Iranian Journal of Microbiology

Volume 14 Number 5 (October 2022) 645-652

ORIGINAL ARTICLE

Streptococcus pseudopneumoniae as an emerging respiratory tract pathogen at Assiut University hospitals

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Received: October 2021**, Accepted:** July 2022

ABSTRACT

Background and Objectives: *Streptococcus pseudopneumoniae* is a member of viridans streptococci. It is non-capsulated, bile insoluble and optochin susceptible in ambient air but resistant in 5% CO_2. This study aimed to isolate *S. pseudopneumoniae* from sputum specimens of patients admitted to Chest Department and Chest ICU of Assiut University hospitals, differentiate it from *Streptococcus pneumoniae* in addition, to evaluate the prevalence of *Streptococcus pseudopneumoniae* in clinical isolates by phenotypic and genotypic methods, to subject the isolates to antimicrobial susceptibility testing using agar disc diffusion method.

Materials and Methods: Isolation of *Streptococcus pseudopneumoniae* from sputum sample and doing phenotypic test (optochin susceptibility test,bile susceptibility test and antimicrobial susceptibility test) and genotypic test by polymerase chain reaction (PCR) for five genes: *CpsA, LytA, AliB-like ORF2*, 16S rRNA and *Spn9802* genes.

Results: Twenty isolates of *S. pseudopneumoniae* were diagnosed phenotypically by optochin susceptibility and bile solubility tests followed by genotypic characterization by polymerase chain reaction (PCR) for five genes: *CpsA, LytA, AliB-like ORF2*, 16S rRNA and *Spn9802* genes. The prevalence of *S. pseudopneumoniae* among studied patients was 10% (20/200).

Conclusion: The pure growth of *S. pseudopneumoniae* from sputum samples together with the great percentage of antibiotic resistance should raise attention to the clinical importance of this organism.

Keywords: *Streptococcus*; Respiratory tract diseases; Polymerase chain reaction

INTRODUCTION

The Mitis group of the genus *Streptococcus* has several phylogenetic clusters, one of which is composed of the three species *Streptococcus pneumoniae*, *Streptococcus mitis*, and *Streptococcus pseudopneumoniae*, all habitats in the human upper respiratory tract (1).

In 2004 *S. pseudopneumoniae* was first reported by Arbique et al. *S. 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. S. pseudopneumoniae* is optochin resistant in the presence of 5% CO_2, bile insoluble and lacks the pneumococcal capsule. However, *S. pseudopneumoniae* is optochin susceptible under ambient atmosphere, often causing

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difficulty in distinguishing it from *S. pneumoniae* in our laboratories (2).

S. pseudopneumoniae is usually misdiagnosed 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* (3). *S. pseudopneumoniae* has greater resistance rate to several antimicrobial agents than typeable pneumococci (4).

The phenotypic features that characterize *S. pseudopneumoniae* are consistent among strains that have been classified as ‘‘atypical’’ or ‘‘unusual’’ streptococci, such as hemolysis patterns on blood agar media (5).

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 (6), and the *Capsular polysaccharide biosynthesis gene A (CpsA)* which is a conserved pneumococcal capsular polysaccharide gene (7), also *AliB-like ORF2* (a gene that usually found in the capsular region of non-capsulated pneumococci) (8).

In this work, as part of an ongoing effort to isolate *S. pseudopneumoniae* from sputum specimens of patients admitted to Chest Department and Chest ICU of Assiut University Hospitals, differentiate it from *Streptococcus pneumoniae*, determine associated risk factors and differentiate between the patients in Chest Department and in Chest ICU. Also to evaluate the prevalence of *Streptococcus pseudopneumoniae* in clinical isolates by phenotypic and genotypic methods and to subject the isolates to antimicrobial susceptibility testing using the agar disc diffusion method.

MATERIALS AND METHODS

Design of the study. Cross sectional descriptive study (Clinical Trials No. 03528759) was carried out from February 2019 to February 2020, and was conducted on 200 patients showing signs of lower respiratory tract infection.

Sample collection. Sputum samples were collected from all studied patients under complete aseptic condition. The samples were processed in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University.

Microscopic examination. Prepared films stained with Gram stain from sputum specimens were microscopically examined for the presence of Diplococci.

