18/312, 5.8%), *Enterococci* (12/312, 3.8%), MRSA (10/312, 3.2%), *Citrobacter* species (6/312, 1.9%), *Haemophilus influenzae* (6/312, 1.9%) and *Moraxella catarrhalis* (4/312, 1.3%).

Among the isolated 62 alpha hemolytic Streptococci, 16 isolates were presumptively recognized as being *S. pseudopneumoniae* by phenotypic tests, as all these isolates lacked the pneumococcal capsules, showed resistance to optochin (zone diameter <14 mm) in 5% $CO_2$ incubation, but they were sensitive to optochin (zone diameter between 18 to 25 mm) when incubated in ambient air; also they were found to be bile insoluble. Regarding the colony morphology of the *S. pseudopneumoniae* on 5% sheep blood agar, they were observed to be small (about 1 mm in diameter), shiny, smooth, and domed, with entire edges. Few colonies having depressed centers, seem to be a smaller version of the *S. pneumoniae* draftsmen colonies. The remaining 46 alpha hemolytic Streptococci were identified as *S. pneumoniae* (21 isolates) and other viridans group Streptococci (25 isolates) accounted for 6.7 (21/312) and 8.0% (25/312) of the isolated respiratory pathogens, respectively. The percentage of the isolated *S. pseudopneumoniae* in relation to other detected respiratory pathogens was 5.1% (16/312) and its prevalence among studied patients was 4.9% (16/329).

The multiplex PCR assay which was conducted on the studied alpha hemolytic Streptococci showed that DNA from 2/16 of the putative *S. pseudopneumoniae* isolates and 5/25 of the viridans group Streptococci were negative for 16S rRNA gene, so these extracts must have been inhibitory. *S. pseudopneumoniae* PCR results for the other targets therefore were determined out of the 14 (non-inhibitory) samples. All of the phenotypically identified *S. pseudopneumoniae* isolates were negative for *cpsA* and *lytA* genes, but they were positive for *aliB*-like ORF2 (Table 2 and Figure 1).

Antibiotic sensitivity testing of the studied 16 *S. pseudopneumoniae* isolates showed that all of them were sensitive to linezolid, vancomycin and teicoplanin, on the other hand, co-trimoxazole, penicillin and erythromycin recorded the lowest sensitivity for those isolates (Table 3).

The distinctive data of patients with culture positive sputum for *S. pseudopneumoniae* (16 patients) showed that their mean age was 58.06 years (range 17-76 years); 56.2% were males, 68.8% were presented with COPD with infected exacerbation. On the other hand, only 37.5% of them showed positive X-ray for infiltration and 31.2% recorded elevated leukocyte counts (>11 × $10^9$ cells/L). *S. pseudopneumoniae* was the only detected pathogen in 68.8% of sputum cultures of those patients, whereas *H. influenza* was the most common co-pathogen detected in sputum culture of patients that showed other bacterial growth along with *S. pseudopneumoniae* (Table 4).

On comparing patients with culture positive sputum for *S. pseudopneumoniae* with patients who showed the carriage of other alpha hemolytic Streptococci (*S. pneumoniae* and viridans group Streptococci) in their sputum samples, COPD with infected exacerbation and previous hospitalization with COPD were found to be more significantly associated with the acquisition of *S. pseudopneumoniae* infections (P= 0.006 and 0.01, respectively). On the other hand, there was no statistically significant difference regarding presence of asthma, pneumonia, bronchiectasis, bronchogenic carcinoma or smoking history as risk factors (Table 5).

## DISCUSSION

*S. pseudopneumoniae* may often be overlooked during examination of sputum samples of patients with respiratory disorders particularly in our locality. Few studies have been conducted on the isolation and description of this organism; the prevalence of *S. pseudopneumoniae* is determined in few researches that have been considered to be unique for certain areas of the world and this research was one of the first attempts to isolate this organism from patients with respiratory diseases in our locality.