Isolation of *S. Pseudopneumoniae*. Samples were cultured for 24 hours at 37°C in a humidified atmosphere supplemented with 5% CO_2 on Columbia blood agar plates (Oxoid), which were poured using 10% defibrinated sheep blood. Selectivity of media was verified by adding 5 µg/ml gentamicin, 10 µg/ml colistin and 10 µg/ml amphotericin B (9).

Alpha haemolytic colonies on the culture plates, that were Gram positive cocci in pairs by staining, were identified as *S. pseudopneumoniae* and differentiated from *S. pneumoniae* on the basis of the tests for bile solubility and optochin susceptibility (10).

Identification of *S. Pseudopneumoniae* isolates: optochin susceptibility test. Optochin susceptibility was tested by disc diffusion, using commercially available optochin discs (5 mg; 6 mm; Oxoid, Hampshire, England) applied into blood agar plates. 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 discs were measured and interpreted according to the recommended standards of CLSI (2014) (11).

Bile solubility test. Tube bile solubility testing was performed: fresh growth of the organism from sheep blood agar was suspended in phosphate buffered saline, to obtain turbidity equivalent to that of 0.5 McFarland standard, and equal volumes (0.5 ml) were transferred into two tubes.

0.5 ml of 2% sodium deoxycholate was added to one tube, and an equal volume of phosphate buffered saline was added to the control tube; the samples then were incubated at 37°C in an ambient atmosphere for up to 2 hours (12).

Testing for antimicrobial susceptibility. *S. pseudopneumoniae* isolates were subjected to antimicrobial susceptibility testing as recorded by the recommendations of CLSI using agar disc diffusion method using the following antibiotics: penicillin, amoxicilline/clavulinic acid, erythromycin, clindamycin, cefotaxime, co-trimoxazole, ciprofloxacin, linezolid, cefepime, vancomycin and meropenem. The result was interpreted as either sensitive, intermediate or resistant as per guidelines.

Preservative medium. For isolates preservation, skim milk tryptone glucose glycerol (STGG) medium was used. 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 (13).

PCR for genotypic characterization of *S. Pseudopneumoniae* isolates. DNA was extracted from bacterial isolates using Thermo Scientific Gene JET Genomic DNA Purification Kit according to the manufacturer's instructions. The PCR was performed for all of studied alpha hemolytic streptococci for five genes: *CpsA, LytA, AliB-like ORF2,* 16S rRNA and *Spn9802* genes. The primer sequences and expected band sizes have been used in accordance with previous studies (Table 1).

PCR assays. PCR was done in a 20 µl volume (Distalled Water11.3 µl. 5× Green Go Taq Buffer 4 µl. dNTPs mix (2.5Mm) 0.4 µl. Taq DNA polymerase 0.1 µl. Primer (F) (10 mM) 1 µl, Primer (R) (10 mM) 1 µl. DNA template 1 µl. $MgCl_2$, 1.2 µl).

16S rRNA primers. A pre-denaturation step of 4 min at 94°C. 35 cycles of: denaturation at 94°C for 30 s, annealing at 52°C for 45 s, and extension at 72°C for 1 min; a final extension step of 5 min at 72°C.

***AliB-like ORF2* primers.** A pre-denaturation step of 5 min at 95°C. 30 cycles of:denaturation at 95°C for 30 s, annealing at 55°C for1 min, and extension at72°C for 1 min; a final extension step of 5 min at 72°C.

***Spn9802* primers.** A pre-denaturation step of 4 min at 94°C. 30 cycles of: denaturation at 94°C for 30 s, annealing at 54°C for 45 s, and extension at 72°C for 1 min; a final extension step of 5 min at 72°C.

***7LytA* primers.** A pre-denaturation step of 2 min at 94°C. 30 cycles of: denaturation at 94°C for 15 s, annealing at 53°C for 15 s, and extension at 72°C for 15 s; a final extension step of 2 min at 72°C.

***CpsA* primers.** A pre-denaturation step of 4 min at 94°C. 30 cycles of: denaturation at 94°C for 30 s, annealing at 53°C for 45 s, and extension at 72°C for 1 min; a final extension step of 5 min at 72°C.

Detection of the amplified product. The resulting PCR amplicons were screened on 1.5% agarose gel, stained with ethidium bromide and visualized under UV light at 80 volts for about 2 h. Distilled water was incorporated in every run of the PCR as a negative control.

Statistical analysis. Categorical variables were described by number and percent (N, %), Chi-square test and fisher exact test used to compare between categorical variables. A two-tailed $p < 0.05$ was considered statistically significant. All analyses were performed with the IBM SPSS 26.0 software.

Sample size calculation. Sample size was calculated using statcalc program of EPI-info version 7.2 using population survey or descriptive observational study calculation, according to the prevalence of *S. pseudopneumoniae* infection among admitted patients in chest department was 4% (16), population size 800, acceptable margin of error was 2%, confidence interval 90%, the minimum required sample

Table 1. Sequence of primers used for molecular characterization of *S. pseudopneumoniae*

Gene	Primer name	Primer sequence	Size	References
CpsA	CpsA F2	AGCAGTTTGTTGGACTGACC	613 bp	(14)
	CpsA R2	GTGTGAATGGACGAATCAAC		
LytA	LytA 1145	AATCAAGCCATCTGGCTCTA	395 bp	(14)
	LytA 750	GGCTACTGGTACGTACATTC		
AliB-like ORF2	104_FI3.6	AGATGCCAAATGGTTCACGG	290 bp	(8)
	4_b832.10	GAAACTCTTCGTTTACTGGG		
16S rRNA	16S rRNA F2	ACATGCTCCACCGCTTGTG	522 bp	(14)
	16S rRNA R2	GCTCTGTTGTAAGAGAAGAACG		
Spn9802	Spn9802 forward	CAAGTCGTTCCAAGGTAACAAGTCT	162 bp	(15)
	Spn9802 reverse	CTAAACCAACTCGACCACCTCTTT		

size will be 196 patients. Sample size was raised to 200 to avoid dropout.

Ethical statement. The study was permitted by the Ethical Committee of the Faculty of Medicine, Assiut University, Assiut, Egypt, according to the code of ethics of the World Medical Association (Declaration of Helsinki). Informed consent was taken from all participants.

RESULTS

Out of the studied 200 sputum samples, 50 isolates were alpha hemolytic streptococci. Among the isolated 50 alpha hemolytic streptococci, 30 isolates were presumptively recognized as being *S. pneumoniae* and 20 isolates were presumptively recognized as being *S. pseudopneumoniae* by phenotypic and genotypic methods. The percentage of the isolated *S. pseudopneumoniae* in relation to other detected respiratory pathogens was 10% (20/200).

The phenotypic test results. All 20 studied *S. pseudopneumoniae* isolate were resistance to optochin (zone diameter <14mm) in 5% CO_2 incubation, but they were sensitive to optochin (zone diameter≥14 mm) when incubated in ambient air, also they were found to be bile insoluble. Regarding the colony morphology of the *S. pseudopneumoniae* on 10% 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.

The PCR results of the examined alpha hemolytic streptococci. PCR assay which was conducted on the studied alpha hemolytic streptococci showed that all alpha hemolytic streptococci isolates were positive for the 16S rRNA gene. All *S. pneumoniae* and some of *S. pseudopneumoniae* 35% (7/20) were positive for *Spn9802* gene. All of the phenotypically identified *S. pseudopneumoniae* isolates were negative for *CpsA* and *LytA* genes, but they were positive for *AliB like ORF2* (Figs. 1-4).

Antimicrobial susceptibility pattern of the studied 20 *S. pseudopneumoniae* isolates. *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 10% sheep blood.

Antimicrobial susceptibility testing of the studied 20 *S. pseudopneumoniae* isolates showed that all of them were resistant to penicillin, erythromycin, clindamycin, ciprofloxacin, cefopime, co-trimoxazole. On the other hand, 50% were sensitive to Linezolid, 40% were sensitive to vancomycin, 25% were sensitive to meropenem, 15% were sensitive to amoxicillin/ clavulinic acid, and 5% were sensitive to cefotaxime.

Predisposing risk factors for *S. pseudopneumoniae* are summarized in Table 2 which shows that *S. pseudopneumoniae* was significantly associated with COPD with infected exacerbation 85% (17/20) and previous hospitalization with COPD 85% (17/20).

In the differentiation between the patients in Chest Department and in Chest ICU there were no statistical significant difference between the 2 groups regarding the prevlance, risk factors, antimicrobial susceptibility test.