*S. pseudopneumoniae* could be easily isolated and characterized by few phenotypic and genotypic tests

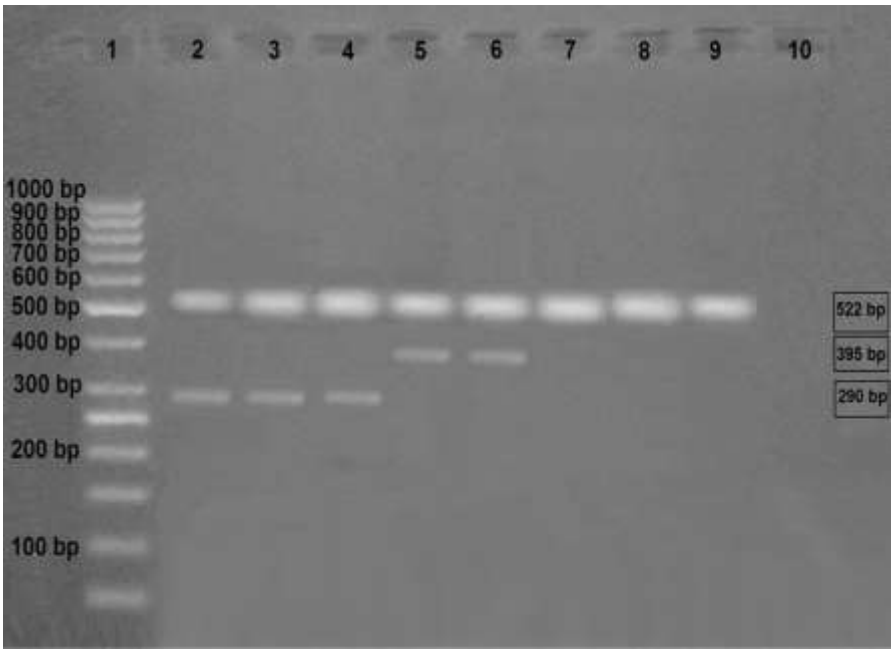


**Figure 1.** Multiplex PCR results of the studied alpha hemolytic Streptococci including *S. pseudopneumoniae* isolates. Lane 1 shows the DNA standard marker. All lanes (2 to 9) show 522 bp fragments of positive internal control. Lane 10 is the negative control (distilled water). Lanes 2, 3 and 4 show the 290 bp bands from positive *S. pseudopneumoniae* isolates to *aliB*-like ORF2 gene. Lanes 5 and 6 show the 395 bp bands from positive *S. pneumoniae* isolates to *lytA* gene. Lanes 7, 8 and 9 show only the bands of positive internal control with absence of bands of other target genes from negative isolates of viridans group Streptococci.

**Table 3.** Antibiotic sensitivity pattern of the studied 16 *S. pseudopneumoniae* isolates.

| Antibiotic tested | *S. pseudopneumoniae* (number=16) | |
|---|---|---|
| | % (number sensitive/screened) | % (number resistant/screened) |
| Penicillin | 37.5 (6/16) | 62.5 (10/16) |
| Amoxicilline/clavulinic acid | 56.2 (9/16) | 43.8 (7/16) |
| Erythromycin | 43.8 (7/16) | 56.2 (9/16) |
| Clindamycin | 56.2 (9/16) | 43.8 (7/16) |
| Cefotaxime | 87.5 (14/16) | 12.5 (2/16) |
| Co-trimoxazole | 25.0 (4/16) | 75.0 (12/16) |
| Ciprofloxacin | 62.5 (10/16) | 37.5 (6/16) |
| Linezolid | 100 (16/16) | 0.0 (0/16) |
| Cefepime | 93.8 (15/16) | 6.2 (1/16) |
| Vancomycin | 100 (16/16) | 0.0 (0/16) |
| Meropenem | 93.8 (15/16) | 6.2 (1/16) |
| Teicoplanin | 100 (16/16) | 0.0 (0/16) |

particularly the optochin sensitivity test and specific genes detection by multiplex PCR assay.