DISCUSSION

During examination of sputum samples of patients with lower respiratory tract disease, *S. pseudopneumoniae* may be overlooked particularly in our locality. Few studies have been interested in the isolation and identification of *S. pseudopneumoniae*, the prevalence of this organism is determined in few researches that have been considered to be unique for certain areas of the world.

In the present study, 200 patients showing signs of lower respiratory tract infection were tested for the presence of *S. pseudopneumoniae* pathogen, the study aimed to evaluate the different diagnostic tests for detection of *S. pseudopneumoniae*.

In this study *S. pseudopneumoniae* were isolated at a rate of 10% (20/200), this percentage is higher than what has been recorded in previous studies at which this pathogen was isolated from 4% of the studied cases (10). The variability of these results among various studies may be due to the difference in patient health care and the associated manner of infectious agent distribution in different localities.

In the present study, all *S. pseudopneumoniae* isolates were resistance to optochin (zone diameter <14 mm) in 5% CO_2 incubation, but they were sensitive to optochin (zone diameter≥14 mm) when incubated in ambient air. Also, all *S. pseudopneumoniae* isolates were bile insoluble which agreed with what has been

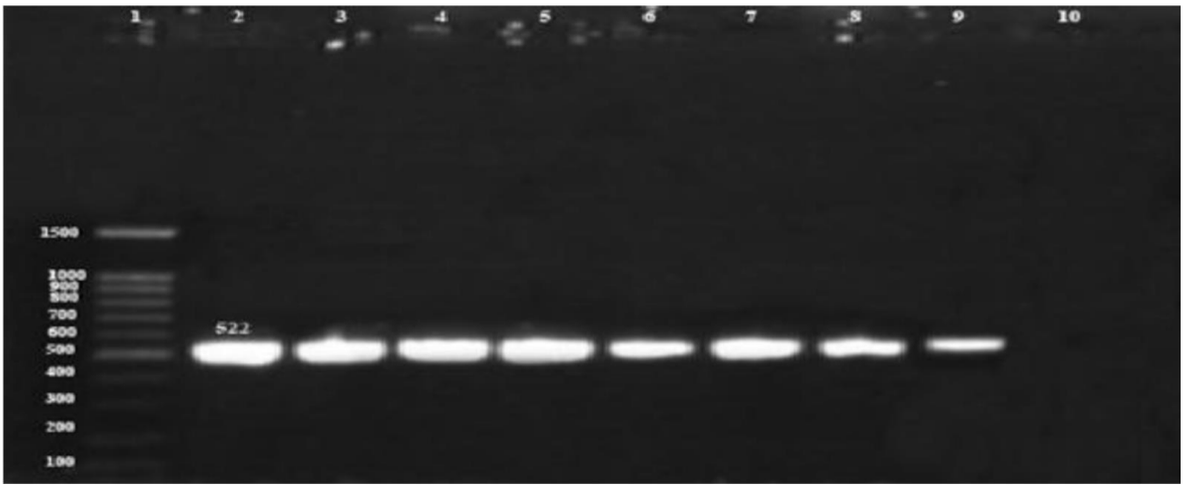

Fig. 1. Agarose gel electrophoresis of the products of PCR to detect 16S rRNA gene (522 bp).
Lane (1) shows the DNA standard marker, All lanes (2 to 9) show 522 bp, Lane (10) is the negative control.

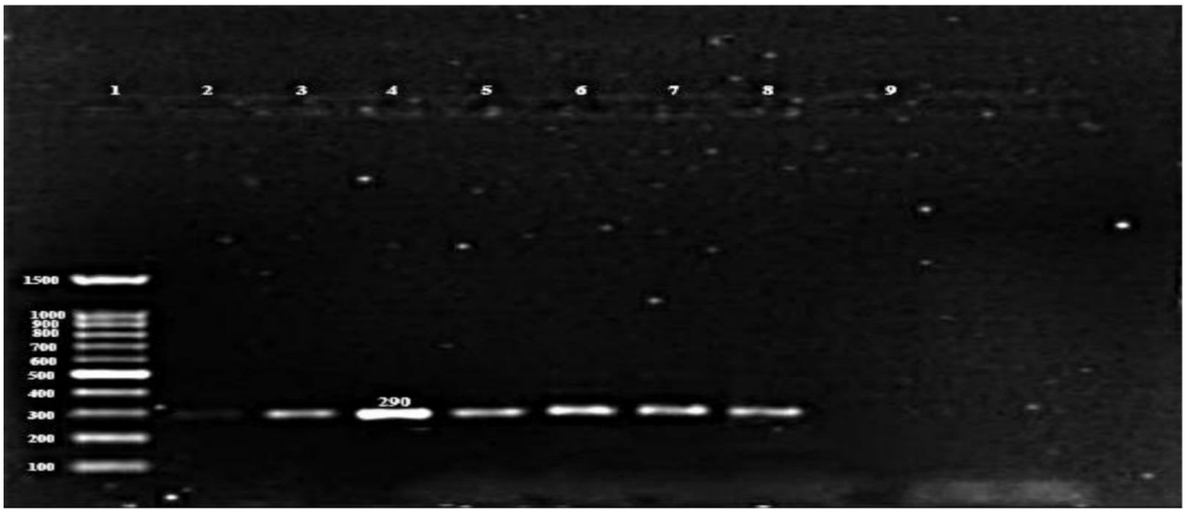

Fig. 2. Agarose gel electrophoresis of the products of PCR to detect *aliB-like ORF2* gene (290).
Lane (1) shows the DNA standard marker,
All lanes (2 to 8) show 290 bp, *S. pseudopneumoniae* positive isolates, Lane (9) is negative.

previously reported by a previous study (16).

In this study, checking for specific genetic markers by PCR revealed the absence of *cpsA* and *lytA* genes in all studied isolates of *S. pseudopneumoniae*, whereas they were found to be, positive in 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 (17).

In our study Spn9802 gene cannot differentiate *S. pneumoniae* from *S. pseudopneumoniae*, as it present in all *S. pneumoniae* and some of *S. pseudopneumoniae* (35%), which was consistent with a previous studies ensured that *spn9802* present in both *S. pneumniae* and *S. pseudopneumoniae* (3).

In our study *AliB-like ORF2* gene was detected in all studied *S. pseudopneumoniae* isolates supporting the observations recorded in previous study that have mentioned this gene as being specific for non-capsulated pneumococci and hence *S. pseudopneumoniae* as being non capsulated (8).

In the present study, there were a great antibiotic resistance rate of the studied *S. pseudopneumoniae*, all

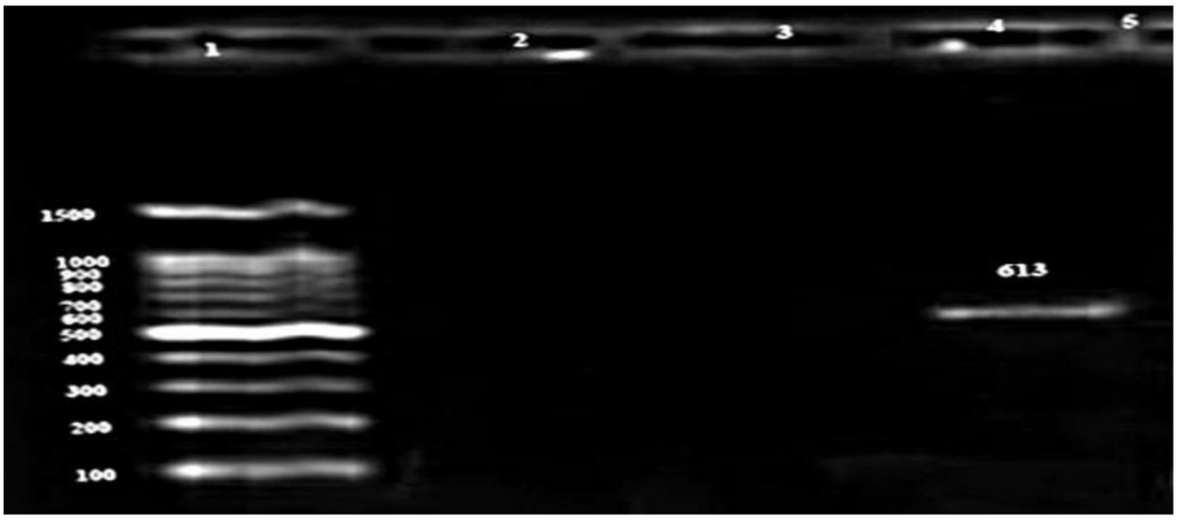

Fig. 3. Agarose gel electrophoresis of the products of PCR to detect *cpsA* gene (613 bp)
Lane (1) shows the DNA standard marker, Lane (4) shows 613 bp, *S. pneumoniae*, Lanes (2) and (3) show *S. pseudopneumoniae* (negative for this gene).