The prevalence of *S. pseudopneumoniae* among studied patients with respiratory tract disorders was 4.9%; this percentage approximates what has been recorded in previous studies at which this pathogen was isolated from nearly 4% of the studied cases (Keith et al., 2006; Mohammadi and Dhanashree, 2012). On the other hand, lower percentage of *S. pseudopneumoniae* (nearly 1%) has been observed by other researchers (Harf-Monteil et al., 2006; Swathi et al., 2015). The variability of results among various studies may be due to the

**Table 4.** distinctive features of patients with culture positive sputum for *S. pseudopneumoniae*.

| Item | Study group (n=16) | |
|---|---|---|
| | (Number positive/screened) | % |
| **Age (years)** | | |
| Mean ± SD | 58.06±15.03 | - |
| Min-Max | 17-76 | - |
| **Sex** | | |
| Male | (9/16) | 56.2 |
| Female | (7/16) | 43.8 |
| **Clinical diagnosis** | | |
| COPD with infected exacerbation. | (11/16) | 68.8 |
| Lung abscess | (1/16) | 6.2 |
| Bronchogenic carcinoma, COPD | (1/16) | 6.2 |
| Pneumonia | (1/16) | 6.2 |
| Asthma | (1/16) | 6.2 |
| Bronchiectasis | (1/16) | 6.2 |
| **Chest X ray for infiltration** | | |
| Positive | (6/16) | 37.5 |
| Negative | (10/16) | 62.5 |
| **Elevated leukocyte** | | |
| Positive | (5/16) | 31.2 |
| Negative | (11/16) | 68.8 |
| **Acompanied bacteria in sputum culture** | | |
| No other bacteria | (11/16) | 68.8 |
| *H. influenzae* | (4/16) | 25 |
| *E. coli* | (1/16) | 6.2 |

**Table 5.** Patients risk factors for acquisition of *S. pseudopneumoniae* in relation to patients infected with other alpha hemolytic Streptococci.

| Risk factor | Patients with culture positive sputum for *S. pseudopneumoniae* (number=16) % (no. positive/screened) | Patients with culture positive sputum for other alpha hemolytic Streptococci (number=46) % (no. positive/screened) | P value | Odds ratio and confidence interval |
|---|---|---|---|---|
| Previous hospitalization with COPD | 62.5 (10/16) | 23.9 (11/46) | 0.01* | 5.30 (1.36-21.66) |
| COPD with infected exacerbation | 68.8 (11/16) | 26.1 (12/46) | 0.006* | 6.23 (1.55-26.42) |
| Asthma | 6.2 (1/16) | 21.7 (10/46) | 0.2 | 0.24 (0.01-2.15) |
| Pneumonia | 6.2 (1/16) | 26.1 (12/46) | 0.1 | 0.19 (0.01-1.65) |
| Bronchiectasis | 6.2 (1/16) | 17.4 (8/46) | 0.4 | 0.32 (0.01-2.94) |
| Smoking | 56.2 (9/16) | 58.7 (27/46) | 0.9 | 0.90 (0.25-3.31) |
| Bronchogenic carcinoma | 6.2 (1/16) | 15.2 (7/46) | 0.6 | 0.37 (0.02-3.53) |

difference in patient health care and the associated manner of infectious agent distribution in different localities.

Checking for specific genetic markers by multiplex PCR revealed the absence of *cpsA* and *lytA* genes in all studied isolates of *S. pseudopneumoniae,* whereas they were found to be, respectively positive in 90.5 and 100% of the isolated *S. pneumoniae*. These findings support a previous published study at which *cpsA* was confirmed to be a specific capsular polysaccharide gene exclusively harbored by *S. pneumoniae* as it is commonly linked with the capsular operon and absent in *S. pseudopneumoniae*

isolates as being non capsulated (Park et al., 2010). Similarly, *lytA* gene which has been considered to be one of the diagnostic markers that differentiate *S. pneumoniae* from other closely related species including *S. pseudopneumoniae* was also found to be absent in all isolates of *S. pseudopneumoniae* that have been examined in other researches (Rolo et al., 2013).

*aliB*-like ORF2 gene was detected in all studied *S. pseudopneumoniae* isolates supporting the observations recorded in previous researches that have mentioned this gene as being specific for non-capsulated Pneumococci and hence *S. pseudopneumoniae* as being non capsulated (Hathaway et al., 2004). There were three isolates of the examined *S. pneumoniae* found to be positive for *aliB*-like ORF2 gene; our results are in agreement with previous findings that have recorded the same observation (Simo˜es et al., 2011) which could be explained by the lack of ability of those isolates to produce the capsule *in vitro* (Scott et al., 2012) as a result of capsular genes mutation (Melchiorre et al., 2012).