Fig. 4. Agarose gel electrophoresis of the products of PCR to detect *lytA* gene (395 bp)
Lane (1) shows the DNA standard marker, Lane (4) show 395 bp, *S. pneumoniae*, Lanes (2) and (3) show *S. pseudopneumoniae* (negative for this gene).

of them were resistant to penicillin, erythromycin, ciprofloxacin, cefopime, co-trimoxazole and clindamycin which was similar to resistance pattern has been observed among studied isolates (18). On the other hand, 50% were sensitive to lienozolid, 40% were sensitive to vancomycin, 25% were sensitive to meropenem, 15% were sensitive to amoxicillin/ Clavulinic acid, and 5% were sensitive to cefotaxime. These findings supported by a previous published study (15). Other studies have also documented a high resistance rate among *S. pseudopneumoniae* isolates to erythromycin (60%), (77%) to tetracyclin, (11%) to cotrimoxazole without any recorded resistance to penicillin (10), 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.

On studying some of the risk factor of *S. pseudopneumoniae*, we demonstrated that COPD with infected exacerbation and previous hospitalization with COPD (85%) were significantly associated with increase in the acquisition of *S. pseudopneumoniae.*

The association of COPD with *S. pseudopneumoniae* infection has been documented by previous studies (19).

To identify the role of *S. pseudopneumoniae* in the exacerbation of COPD, it will be essential to examine COPD exacerbations patients and compare them to those who do not with regard to isolation of this organism. Ideally, it would be important to monitor a cohort of patients with COPD and examine them during both times of clinical stability and exacerbations. The appearance of *S. pseudopneumoniae* or a new strain of *S. pseudopneumoniae* during exacerbations would support the more accepted hypothesis that acquisition of a new strain of bacteria plays a causative role in exacerbation of COPD (20).

Table 2. Patients risk factors for acquisition of *S. pseudopneumoniae*

Risk factor	Patients with culture positive sputum for *S. pseudopneumoniae* (number=20) % (no. positive/screened)	Patients with culture positive sputum for *S. pneumoniae* (number=46) % (no. positive/screened)	P value	Odds ratio and confidence interval
Previous hospitalization with COPD	85 (17/20)	16.6(5/30)	0.005**	5.1 (1.62-16.05)
COPD with infected exacerbation	85 (17/20)	16.6(5/30)	0.005**	5.1 (1.62-16.05)
Asthma	0 (0/20)	23.3(7/30)	0.121	0.099 (0.005-1.834)
Pneumonia	5 (1/20)	50(15/30)	0.032*	0.1 (0.012-0.818)
Bronchiectasis	10 (2/20)	10(3/30)	1.000	1 (0.153-6.531)
Smoking	90 (18/20)	56.6(17/30)	0.298	1.59 (0.664-3.797)
Bronchogenic carcinoma	0 (0/20)	0(0/30)	0.844	1.488 (0.028-78.03)

CONCLUSION

The pure growth of *S. pseudopneumoniae* from sputum samples together with the great percentage of antibiotic resistance of those isolates observed in this study should raise our attention to the clinical importance of this organism. Future studies should be encouraged to allow further description of *S. pseudopneumoniae* especially in relation to lower respiratory tract diseases.

REFERENCES

1. Kilian M, Tettelin H. Identification of virulence-associated properties by comparative genome analysis of *Streptococcus pneumoniae, S. pseudopneumoniae, S. mitis,* three *S. oralis* subspecies, and *S. infantis. mBio* 2019; 10(5): e01985-19.
2. Arbique JC, Poyart C, Trieu-Cuot P, Quesne G, Carvalho Mda G, Steigerwalt AG, et al. Accuracy of phenotypic and genotypic testing for identification of *Streptococcus pneumoniae* and description of *Streptococcus pseudopneumoniae* sp. nov. *J Clin Microbiol* 2004; 42: 4686-4696.
3. Rolo D, Simoes AS, Domenech A, Fenoll A, Linares J, De Lencastre H, et al. Disease isolates of *Streptococcus pseudopneumoniae* and non-typeable *S. pneumoniae* presumptively identified as atypical *S. pneumoniae* in Spain. *PLoS One* 2013; 8(2): e57047.
4. Leegaard TM, Bootsma HJ, Caugant DA, Eleveld MJ, Mannsåker T, Frøholm LO, et al. Phenotypic and genomic characterization of pneumococcus-like streptococci isolated from HIV-seropositive patients.

Microbiology (Reading) 2010; 156: 838-848.
5. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev* 2002; 15: 613-630.
6. Simões AS, Sá-Leão R, Eleveld MJ, Tavares DA, Carriço JA, Bootsma HJ, et al. Highly penicillin-resistant multidrug-resistant pneumococcus-like strains colonizing children in Oeiras, Portugal: genomic characteristics and implications for surveillance. *J Clin Microbiol* 2010; 48: 238-246.
7. Bentley SD, Aanensen DM, Mavroidi A, Saunders D, Rabbinowitsch E, Collins M, et al. Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. *PLoS Genet* 2006; 2(3): e31.
8. Hathaway LJ, Stutzmann Meier P, Bättig P, Aebi S, Mühlemann K. A homologue of AliB is found in the capsule region of nonencapsulated *Streptococcus pneumoniae*. *J Bacteriol* 2004; 186: 3721-3729.
9. Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, et al. Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae:* updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* 2013; 32: 165-179.
10. Keith ER, Podmore RG, Anderson TP, Murdoch DR. Characteristics of *Streptococcus pseudopneumoniae* isolated from purulent sputum samples. *J Clin Microbiol* 2006; 44: 923-927.
11. Ktari S, Ben Ayed NEH, Maalej S, Mnif B, Rhimi F, Hammami A. Clinical optochin resistant *Streptococcus pneumoniae* and *Streptococcus pseudopneumoniae* strains in Tunisia. *J Infect Dev Ctries* 2021; 15: 672-677.
12. Ercibengoa M, Morales M, Alonso M, Ardanuy C, Marimón JM. Variants of the bile: solubility test to differentiate *Streptococcus pneumoniae* from other viridans group streptococci. *Future Microbiol* 2019; 14: 949-955.
13. O'Brien KL, Bronsdon MA, Dagan R, Yagupsky P, Janco J, Elliott J, et al. Evaluation of a medium (STGG) for transport and optimal recovery of *Streptococcus pneumoniae* from nasopharyngeal secretions collected during field studies. *J Clin Microbiol* 2001; 39: 1021-1024.
14. Simões AS, Valente C, De Lencastre H, Sá-Leão R. Rapid identification of noncapsulated *Streptococcus pneumoniae* in nasopharyngeal samples allowing detection of co-colonization and reevaluation of prevalence. *Diagn Microbiol Infect Dis* 2011; 71: 208-216.
15. Wessels E, Schelfaut JJG, Bernards AT, Claas ECJ. Evaluation of several biochemical and molecular techniques for identification of *Streptococcus pneumoniae* and *Streptococcus pseudopneumoniae* and their detection in respiratory samples. *J Clin Microbiol* 2012; 50: 1171-1177.
16. Mohammadi JS, Dhanashree B. *Streptococcus pseudopneumoniae:* an emerging respiratory tract pathogen. *Indian J Med Res* 2012; 136: 877-880.
17. Park HK, Lee S-J, Yoon JW, Shin JW, Shin H-S, Kook J-K, et al. Identification of the *CpsA* gene as a specific marker for the discrimination of *Streptococcus pneumoniae* from viridans group streptococci. *J Med Microbiol* 2010; 59: 1146-1152.
18. Keith ER, Murdoch DR. Antimicrobial susceptibility profile of *Streptococcus pseudopneumoniae* isolated from sputum. *Antimicrob Agents Chemother* 2008; 52: 2998.
19. Harf-Monteil C, Granello C, Le Brun C, Monteil H, Riegel P. Incidence and pathogenic effect of *Streptococcus pseudopneumoniae*. *J Clin Microbiol* 2006; 44: 2240-2241.
20. Sethi S, Evans N, Grant BJB, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. *N Engl J Med* 2002; 347: 465-471.