A great resistance rate of the studied *S. pseudopneumoniae* isolates was recorded for co-trimoxazole (75.0%), penicillin (62.5%) and erythromycin (56.2%) which was consistent with previous studies at which the same resistance pattern has been observed among studied isolates (Keith and Murdoch, 2008; Mohammadi and Dhanashree, 2012). Other studies have also documented a high resistance rate among *S. pseudopneumoniae* isolates to erythromycin (60%) without any recorded resistance to penicillin (Keith et al., 2006) that was in contrast with our finding and may be due to different antibiotic policy in other countries at which penicillin is used within limits. Unfortunately, there is no recorded information about isolation and antibiotic sensitivity profile of *S. pseudopneumoniae* in our locality to be compared with our findings. The observed high resistance of this organism to penicillin and other classes of antibiotics highlights its role as one of the respiratory tract pathogen that should not be neglected particularly during selection of the appropriate antibiotic that could be used in dealing with respiratory tract infection.

In this study, *S. pseudopneumoniae* was the only pathogen recovered from 11/299 (3.7%) of specimens that were positive both by smear-microscopy and culture for bacterial pathogens and it was the predominant one in samples that showed growth of other co pathogen. This observation should not be neglected as it may be an indirect evidence of the important clinical role of this pathogen in the acquisition of respiratory tract infection. This observation was also supported by the presence of pulmonary infiltrate in 37.5% and elevated leukocyte count in 31.2% of cases with culture positive *S. pseudopneumoniae*. Although a high resistance rate of *S. pseudopneumoniae* to various classes of antibiotics has been documented in previous studies, most of them suspected this organism as a colonizing bacterium of the respiratory tract or a weak opportunistic pathogen that is usually associated with potentially pathogenic organisms in patients with chronic conditions (Laurens et al., 2012). Subsequent studies have supported our finding that this organism may have a real clinical importance depending on the observed high resistance rate for this organism together with its involvement in various types of invasive diseases (Rolo et al., 2013).

In this study, the actual risk factors associated with *S. pseudopneumoniae* infection were assessed by comparing patients with culture positive sputum for *S. pseudopneumoniae* with patients who revealed other alpha hemolytic Streptococci (*S. pneumoniae* and viridans group Streptococci) in their sputum cultures. COPD with infected exacerbation and previous hospitalization with COPD were significantly associated with increase in the acquisition of *S. pseudopneumoniae* in spite of large number of studied cases suffering from COPD in this research. The association of COPD with *S. pseudopneumoniae* infection has been documented by previous studies (Keith et al., 2006; Harf-Monteil et al., 2006), however other studies have recorded that *S. pseudopneumoniae* were more frequently associated with bronchitis and pneumonia rather than COPD (Laurens et al., 2012). To determine the actual role of *S. pseudopneumoniae* in COPD exacerbation, cohort studies for patients during periods of exacerbation and stability of COPD is recommended to observe the appearance of new *S. pseudopneumoniae* isolates in the exacerbation periods that could confirm the association of infection with new bacterial strains and COPD exacerbation (Sethi et al., 2002).

Future studies seems to be necessary to determine the actual habitat, epidemiology, colonization of *S. pseudopneumoniae* and if it is associated with respiratory diseases other than COPD particularly pneumonia.

## Conclusion

The pure growth of *S. pseudopneumoniae* from sputum samples together with the great percentage of antibiotic resistance exhibited by those isolates observed in the present study should raise our attention to the clinical importance of this organism. The initial data recorded in this research concerning the prevalence, the genetic characterization, the antibiotic sensitivity pattern and the associated risk factors of *S. pseudopneumoniae* were the first in our locality as regarding this organism. Future studies should be encouraged to allow further description of *S. pseudopneumoniae* especially in relation to lower respiratory tract diseases; this may change the notion that they are just atypical pneumococci as it has been previously believed in our area.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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